

Effect of a Velogenic Newcastle Disease Virus on Testosterone Concentration and Gonadal Sperm Reserves of Vaccinated Shikabrown Cocks

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Abstract: About fifty 20 weeks old Shika Brown (SB) cocks were used in this study. Five cocks consisting of three Red Shika-Brown (RSB) and two White Shika-Brown (WSB) were bled for serum samples for testosterone assay at weeks 1, 3 and 6 pre and post-infection with a Velogenic Newcastle disease virus. Blood samples were collected at 30 min interval for 3 h from each cock on the days of sampling. The blood samples were centrifuged in a Hermle Z364 centrifuge at $251.6 \times g$ for 15 min with the sera obtained stored in serum vials and kept in a deep freezer at -20°C until analysis using the Enzyme Linked Immunosorbent Assay (ELISA) technique. At the end of the study, twenty control ($n = 20$) and twenty infected ($n = 20$) cocks were slaughtered. Their testicles were removed, measured, minced and ground for the determination of gonadal sperm reserves. The mean testosterone concentration of both the control SB cocks and the pre-infected SB cocks had no particular pattern. The mean testosterone concentration post infection showed a decrease from week 1-6. The mean testosterone concentration peak for the control red SB cocks was 1, 2 and $2.5 \text{ nmols mL}^{-1}$ at weeks 1, 3 and 6, respectively while the white SB cocks had 12.5, 5.5, 3 nmols mL^{-1} at weeks 1, 3 and 6, respectively. The infected red SB cocks had mean testosterone concentration peaks of 9.7, 6.3 and $2.7 \text{ nmols mL}^{-1}$ at weeks 1, 3 and 6 post-infection, respectively while the white SB cocks had a mean testosterone concentration peak of 6.5, 14.5 and $6.5 \text{ nmols mL}^{-1}$ at weeks 1, 3 and 6 post-infection, respectively. The gonadal sperm reserves of the control red and white SB cocks were not significantly different but the gonadal sperm reserves of the control white SB cocks was significantly ($p > 0.05$) higher than the gonadal sperm reserves of the infected red and white SB cocks. The total gonadal sperm reserve of the control white cocks was significantly ($p > 0.05$) higher than the total gonadal sperm reserves of the infected red and white SB cocks.

Key words: Shikabrown, sperm, testosterone concentration, infected, peak, centrifuged

INTRODUCTION

The general study of hormones such as gonadotropin releasing hormone, follicle stimulating hormone, luteinizing hormone, estrogen, prostaglandin, testosterone and progesterone has clarified the fundamental mechanisms which regulate puberty, sexual (function) cycles, gestation and lactation in domestic animals (Edqvist *et al.*, 1976; Peters and Ball, 1995; Rekwot, 2000). The association between onset of spermatogenic activity and increased testosterone concentration has been reported (Karg *et al.*, 1976). Circadian concentration rhythm and well defined episodic peaks have been documented in serum testosterone profiles (Agarwal *et al.*, 1983; Rekwot *et al.*, 1997). In cockerels and other avian species, acute or long term stress reduces plasma luteinizing hormone and testosterone (Deviche, 1983). Wilson *et al.* (1979) reported

changes in plasma testosterone levels in repeated samples drawn every 15 min and since the dynamics of the changes varied in different individuals, this was attributed the changes to a pulsatile release of the hormone. Plasma testosterone levels in cockerels increase with the progress of sexual maturation (Sharp *et al.*, 1977). Several mechanisms could be involved in the increase of testicular testosterone in response to stress (Heiblum *et al.*, 2000). In red jungle fowl after aggressive encounters between pairs of males, testosterone levels increased in individuals that attacked first and decreased in their opponents (Johnson and Zuk, 1995). Heiblum *et al.* (2000) found that the greatest increase in testosterone in birds occurred during repeated blood sampling without restraint. There is a possible relationship between testosterone elevation during stress, aggressiveness and/or sexual behavior in domestic fowl. Studies have shown that larger and heavier testes produced more spermatozoa than smaller and

lighter ones (Coulter and Foote, 1979). Osinowo *et al.* (1981) reported that testicular size is highly correlated with seminiferous tubule diameter and gonadal sperm reserve in Bunaji bulls.

Semen production is found to be related to testicular development by a positive correlation between testicular width, gonadal and extragonadal sperm reserves and sperm concentration (Amann, 1970).

Newcastle disease virus is widespread in Nigeria and enzootic in Zaria. It is known to cause a decrease in quantity and quality of egg production and substantial losses in poultry production through death of hens. The effect of NDV on the reproductive parameters of SB cocks including testosterone concentration and gonadal spermatozoa reserves has not been properly documented. Therefore, the aim of this study was to determine testosterone profiles and gonadal sperm reserves in SB breeder cocks following challenge with Velogenic Kudu 113 strain of Newcastle disease virus.

MATERIALS AND METHODS

Location: This study was carried out at the Poultry Research Programme of the National Animal Production Research Institute (NAPRI), Shika, Zaria (11 and 12° N, 7 and 8° E) at an elevation of 650 m above sea level in the Northern Guinea Savannah zone of Nigeria. An average annual maximum and minimum temperature of 31.8+3.2°C and 18.0+3.7°C, respectively characterize the climate of the area. The monthly average rainfall during the rainy season (May-October) is 148.1+68.4 mm (69.2-231.9 mm) while mean monthly relative humidity is 71.1+9.7% (Rekwot, 2000).

Experimental cocks and management of cocks: Fifty 20 weeks old SB cocks consisting of 28 white and 22 red strains were used for this study. The cocks had been routinely vaccinated against ND using the Vom produced vaccine.

The cocks were kept in pairs within cages and fed layers mash containing 18% crude protein, 95.6% dry matter, 17% crude fibre and 3% nitrogen *ad libitum*.

Water was provided *ad libitum*. All the necessary screening and treatment for ecto, endo and hemoparasites were carried out. For a period of 6 weeks the cocks had their cloacal temperatures taken using a digital thermometer and were weighed weekly (Plate III). The cocks were infected with 2 mL of $10^{6.0}$ EID₅₀ of a velogenic 113 strain Kudu of Newcastle disease virus intranasally and orally after screening.

Blood sampling and testosterone assay: Five cocks consisting of 3 red SB and 2 white SB were randomly chosen and bled for testosterone studies. These five cocks were among the 50 cocks sampled pre-infection. About 2 mL of blood was taken from the wing vein of each of the cocks every 30 min for 3 h starting at 9 h on each day of sampling at 6, 3 and 1 week before infection. The same five cocks were among the twenty five infected cocks. They were sampled again at 1, 3 and 6 weeks post infection. The blood samples collected were centrifuged at 251.6× g for 15 min and the sera put in properly labelled plastic tubes and stored at -20°C in a deep freezer until analysis.

A total of 200 sera samples were collected and stored. Testosterone assay was carried out using the Enzyme Linked Immunosorbent Assay (ELISA). The microwell testosterone is a solid immunoassay utilizing the competitive binding principle. Testosterone present in serum sample competes with enzyme-labelled testosterone for the binding sites on anti-testosterone antibody immobilized on the microwell surface. The testosterone kit was bought from the Dialab GMBH of Austria. The kit consisted of the following reagents and material, five calibrator standard reagents C_A and C_E. These standard reagents contained 0, 0.2, 1.0, 4.0 and 16.0 ng mL⁻¹ of testosterone, respectively and enzyme conjugate which contained 12 mL testosterone-HRP conjugate; microwell plate with 8×12 strips anti-testosterone Ig G adsorbed on microtitre plate; substrate solution containing 12 mL of H₂O₂-TMB 0.25 g L⁻¹; stop solution which contained 12 mL of 0.15 mol L⁻¹ of sulphuric acid.

About 25 mL of the serum samples were placed in each well of the microwell plate and 25 µL of each of the five calibrator standard solutions were added to the serum samples in the microtitre plate well. About 100 µL of the enzyme conjugate was also added to each serum sample. The serum samples were properly mixed and incubated for 1 h at 37°C in an oven. After incubation the contents of the wells were decanted and the wells washed with 300 µL of distilled water. Washing was repeated and the water was drained completely from the wells. About 100 µL of substrate solution was added to each of the wells containing the sera samples and incubated at room temperature for 15 min in the dark. This resulted in a colour change (the solution turned blue). About 100 µL of stop solution was added to each of the wells containing the blue solution and it turned yellow. The microwell plate with the yellow solution was taken to the microwell reader (Multiskan Ascent, Thermo Electron Corporation, Finland) and the absorbance (E) read at 450 nm against

blank. The sensitivity of the assay was 5 pg mL⁻¹ and inter and intra assay coefficients of variation were 3.9 and 6.2, respectively.

Determination of gonadal sperm and spermatid reserves:

At the end of the study 6 weeks post infection, 20 control cocks consisting of 11 red SB and 9 white SB and 20 infected cocks consisting of 7 red SB and 13 white SB were weighed and slaughtered. The right and left testes were removed and their lengths and weights measured. Gonadal sperm and spermatid reserves were determined as described. Each testis was homogenized in 50 mL of physiological saline containing streptomycin sulphate (at 1 mg mL⁻¹) and sodium penicillin (G 100 I.U. mL⁻¹) using a mortar and pestle.

The volume of the homogenate was measured after rinsing the mortar and pestle with 20 mL of physiological saline and adding the effluent. About 5 mm of the homogenate were transferred to a conical flask and diluted with 80 mL of saline. The diluted testicular homogenate sample was stored overnight in a refrigerator and filtered through gauze and the volume of the filtrate measured. The number of spermatozoa and spermatid in the homogenate sample were determined using a hemocytometer.

Both chambers of an improved Neubauer bright line hemocytometer were filled with the testicular homogenate and spermatozoa were counted in five of the small centre boxes in both chambers. The total counts of both chambers were averaged and multiplied by 10⁹ the resultant figure was recorded as spermatozoa per cu mm. Spermatozoa counted in the testicular homogenates included elongated spermatids and spermatozoa in stages vi, vii and viii of the seminiferous epithelium (Hafez, 1987).

Statistical analysis: The data on body weights, length and testicular weight, gonadal sperm and spermatid reserves were subjected to statistical analysis.

RESULTS AND DISCUSSION

The mean testosterone concentration of the infected SB cocks showed a decrease from week 1 post infection to week 6 post infection (Fig. 1). The mean testosterone concentrations of the cocks pre-infection did not show a clear pattern. The mean testosterone concentration 6 weeks pre-infection was higher than that of week 3 and 1 with week 3 mean testosterone concentration being the lowest (Fig. 1). The mean testosterone concentration of the infected cocks shows that the velogenic Newcastle disease virus affected the testosterone secretion of the cocks.

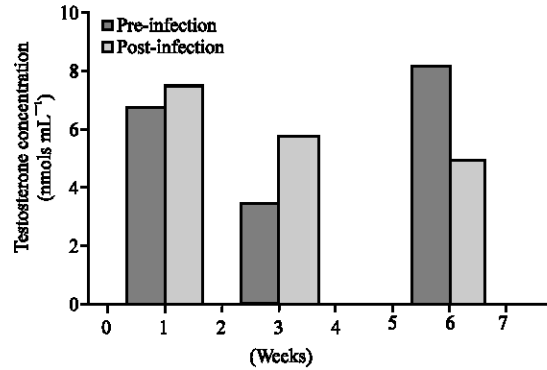


Fig. 1: Mean testosterone concentration of Shikabrown cocks pre and post-infection

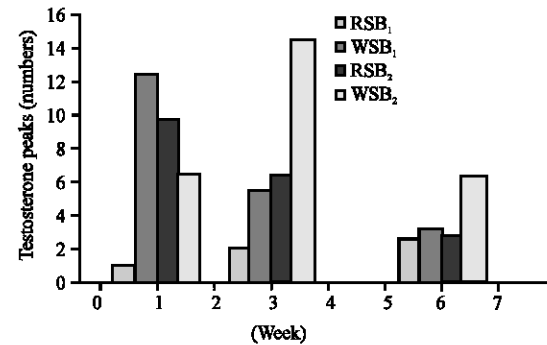


Fig. 2: Mean number of testosterone peaks of Shika brown cocks

The challenge of the cocks with the velogenic Newcastle disease stressed the cocks leading to increased production of corticosteroids which affected the interstitial cells which produce testosterone in the testes. The virus used in this experiment is velogenic and pantropic therefore it is likely that it has adversely affected the interstitial cells responsible for the production of testosterone thus the decrease in testosterone concentration in infected cocks. The mean of the lowest testosterone concentration values observed during the 3 h sampling period were taken as basal testosterone concentration. A peak was defined as each single value or series of values two-fold above mean basal concentration.

The mean number of testosterone concentration peaks of the control red Shika-brown cocks increased from weeks 1-6. In the white Shika-brown cocks the reverse was the case, the mean testosterone concentration peaks decreased from weeks 1-6. The pre-infection mean testosterone concentration peaks of all the cocks (red and white) was about the same value for week 1 and 6 while week 3 was lower in value than both of them (Fig. 2 and 3).

The mean number of testosterone concentration peaks for the red cocks post infection showed a decrease in the number of peaks from weeks 1-6. In the white cocks, week 3 had the highest mean testosterone concentration peaks while the value for week 1 and 6 was the same. All the cocks (red and white) post infection did not show a clear pattern in the mean number of testosterone concentration peaks. Weeks 1 and 3 had high values compared with a low value for week 6 (Fig. 2 and 3).

There was no significant difference ($p>0.05$) in the mean body weight and right testis weight of both control and infected cocks. There was no significant difference ($p>0.05$) in the left testis weight of the control red and white cocks, similarly the left testis weight of the infected red and white cocks was not significantly different. The left testis weight of the control red and white cocks was significantly different ($p<0.05$) from that of the infected red and white cocks (Table 1).

The gonadal sperm reserves of the right testes of the control red and white cocks was not significantly different but the gonadal sperm reserve of the control white cocks was significantly higher than that of the infected white and red cocks (Table 1). The gonadal sperm reserve of the left testes was not significantly different ($p>0.05$) for both the control and infected red and white cocks (Table 1). The total gonadal sperm reserve of the control white

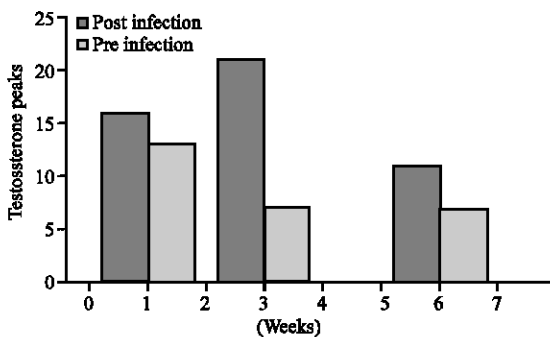


Fig. 3: Mean number of testosterone peaks of Shika-brown cocks pre and post-infection

cocks was significantly higher ($p<0.05$) than that of the infected red and white cocks. The presence of basal and peak testosterone concentrations agreed with the reports of Sanwal *et al.* (1974) in exotic bulls and Rekwot *et al.* (1997) in Zebu bulls. The testosterone profiles in this study were episodic, pulsatile or temporal in nature. This confirms earlier report in bulls by Thibier (1976) and Agarwal *et al.* (1983). The testosterone episodic peaks observed in this study agreed with earlier observations of Sanwal *et al.* (1974); Agarwal *et al.* (1983) and Rekwot *et al.* (1997) in bulls.

The exact significance of these testosterone episodic peaks is not clear but may be related to the sexual and behavioural states of animals to photoperiodicity, temperatures and postural states of the animal (Sanwal *et al.*, 1974). According to Karg *et al.* (1976) and Rekwot *et al.* (1997) the higher the testosterone concentration the better the semen characteristics. In the present study the control white Shikabrown cocks had higher mean testosterone concentration peaks than the control red Shikabrown cocks. The mean testosterone concentration peaks of the infected red and white cocks did not follow any clear pattern.

The infected red cocks had high mean testosterone concentration peaks at weeks 1 and 3 while the infected white cocks had high mean testosterone concentration peaks at weeks 3 and the same mean testosterone concentration peaks at weeks 1 and 6. The mean testosterone concentration of Shikabrown cocks post infection shows a decline from weeks 1-6 indicating that the velogenic Newcastle virus affected testosterone production.

In the cock testosterone also plays a major role in the onset of puberty such as development of genital tract, libido, initiation and potentiation of spermatogenesis in conjunction with androgen building proteins and follicle stimulating hormone (Etches, 1996; Hafez, 1987, 1990). This study showed that there was a decrease in the mean testosterone concentration of infected cocks due to the stress of infection hence a delay in the onset of puberty

Table 1: Gonadal sperm and spermatid reserves of control and infected red Shikabrown and white Shika brown cocks

Parameters	Control		Infected	
	RSB (n = 11)	WSB (n = 9)	RSB (n = 7)	WSB (n = 13)
Body weight (kg)	2.4±0.2 ^a	2.4±0.1 ^c	2.1±0.40 ^b	2.2±0.1 ^d
Weight of right testis (g)	12.7±2.4	12.1±2.9	12.5±2.30	12.0±2.3
Weight of left testis (g)	13.7±2.4 ^a	13.3±1.8 ^c	11.5±4.90 ^a	11.5±5.0 ^d
Length of right testis (cm)	4.6±0.4	4.4±0.5	4.7±0.2	4.7±0.5
Length of left testis (cm)	4.8±0.4	4.6±0.6	4.9±0.1	4.8±0.3
Gonadal sperm reserves (×10⁶ g/testis)				
Gonadal sperm reserve of right testes	1.7±0.2	2.1±0.2	1.5±0.20 ^d	1.7±0.6 ^d
Gonadal sperm reserve of left testes	1.7±0.2	2.1±0.3	1.6±0.30	2.0±0.5
Total gonadal sperm and spermatid reserves	3.4±0.2	4.2±0.3 ^a	3.1±0.40 ^f	3.2±0.3 ^e

^{Abcdef} = Means±SD within rows with different letter superscripts are significantly different ($p<0.05$)

in cocks infected with Newcastle disease. Daily sperm production and daily production per gram of parenchyma of testis have been estimated in dairy and beef *Bos taurus* bulls (Almquist and Amann, 1961; Weisgold and Almquist, 1979) in several breeds of *Bos indicus* as well as in crossbreed *Bos indicus* (Wildeus and Entwistle, 1982; Tegegne *et al.*, 1992). Reports have shown strong association between plane of nutrition, body weight and scrotal growth (Van Demark and Mauger, 1964; Reeves and Johnson, 1976; Rekwot *et al.*, 1988). The differences in the right and left testicular weight in the two breeds in the present study is consistent with previous studies in cattle (Fields *et al.*, 1979, 1982; Entwistle, 1983; Tegegne *et al.*, 1992, 1994).

As it is generally in most animal species and poultry the left testis is always heavier than the right (Roberts, 1971; Etches, 1996). This study confirmed the earlier studies (Etches, 1993). The Newcastle disease virus causes decrease in testosterone concentration and gonadal sperm reserves in Shikabrown cocks. Since the velogenic Newcastle disease virus caused a decrease in the mean testosterone concentration of the Shikabrown cocks they should be adequately and routinely vaccinated against Newcastle disease.

It is assumed that NDV causes a decrease in P4 concentration apparently by disruption of the hypothalamic pituitary testicular axis resulting in P4 depression. This is further elucidated by the lowering of Gonadal sperm/spermatid reserves. In view of this, it is suggested that breeder cocks be routinely vaccinated against ND.

CONCLUSION

From this study, the weights of the left testicles of the control red and white SB cocks were significantly heavier than those of the infected red and white SB cocks.

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