RESPONSES OF NIGERIAN LOCAL COCKERELS TO DIETARY VITAMIN A SUPPLEMENTATION

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ABSTRACT
A five-week experiment was conducted to investigate the effect of dietary vitamin A (VA) supplementation on blood testosterone and semen quality characteristics of Nigerian local cockerels. Sixty-four, pubertal local cockerels were divided into 4 groups of 16 birds, and each group replicated four times. Each group was assigned to one experimental diet in a completely randomized design designated Q_0 (0 mg VA per 300 g dry matter (DM feed)), Q_1 (0.75 mg VA per 300 g dry matter (DM feed)), Q_2 (1.50 mg VA per 300 g dry matter (DM feed)) and Q_3 (2.25 mg VA per 300 g dry matter (DM feed)). Semen and blood samples for analysis were randomly obtained from 2 birds in each replicate and analysed statistically. A quadratic equation was used to determine the VA levels for optimum serum testosterone and semen values. Daily VA supplementation at 0.75 mg/ 300g DM feed increased ($P<0.05$) ejaculate volume and sperm concentration value. In addition, daily VA supplementation at 1.15 mg / 300 g DM feed increased normal sperm and active motile cells by 1.78% and 24.51%, respectively while, daily VA administration at 2.25 mg / 300 g DM feed reduced ($P<0.05$) sluggish motile cells by 57.32% and increased ($P<0.05$) serum testosterone by 49.00%. Results of quadratic function revealed that VA levels of 2.03, 3.27, 2.24, 1.22, 2.57 and 2.08 mg / 300 g DM feed supported optimum ejaculate volume, pH, sperm concentration, active motile sperm and normal sperm production while the highest ratio of 4.16 supported optimum testosterone level. There are significant ($P<0.01$) regression effect on sluggish motile sperm and serum testosterone value of local cockerels on daily VA. It is concluded that daily vitamin A supplementation at 0.75 – 2.25 mg /300 g DM feed positively influenced blood testosterone and some semen traits of local cockerels.
Keywords: Local cockerels, vitamin A, semen, testosterone.

INTRODUCTION
Indigenous chickens are predominant flock composition and make up about 98% of the total poultry numbers (chickens, ducks and turkeys) kept in Africa (Gueye, 2003). They are widely distributed in the rural areas of tropical countries where they are kept by women, the main managers of family poultry. Indigenous poultry production in Nigeria and elsewhere in Africa fulfills important social and economic functions. They are generally hardy, adaptive to rural environments, survive on little or no care and adjust to changes in feed resources. They have the ability to hatch their eggs and brood their chicks. Despite this attributes, their productivity is low and thus they have slow growth rate, low feed efficiency and poor reproductive rate when compared to other exotic chicken breeds in commercial systems (Molekwa, 2007). The solution is therefore to explore the use of vitamins which hitherto is under exploited by resource poor smallholder poultry farmers. However, limited information exists on the effect of vitamin A administration on reproductive physiology of local cockerels. This study therefore was conducted to determine the effect of graded doses of vitamin A administration on semen quality characteristics and serum testosterone concentration of Nigerian local cockerels.

MATERIALS AND METHODS
This study was conducted at the Teaching and Research Farm of the Federal University of Technology Owerri, Imo State, Nigeria. Imo State lies between latitude 4°4' and 6°3' N and longitude 6°15' and 8°15'E (Google Earth, 2015). Local cockerels were sourced from Relief Market Owerri. The birds used were Nigerian indigenous normal feathered cockerels. Vitamin A (soft gel capsules filled with pale yellow oily solution manufactured by Mason Vitamins, Inc, USA) were bought from Milan Pharmacy at Owerri, Nigeria. A capsule that weighed 1.48 g was cut open and the content extracted using 10 g of corn starch. The animals used in this study were 64 local cockerels aged 40 – 44 weeks with an average live weight of 1.18 kg. Two weeks pre-treatment periods was used to acclimatize the animals to their new environment and to get them used to the experimental procedures. The 64 local cockerels were divided into 4 groups of 16 birds each. Each of the four groups were further

divided into four replicates of 4 cocks each on weight equalization basis (Zduńczyk *et al*, 2002). Each replicate was housed in compartment measuring 3.3m x 1.7m in an open sided poultry house measuring 16.8m x 4.5m x 2.25m. The floor was cemented and covered with wood shaving as litter. The crude protein, ether extract, crude fibre, salt, calcium, phosphorus, lysine and methionine of the experimental diet were 18.00, 6.00, 5.00, 0.30, 1.00, 0.40, 0.85 and 0.35 %, respectively, with a metabolizable energy of 2900 Kcal/kg. The four experimental cock groups were randomly assigned to the four vitamin A treatments designated Q_0 (0 mg VA / 300g DM feed), Q_1 (0.75 mg vitamin A per 300g DM feed), Q_2 (1.5 mg vitamin A per 300g DM feed) and Q_3 (2.25 mg vitamin A per 300g DM feed) in a completely randomized design experiment. Feed and water were offered *ad libitum* and the experiment lasted 5 weeks (35 days).

Semen was collected once a week for two weeks from two cocks in each replicate through abdominal massage technique (Lake, 1957) and evaluated for semen quality parameters following standard procedures. Blood samples for determination of testosterone concentration were obtained from four birds selected from each treatment group and analyzed with the aid of the tube based enzyme immunoassay method, according to the method of Micaleft *et al.* (1995) as described for the kit (BioCheck ELISA Assay, USA).

Data on semen quality and testosterone were analyzed using the general linear model procedure of the statistical analysis of variance (SAS, 2008). Optimal response in testosterone and semen quality characteristics to the dietary vitamin A supplementation level for the cockerels was modeled using the following quadratic equation $Y = a + b_1x + b_2x^2$. Where, Y is semen quality and testosterone; a is the intercept; b_1 and b_2 is the coefficients of the quadratic equation; x is the daily vitamin A dose level and $-b_1/2b_2$, which is the x value for the optimum response. The quadratic model was fitted to the experimental data by means of the non-linear model (NLIN) procedure of SAS (2008).

RESULTS AND DISCUSSION

Daily vitamin A administration had no significant effect ($P<0.05$) on sperm cells with swollen neck (Table 1). Ejaculate volume increased from Q_0 and Q_1 and the observed difference was significant ($P<0.05$). However, this fall is within the normal range (7.2 – 7.6) reported by Hafez and Hafez (2000) for clinically healthy local cockerels. Local cockerels administered daily vitamin A at 0.75 mg per 300 g DM feed had increased ($P<0.05$) ejaculate volume (ml) and sperm concentration (10^6/ml) values than those at 0 mg, 1.5 mg and 2.25 mg of vitamin A per 300 g DM feed. Observed semen volumes were higher than values of 0.20 – 0.50 mL reported for domestic cockerels by Hafez and Hafez (2000). Number of sluggish motile sperm cells were significantly reduced ($P<0.05$) for birds administered vitamin A at 1.5 mg and 2.25 mg per 300 g DM feed in comparison to those on the other two vitamin A treatment levels. This is an indication that vitamin A at these levels of administration support the production of motile sperm cells. Normal sperm cells ranged between 92.33 and 95.67%, this was slightly above the range (85 – 90%) reported by Hafez and Hafez (2000). Higher ($P<0.05$) testosterone value was obtained in the group administered 2.25 mg of vitamin A per 300g DM feed. Daily administration of vitamin A at 0.75 mg per 300g DM feed increased ($P<0.05$) ejaculate volume and sperm concentration values by 8.24% and 259.74%, respectively (Table 2). Similarly, daily vitamin A administration at 1.5 mg and 2.25 mg per 300 g DM feed increased ($P<0.05$) sperm with active motile cells 19.12 – 24.51%, while testosterone level was increased by 49.00%, at 2.25 mg of vitamin A administration. The observed 1.18 – 8.24% significant increase in ejaculate volume as observed was in harmony with the findings of Etchu and Egbunike (2002), that secretory functions of the accessory sex glands are very sensitive to dietary changes. The observed 19.12 – 24.51% increase in the number of active motile sperm as observed in this study could be attributed in part to the ability of vitamin A to support the production of motile cells in male chickens.

Table 3 presents a series of quadratic regressions that predict optimum vitamin A dose level for sperm quality characteristics and testosterone levels of local cockerels. Results indicate that ejaculate volume was optimized at vitamin A level of 2.03 mg per 300 g DM feed ($r^2 = 0.0891$), pH at 3.27 mg per 300 g DM feed ($r^2 = 0.3152$), sperm concentration at 2.24 mg per 300 g DM feed ($r^2 = 0.4561$), active motile sperm at 1.22 mg per 300 g DM feed ($r^2 = 0.4583$), normal sperm at 2.57 mg per 300 g DM feed ($r^2 = 0.0404$) and testosterone at 2.08 mg per 300 g DM feed ($r^2 = 0.8623$). These results have implications when formulating diets for local cocks. Thus dietary vitamin A supplementation level for optimal productivity will depend on the variable in question. The r values were all positive, with the values ranging from low to high. The r^2 values ranged from 0.0404 to 0.8623, which is 4.04 to 86.23% in

percentages of R^2. Furthermore, the blood testosterone (ng/ml) and non motile sperm (%) had very strong coefficient of determination. The significant regression on sluggish motile sperm and blood testosterone values implied that their values can be predicted given a quantity of vitamin A to the cocks. In the present study, vitamin administration level for optimum semen pH, normal sperm cells, bent neck, swollen head and swollen neck was not reached. It is possible that the levels of vitamin A required for optimum parameters listed above in the present study were higher than the values used in the present study. However, the result shows that minimum active motile sperm cell (%) increase was achieved at optimum vitamin A level of 1.22 mg per 300 g DM feed ($Y = 67.915 - 6.718x + 2.75x^2$; $r^2 = 0.4583$; $p = 0.0984$). This suggests that the dose level of 1.22 mg per 300 g DM feed could ensure production of motile sperm cells and hence support spermatogenesis in local cockerels. Thus it is clear from our result that administration of vitamin A above 1.22 mg per 300 g DM feed will reduce sperm with active motile cells and have a negative effect on quality of the spermatozoa produced by local cockerels.

CONCLUSION

This study reveals that vitamin A supplementation to the diet of local cockerels has positive effect on semen quality and testosterone levels. Vitamin A requirements of local cocks for optimal productivity were variable and depended on the production parameters under investigation. These results have a lot of implications on ration formulation for local cocks. However, more study should be carried out to ascertain these responses.

REFERENCES

Etchu, K.A. and Egbunike, G.N. (2002). Effect of processed sweet potato on the performance of broiler birds in the humid tropics during the rainy season. *Trop. Anim. Prod. Invest.*, 5: 67 -78, 2002.

Google Earth (2015). Owerri google earth 3D map, 2015. www. Maplandia.com/Nigeria/imo/Owerri-google-earth.html.

Gueye, E.F. (2003). Production and consumption trend in Africa. *World Poult.*, 19: 12-14.

Hafez, B. and Hafez, E.S.E. (2000). Reproduction in farm animals. 7th ed. Lippincoh Williams and Wikkins, USA, New York.

Lake, P.E. (1957). The male reproductive tract the fowl. *J. Anatomy* 91:116-129.

Micaleft, J.V., Hays, M.M., Latif, A., Alhasan, R. and Sufi, S. (1995). Serum binding of steroid tracers and its possible effects on direct steroid immunoassay. *Ann. Clin. Biochem.*, 32 : 566 - 574.

Molekwa, J.T. (2007). Relationships between cock semen viability and the fertility of artificially inseminated South African indigenous chicken breeds. Dissertation M. Tech. Degree, CUT, Free State. Bloemfontein.

SAS (2008). Institute Inc. SAS Technical Report Package 234 SAS/STAT Software. The GEMOD Procedure. Release 6.09.SAS Institutes Inc. Cary, NC. USA.

Zduñczyk, Z., Jankowski, J. and Koncicki, A. (2002). Growth performance and physiological state of turkeys fed diets with higher content of lipids oxidation products selenium, vitamin E and vitamin A. *World's Poult. Sci. J.*, 58 (3): 357 – 364.

Table 1: Effect of vitamin A administration on semen and testosterone values of Nigerian local cockerels

Parameters		Vitamin A administration levels				SEM
		Q_0	Q_1	Q_2	Q_3	
Semen pH (pH)	-	7.07^{ab}	7.13^{b}	6.80^{a}	7.00^{ab}	0.0953
Ejaculate volume (EV)	ml	0.85^{ab}	0.92^{c}	0.82^{a}	0.86^{b}	0.0166
Sperm concentration (SC)	10^6	0.77^{a}	2.77^{b}	0.67^{a}	1.53^{a}	0.3304
Active motile sperm (AM)	%	68.00^{b}	53.00^{a}	84.67^{b}	81.00^{b}	5.1200
Sluggish motile sperm (SM)	%	25.00^{b}	35.00^{b}	13.67^{a}	10.67^{a}	3.5515
Non motile sperm (NM)	%	2.00^{a}	2.67^{a}	0.00^{a}	8.33^{b}	1.3769
Normal sperm (NS)	%	94.00^{b}	92.33^{a}	95.67^{c}	93.00^{ab}	0.5094
Bent neck (BN)	%	1.00^{a}	2.67^{b}	1.33^{a}	2.67^{b}	0.3361
Swollen head (SH)	%	2.33^{ab}	2.67^{b}	2.00^{a}	2.33^{ab}	0.1880
Swollen neck (SN)	%	2.33	2.65	2.00	2.33	0.2752
Testosterone	ng/ml	4.00^{b}	1.93^{a}	4.16^{b}	5.97^{c}	0.4724

^{a,b,c} Means in the same row with different superscripts are significantly different ($p<0.05$); Q_0 = 0 mg vitamin A / 300 g DM feed; Q_1 – 0.75 mg vitamin A per 300 g DM feed; Q_2 – 1.5 mg vitamin A per 300 g DM feed; Q_3 – 2.25 mg vitamin A per 300 g DM feed; SEM - Standard error of the mean.

Table 2: Percentage changes from control and vitamin A dose levels on significant testosterone and semen traits in local cockerels.

Traits	Q1			Q2			Q3		
	(%)	Change	(P<0.5)	(%)	Change	(P<0.5)	(%)	Change	(P<0.5)
pH	0.85	+ve	Ns	3.82	-ve	ns	0.99	-ve	Ns
EV	8.24	+ve	*	3.53	-ve	ns	1.18	+ve	Ns
SC	259.74	+ve	*	12.99	-ve	ns	98.70	+ve	Ns
AM	22.06	-ve	*	24.51	+ve	*	19.12	+ve	*
SM	40.00	+ve	ns	45.32	-ve	*	57.32	-ve	*
NM	33.50	+ve	ns	100.00	-ve	ns	433.00	+ve	*
NS	1.78	-ve	*	1.78	+ve	ns	1.06	-ve	Ns
BN	126.00	+ve	*	33.00	+ve	ns	126.00	+ve	*
SH	14.59	+ve	ns	14.16	-ve	ns	0.00	nil	Ns
TS	51.75	-ve	*	4.00	+ve	ns	49.00	+ve	*

+ve = positive; -ve = negative; * = Significant difference (P<0.05) between Q_0 and Q_1, Q_2, Q_3; ns – not significant different (P<0.05) between Q_0 and Q_1, Q_2, Q_3.

Table 3: Effect of vitamin A dose level on optimal semen and testosterone values of Nigerian local cockerels

Parameters	Formula	VA	r^2	p-value	Significance
Ejaculate volume	$Y = 0.8425 + 0.0305x - 0.0075x^2$	2.03	0.0891	0.5476	Ns
Semen pH	$Y = 7.31 - 0.229x + 0.035x^2$	3.27	0.3152	0.6981	Ns
Sperm concentration	$Y = 0.8075 + 2.4085x - 0.5375x^2$	2.24	0.4561	0.0584	Ns
Non motile sperm cells	$Y = 8.745 - 7.943x + 1.915x^2$	2.07	0.7313	0.1581	Ns
Sluggish motile sperm cells	$Y = 20.915 + 9.818x - 3.25x^2$	1.51	0.6689	0.0247	*
Active motile sperm cell	$Y = 67.915 - 6.718x + 2.75x^2$	1.22	0.4583	0.0984	Ns
Normal sperm cell	$Y = 92.415 + 1.284 - 0.25x2$	2.57	0.0404	0.0532	Ns
Bent neck	$Y = 0.5875 + 0.7795x - 0.0825x^2$	4.72	0.3021	0.1534	Ns
Swollen head	$Y = 2.4875 - 0.054x - 0.00325x^2$	8.38	0.1001	0.7278	Ns
Swollen neck	$Y = 4.51 - 2.009x + 0.335x^2$	2.99	0.6462	0.1504	Ns
Testosterone	$Y = 6.8175 - 4.0225x + 0.9675x^2$	2.08	0.8623	0.0018	*

r^2: Regression coefficient; P: Probability; VA - Vitamin A administration (mg / kg dry matter feed) for optimum variable.