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TESTICULAR MORPHOMETRY AND SPERMATOZOA CHARACTERISTICS OF COCKS SERVED FLUTED PUMPKIN LEAF EXTRACT

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ABSTRACT

The study was conducted to determine the effects of fluted pumpkin leaf on the testicular morphometry characteristics and volumetric proportion of testicular elements of cocks administered the extract. A total of 180 14-day old cockerel chicks were randomly allotted to six treatments each with 3 replicate groups containing 10 chicks per replicate. Fluted pumpkin was added at 0, 30, 60, 90, 120 and 150mL/liter of drinking water for treatments 1, 2, 3, 4, 5 and 6 respectively. Result showed that there was no significant ($p>0.05$) difference in the testicular morphometric characteristics of cocks across treatments. The round spermatids for cocks on treatments 1 - 4 were within a range of 15.60% - 17.33% while birds on treatment 6 had the lowest value of 12.93%. The proportions of spermatozoa for birds on treatments 1 (2.67%), 2 (2.93%) and 4 (1.47%) were similar but significantly higher ($p < 0.05$) than what obtained for cocks on treatment 3 (0.67%), 5 (0.13%) and 6 (0.27%). Cocks on treatment 1 had the highest value (4.8%) for Sertoli cells proportions and the lowest value (3.72%) for Leydig cell proportion while cocks on treatments 2, 3, 4 and 5 were similar (4.27 - 6.67%) Administering of fluted pumpkin leaf extract to cocks at high levels may lead to reproductive defects and therefore it should not be administered in excess of 90mL fluted pumpkin leaf extract per liter of water.

Keywords: Fluted pumpkin, Leaf extract, Testicular morphometry, Testicular elements, Cocks
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INTRODUCTION

The use of supplements (organic and inorganic) to produce special effect in animal production is on the increase. These supplements are growth promoters and they exist in different forms (Dairo, 2006). Herbs and spices have been found and widely used as alternative therapies in both human and animal medicines. Therefore, more attention needs to be paid to widely cultivated vegetables in the tropics and sub-tropics exploit their leaf extracts as protein and mineral supplement in poultry nutrition. Herbal medicines entail the use of plant seed, berries, roots, leaves, bark or flowers for medicinal purposes.

Fluted pumpkin (*Telfairia occidentalis* Hook F.) of the family Cucurbitaceae is a common

vegetable consumed by many ethnic group in Nigeria. The nutritive value of the leaves of fluted pumpkin (FP) has been evaluated chemically and is found to be rich in protein, oil and minerals (Aletor *et al.,* 2002). Studies have shown that FP leaf is rich in minerals (iron, potassium, sodium, phosphorus, calcium and magnesium) antioxidant, vitamins (thiamine, riboflavin, nicotinamide and ascorbic acids), phytochemicals such as phenols (Fasuyi, 2006, Oboh and Akindahunsi, 2004). Fluted pumpkin has also been found to suppress or prevent the production of free radicals and scavenges already produced free radicals, lower lipid preoxidation status and elevates antioxidant enzymes (Iweala and Obidoa 2009, Adaramoye *et al.,* 2007). Many medicinal plants have been reported to have anti-spermatogenic effects. Testicular damage was observed with the use of *Curcuma comasa* extract (Piyacchaturawati *et al.,* 1998) and *Vernonia amygdalina* extract (Oyeyemi *et al.,* 2007). Alkaloids, tannins, saponins and flavonoids have been reported to be some of the photochemical constituent of this plant (Akubue *et al.,* 1980). Nwangwa *et al.* (2007) reported that FP have the potential to regenerate testicular damage and also increase spermatogenesis in rats, while Adisa *et al*. (2014) reported that FP have potential of reducing male sexual functions because of its observed effect on semen quality, plasma testosterone level and histological presentation of the testis.

There has not been any documented report on the effect of aqueous extract of FP on the reproductive parameters of cocks. This study was carried out to investigate the effects of the administration of aqueous extract of FP on the testicular morphometric characteristics and volumetric proportions of testicular elements of cocks.

MATERIALS AND METHODS

Preparation of Fluted pumpkin leaf extract: One kilogram of freshly cut fluted pumpkin leaves with the stalk was separated from the stem, washed with clean water to remove dirt and sand, drained and chopped. This was then squeezed and filtered with cheese cloth to obtain a homogenous extract of the fluted pumpkin leaves. The homogenous leaf extract was prepared at interval of four days and served to the animals fresh according to treatments.

Experimental animals and management: One hundred and eighty (180) 14-day old Nera black cockerel chicks were randomly allotted to six treatments in a completely randomized design (CRD). Each treatment was replicated 3 times with ten (10) birds per replicate and fed with the same diet at the starter and finisher phase. All diets were formulated to meet the physiological needs of the birds at both phases. Fluted pumpkin leaf extract was added at 30, 60, 90, 120 and 160mL/litre of drinking water representing treatments 2, 3, 4, 5 and 6 respectively. A treatment without TOLE (0%) serves as the control and designated as 1.

Feed was given *ad libitum* and water served freely after the consumption of the FP leaves extract. FP leaves extract were served at four days intervals throughout the experimental period. Routine management practices were carried out.

At the end of twenty fourth weeks two birds per replicate were slaughtered by cutting the jugular vein after they were slaughtered by cutting the jugular vein after they had been stunned mechanically. After evisceration, the left and right testes were removed, weighed and labeled for proper identification. The testes volumes were determined using Archimedes principles of water displacement in a measuring cylinder and the densities were calculated using the mass and volume ratio. The right and left

epididymis were also carefully removed and weighed. The weights were recorded to the nearest 0.01g.

Volumetric proportions of cellular elements in the seminiferous epithelium were determined by the method of Chalkey (1943) as modified by Egbunike and Steinbach (1972). It essentially involved the counting of the number of hits by cellular elements in 20 fields in each of the two slides per bird with an intergrating eye piece (Zeiss oberkochen) having 25 points asymmetrically arranged in a cycle and calculated accordingly. Stages in the cycle of seminiferous epithelium were determined by classifying twenty seminiferous tubules in each of the slides per bird. The frequency of occurrence of each stage was calculated on percentage basis.

$$\text{Volume \% of element} = \frac{\text{Total hits on the element} \times 100}{\text{Total possible hits-no of artifacts hit}}$$

Data analysis: Data collected were subjected to one way analysis of variance of statistical analysis (SAS, 1999). Treatment means were compared using Duncan's New Multiply Range Test (Obi, 1990).

Table 1: Composition (%) of chicks and grower's diets fed to cockerels and cocks

Diets	Chicks mash	Growers mash
Ingredient (%):		
Maize	45.30	39.00
Corn bran	7.00	5.00
Wheat offal	20.0	9.80
Fish meal	2.00	1.00
Soya bean meal	20.0	15.0
Palm kernel cake	-	26.0
Dicalcium phosphate	2.00	2.50
Oyster shell	3.00	1.00
Lysine	0.10	0.10
Methionine	0.10	0.10
Chicks premix	0.25	-
Grower premix	-	0.25
Table salt	0.25	0.25
Total	**100**	**100**
Calculated nutrient (%):		
Crude protein	18.52	16.85
Crude fibre	6.31	8.60
Ether extract	3.46	4.59
Lysine	1.10	0.98
Methionine	0.40	0.43
Calcium	1.80	1.19
Phosphorus	0.79	0.85
Metabolizable energy (kcal/kg)	2748	2774

RESULTS AND DISCUSSION

The testicular morphometric properties of cocks administered FPLE orally is as presented in Table 2 below. There were no significant ($p > 0.05$) differences in the testicular morphometric characteristics across the treatments.

The result of the volumetric proportions of testicular elements is as presented in table 2. The testicular elements that were significant ($p < 0.0.5$) are the round spermatids, elongated spermatids, spermatozoa, sertoli cells, lumen and leydig cells. The round spermatids for birds on treatment 1-4 were similar ($p > 0.05$) with a range between 15.60 and 17.33% while birds on treatment 5 had the least value (12.93%) though it was not significantly ($p > 0.05$) different from those of treatments 2 (16.13%), 4 (15.60%) and 5 (13.20%). Birds on treatment 3 (11.86%) had significantly ($p < 0.05$) higher value for round spermatids though it was similar to the value obtained for birds on treatments 1 (8.27%), 2 (9.20%) and 5 (10.67%) while birds on treatment 6 (4.63%) recorded the lowest value which was similar to the value obtained for treatment 5 (6.67%).

The proportion of the spermatozoa for birds on treatment 1 (2.67%), 2 (2.93%) and 4 (1.47%) were similar but significantly superior to those of cocks on treatments 3 (0.67%), 5 (0.13%) and 6 (0.27%).

Cocks on the treatment 1 (4.80%) had a significantly higher sertoli cells proportion though it was similar to those of cocks on treatment 5 (3.73%). Cocks on treatment 3 (2.26%) had the least value which was similar to the values obtained for cocks on treatments 2 (3.2%), 4 (2.8%) and 6 (3.07%).

The proportion occupied by leydig cells was significant ($p < 0.05$) for birds on treatment 5 (6.67%) though it was similar to the values obtained for birds on treatments 2 (4.55%), 3 (4.27%) and 6 (6.40%) while the least value (3.72%) was obtained for cocks on treatment 1 and it was similar to the value obtained for cocks on treatment 4 (3.60%). The values of leydig cells proportions for cocks on treatments 2, 3, 5 and 6 (4.27 – 6.67%) were similar while the least value (3.72%) was obtained for cocks on treatment 1.

Table 2: Testicular morphometric characteristics of cock served fluted pumpkin leaf extract

Level of FPLE (ml/L)	Treatments						SEM
	1	2	3	4	5	6	
	0	30	60	90	120	150	
Traits:							
Body weight (g)	2180	2190	2290	2216.7	1923.3	1956.7	5.12
Right testis weight (g)	10.64	13.19	14.26	11.24	8.04	7.58	0.98
Left testis weight (g)	10.46	13.07	12.79	9.03	8.54	8.58	0.76
Paired testes weight (g)	21.11	26.27	28.05	11.48	16.57	13.49	1.63
Right testis volume (cm^3)	9.80	13.00	13.67	9.50	8.50	7.67	0.93
Left testis volume (cm^3)	10.50	13.67	13.30	8.17	8.50	8.00	0.73
Right testis density (g/cm^3)	0.05	0.05	0.04	0.05	0.05	0.05	0.01
Left testis density (g/cm^3)	0.05	0.05	0.04	0.05	0.05	0.06	0.01
Right Epididymis weight (g)	0.89	0.73	1.14	0.58	0.56	0.80	0.06
Left Epididymis weight (g)	0.84	0.94	1.05	0.80	0.61	0.82	0.05
Paired Epididymis weight (g)	1.74	1.66	2.04	1.38	1.16	1.63	0.09

Table 3: Volumetric proportions (%) of testicular elements of cock served fluted pumpkin leaf extract

Level of FPLE (ml/L)	Treatments 1 0	2 30	3 60	4 90	5 120	6 150	SEM
Elements (%)							
Spermatogonia	19.73	19.33	17.47	18.27	19.07	20.40	0.48
Primary spermatocyte	18.13	19.07	19.33	20.40	18.67	20.27	0.44
Secondary spermatocyte	16.13	15.87	15.60	17.53	17.20	17.60	0.34
Round spermatids	17.33^{a}	16.13^{ab}	17.33^{a}	15.60^{abc}	12.93^{bc}	12.93^{bc}	0.41
Elongated spermatids	8.27^{abc}	9.20^{ab}	11.87^{a}	10.67^{ab}	6.67^{bc}	4.63^{c}	0.58
Spermatozoa	2.67^{a}	2.93^{a}	0.67^{b}	1.47^{ab}	0.13^{b}	0.27^{b}	0.21
Sertoli cells	4.80^{a}	$3.20b^{c}$	2.26^{c}	2.80^{bc}	3.73^{ab}	3.07^{bc}	0.18
Lumen	3.73^{b}	4.53^{ab}	5.60^{ab}	4.13^{b}	7.20^{a}	7.33^{a}	0.39
Artifacts	5.47	5.33	5.87	5.20	7.33	6.83	0.45
Leydig cells	3.72^{b}	4.53^{ab}	4.27^{ab}	3.73^{ab}	6.67^{a}	6.40^{ab}	0.36

$^{a-c}$Means along the same row with different superscript are significantly ($p< 0.05$) different.
SEM = Standard Error of Means

There were no significant effects of FPLE on all the testicular and epididymal characteristics as shown in Table 2. The paired testes weight obtained in this study were within the range of 11.40-28.05 grammes. The mean weight of the right and left testes across the treatments were statistically similar, except for treatments 5 (120ml/L) and 6 (150ml/L). However, the right testes were observed to be heavier than the left testes. This observation was contrary to earlier reports by Nkanga (1989) and Ogunlade, *et al.,* (2006) but similar to those of King and Mclleland (1975).

The epididymal weights also showed no significant ($p > 0.05$) treatment effects and were reflections of the weight on the testes. The superiority of the left testis reported in literature was not reflected in this experiment.

FPLE did not have any significant effect on the proportions of the spermatogonia, primary spermatocyte, secondary spermatocytes and artifacts. The variations of these testicular elements did not follow any particular trend.

However, the proportions of the round spermatids, elongated spermatids, spermatozoa, sertoli cell and leydig cells were significantly influenced by the FPLE. Cocks on treatments 5 and 6 recorded the lowest values for the round and elongated spermatids and spermatozoa proportions. This could be as a result of the higher doses at the level. This result were similar to earlier report by Saalu *et al*., (2010) who reported a testicular protective effect in rats treated with doses of *Telfaira occidentalis* extract less than 400mg/kg body weight and testicular toxic effect at concentration greater than 400mg/kg body weight. Oyeyemi *et al.,* (2008) reported that *Telfairia occidentalis* leaves have a destructive effect on testis. Adedapo *et al*., (2008) also reported a marked testicular degeneration with severe disorganization of seminiferous tubules which were devoid of spermatic cells when aqueous extract of *Telfairia occidentalis* leaves were administered to rats both at 800 and 1600 mg/kg body weight. Testicular damage was also observed with the use of *Curcuma comasa* extract (Piyacchaturawati *et al.,*

1998) and *Vernonia amygdalina* (Oyeyemi et al., 2007) extract. These observations however, were contrary to the findings of Iweala and Obiodoa (2009) who reported the presence of thick membrane and large spermatogonia for rats to long term consumption of *Telfairia occidentalis* supplemented diet Nwangwa *et al.,* (2007) reported that *Telfairia occidentalis* leaves extract had also been reported to elicit regenerative effect on the histology of rat testes. It was also reported (Akang *et al.,* 2010) that *Telfairia occidentalis* leaves extract reduce lipid peroxidation thereby improving spermatogensis. The proportion of the spermatozoa and sertoli cells were highest for birds on the control treatment 2.67% and 4.8% respectively). , There was a correlation between the sertoli cells proportions and spermatozoa proportion for cocks on the FPLE treatments which was consistent with the findings of Nkanga and Egbunike (1990). The observed correlation between the spermatozoa and sertoli cells can be explained by the fact that morphological transformations of spermatids during spermiogenesis occur with the spermatids embedded within cytoplasmic pockets of individual sertoli cells.

The proportion of the lumen were statistically different and highest for cocks on treatments 2 (4.53%) and 3 (5.6%) while the least values were obtained for cocks on treatment 4 (4.13%) and 1 (3.73%).. The lumen is expected to be a reflection of the spermatozoa and sertoli cells because if the proportions of spermatozoa and sertoli cells are high there must be a corresponding increase in the proportion of and size for the lumen to accommodate the released spermatozoa. The observation in this study however, did not reflect this trend.

Leydig cells proportions were statistically different with cocks on treatment 5 having the highest value of 6.67% though it was not statistically different from treatments 2 (4.53%), 3 (4.27%) and 6 (64.0%) while the proportions of leydig cells for cocks on treatments 1 (3.72%) and 4 (3.60%) were significantly lower than those obtained for cocks on treatment 5.The leydig cells are the sites for testosterone secretion. Testosterone is needed for growth and development of male reproductive organ (Mooradian et al., 1987) and in association with follicular stimulating hormone acts on the seminiferous tubules to initiate and maintain spermatogenesis. This could be the reason why the proportions of spermatogonia, primary spermatocyte and secondary spermatocyte were numerically higher for cocks on FPLE treatments than for cocks on the control.

CONCLUSIONS

- Fluted pumpkin leaf extract had no adverse effects on the testicular morphometric characteristics of cocks.
- Serving Fluted pumpkin leaf extract up to 90mL/liter of water had no deleterious effect on the volumetric proportions of testicular elements of cocks such as the proportions of the round spermatids, elongated spermatids, spermatozoa, sertoli cell and leydig cells.
- Cocks for breeding purpose should not be given fluted pumpkin leaf extract in excess of 90mL/liter of water to prevent any adverse effects on reproductive parameters.

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