

Semen Characteristics of Vaccinated Shikabrown Cocks Challenged with Velogenic Newcastle Disease Virus

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Abstract: Fifty 20 weeks old Shikabrown (SB) cocks consisting of 22 red and 28 white SB cocks were purchased from the National Animal Production Research Institute Shika. The cocks were fed on a diet of layers mash with 18% crude protein, 95.6% dry matter, 17.1% crude fibre and 3% nitrogen. About 25 cocks consisting of 8 red and 17 white SB cocks selected on the basis of body weight and antibody titres were infected with 0.2 mL of $10^{6.0}$ EID₅₀ of velogenic Kudu 113 strain of Newcastle disease virus intranasally and orally. About 25 cocks consisting of 14 red and 11 white SB cocks served as control. Cloacal temperatures, body weights and semen samples of both control and infected cocks were taken weekly for 6 weeks. The semen was evaluated for volume, colour, motility, concentration, percentage live spermatozoa and percentage total spermatozoa abnormalities. Semen colour was graded as creamy (1 = very good); milky (2 = good) and watery (3 = poor). There was no significant difference in the cloacal temperatures and body weights of control and infected red and white SB cocks. The infected red and white cocks had slightly higher cloacal temperatures than the control. The semen volume of infected red cocks showed a general increase over that of the control red cocks. The semen volume of the control white SB cocks was significantly higher than that of the infected white cocks. The white SB cocks had higher semen volume than the red SB cocks. The red SB cocks had slightly better semen colour than the white SB cocks. The control white cocks had higher spermatozoa motility than the infected white cocks while the infected red cocks had higher spermatozoa motility than the control red cocks. The white SB cocks generally had better spermatozoa motility than the red cocks. The spermatozoa concentration of the control white cocks was consistently higher than that of the infected white SB cocks; the reverse was the case with the red cocks where the spermatozoa concentration of the infected red cocks was higher than that of the control red cocks. The white cocks had better spermatozoa concentration than the red cocks. The control white SB cocks had significantly ($p < 0.05$) higher percent live spermatozoa than the infected white cocks. Similarly, the infected red SB cocks had lower percent live spermatozoa than the control red cocks. The control white SB cocks had significantly ($p < 0.05$) higher percent live spermatozoa than the control red SB cocks. The infected red and white SB cocks had higher percentage total spermatozoa abnormalities than the control red and white cocks. It can be concluded from this study that the white SB cocks had better semen quality than the red SB cocks; the non-infected SB cocks had better semen quality than the infected SB cocks. It is recommended that white SB cocks be used for breeding purposes and that breeder cocks should be routinely vaccinated against Newcastle disease to ensure that the level of antibodies is high enough to prevent adverse effect on semen quality.

Key words: Spermatozoa, vaccinated, cloacal temperature, abnormalities evaluated, antibody, concentration

INTRODUCTION

There have been several studies conducted on semen production and quality in a variety of breeds of poultry. Semen production is strongly correlated with testis size, body weight and breed (Williams and McGibbon, 1943; Boone and Hughes, 1969). Seasonal variations in semen

production have also been reported in poultry (Polge, 1951; Kamar and Nadreldin, 1959; Saeid and Al-Soudi, 1975). Qualitative and quantitative characteristics of semen have a marked effect on egg fertility (Kamar, 1960; Chalov, 1970).

Decreased egg production was experimentally produced by infecting breeder turkey hens with Eastern

Equine Encephalitis Virus (EEEV) or Highland J. virus (Guy *et al.*, 1995). The viruses were shed in the semen of Turkey toms infected experimentally (Guy *et al.*, 1995). This proved that these viruses can be transmitted sexually through artificial insemination of Turkey hens using infected semen. It was also observed that a decline in egg production due to Western Equine Encephalitis Virus (WEEV) was accompanied by an increase in semen antibody titres to WEEV between acute and convalescent sera in Turkeys from affected flocks (Cooper and Medina, 1999).

Apart from the general debilitating effects of disease on the health of an animal, reproductive diseases impair reproductive activities and thus reduce productivity in animals (Rekwot *et al.*, 1998). Viral and bacterial diseases such as Newcastle disease, salmonellosis, egg drop syndrome and fowl typhoid affect egg production in poultry (Jordan, 1990). Newcastle disease is widespread in Nigeria and enzootic in Zaria. It is known to cause decrease in egg production. There is a dearth of information on the effect of ND on semen characteristics. This study was therefore designed to determine the effect of a velogenic Newcastle disease virus on the semen characteristics of vaccinated Shikabrown cocks.

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 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 week old Shikabrown (SB) cocks consisting of 22 red and 28 white SB cocks were purchased from the National Animal Production Research Institute Shika. The cocks were fed on a diet of layers mash with 18% crude protein, 95.6% dry matter, 17.1% crude fibre and 3% nitrogen. About 25 cocks consisting of 8 red and 17 white SB cocks selected on the basis of body weight and antibody titres were infected with 0.2 mL of $10^{6.0}$ EID₅₀ of a velogenic Kudu 113 strain of Newcastle disease virus intranasally and orally. About 25 cocks consisting of 14 red and 11 white SB cocks served as controls. Cloacal temperatures, body weights and semen samples of both control and

infected cocks were taken weekly for 6 weeks. The semen was evaluated for volume, colour, motility, concentration, percent live spermatozoa and percentage total spermatozoa abnormalities. Semen colour was graded as creamy (1 = very good); milky (2 = good) and watery (3 = poor).

Infection of cocks with velogenic Kudu 113 strain of Newcastle disease virus: About 25 cocks consisting of 8 red and 17 white SB cocks were infected with velogenic Kudu 113 strain of Newcastle disease virus. The virus was described by Echeonwu and Emeruwa (1993) and had the following characteristics; hemagglutination titre of ($\log_2$ 256); mean lethal dose of ($\log$ 108.00); mean death time of (49.60 h); intracerebral pathogenicity index of (1.56); intravenous pathogenicity index dose of 2.18; embryo infective dose, 50% endpoint mL^{-1} 8.46% adsorption of chicken brain cell (97.66%); thermostability of hemagglutination at 56°C (120 min) and virus elution rate (>26 h). The titre of the NDV was $10^{8.5}$ mL^{-1} (Alexander, 1988). A vial of the NDV was dissolved in 63 mL of phosphate buffered saline (pH 7.4) and each cock was inoculated with 0.2 mL intranasally and intraocularly.

Semen collection: Semen was collected according to the method described by Lake and Stewart (1978). This involved a gentle massage (stroking) of the back feathers two or three times with the palm of the hand and the abdomen towards the tail with the other hand simultaneously. The semen was immediately expressed from the swollen ejaculating papillae into a graduated plastic tube.

Semen evaluation: Immediately after collection, all semen samples were immersed in a flask containing water at 42°C and carried to the laboratory for immediate evaluation. The semen samples were evaluated for volume, colour, motility, concentration, percent live spermatozoa and percentage total sperm abnormalities as described by Zemjanis (1970). This includes visual and gross evaluation of the ejaculate soon after collection with respect to volume, colour and presence or absence of foreign materials and microscopic examination of wave pattern (gross motility) and live-dead counts. The semen colour was graded as creamy (1 = very good), milky (2 = good) or watery (3 = poor). Gross motility was determined by examining a drop of raw and undiluted semen on a pre-warmed slide under a light microscope at 100× magnification. The concentration of the spermatozoa was determined using the red blood cell counting chamber of a hemocytometer that was crossed with microscopic grids containing 25 large squares with each containing

smaller squares. Sperm cells were counted diagonally from the left top to the right bottom in 5 large squares or a total of 80 small squares (Coles, 1986). Semen smears were stained with eosin-nigrosin for the determination of live-dead ratio. Live sperm cells repel the stain and were colorless while dead cells absorb the stain and appeared reddish (Coles, 1986). At least 400 spermatozoa per slide were counted using the phase contrast microscope at $\times 40$ magnification with oil immersion. Fresh raw semen samples were also fixed in buffered formol saline for two to 3 days to determine sperm abnormalities.

Statistical analysis: Data of the cloacal temperatures, body weights and semen parameters were analysed using the analysis of variance procedure and differences between treatment means compared by Duncan's Multiple Range tests (Helwig and Council, 1979).

RESULTS AND DISCUSSION

Cloacal temperatures and body weight: There was no significant difference in the cloacal temperatures of control and infected red SB. Similarly there was no significant difference in the cloacal temperatures of the control and infected white SB cocks. The infected red and white cocks had slightly higher cloacal temperatures (Table 1). The body weights of the control and infected red SB cocks did not differ significantly, the same was also true of the control and infected white SB cocks. The infected red and white cocks were slightly lower in weight than the control cocks (Table 1).

Semen volume and colour: The semen volume of the control and infected red SB cocks did not differ significantly from week 1-6. However, the semen volume of the infected red cocks showed a general decrease over

that of the control red cocks. The semen volume of the control white cocks was significantly higher than that of the infected white cocks at week 1 and 2. There was no significant difference between the semen volume of the control and infected white cocks at week 3-6. The semen volume of the infected white cocks showed a general decrease (Table 1).

The white SB cocks had higher semen volume than the red cocks. The semen colour of the red cocks (control and infected) and white cocks (control and infected) did not show any significant difference (Table 1).

Spermatozoa motility: Spermatozoa motility of the control and infected red SB cocks was not significantly different at week 1 and 6 post infection, although the spermatozoa motility of the control red cocks was significantly higher ($p < 0.05$) than that of the infected red cocks at week 2-5. The control white Shikabrown cocks had significantly higher spermatozoa motility than the infected white cocks at week 1-6. The infected red cocks had lower spermatozoa motility than the control red cocks while the control white cocks had significantly higher spermatozoa motility than the infected white cocks. The white SB cocks generally had better spermatozoa motility than the red Shikabrown cocks (Table 1).

Spermatozoa concentration: The spermatozoa concentration of the control red SB cocks was significantly higher at week 3-5 (Table 1). The infected red cocks had significantly lower spermatozoa concentration than the control red cocks (Table 1). The spermatozoa concentration of the control white SB cocks was significantly higher than that of the infected white cocks at week 1-3. There was no significant difference

Table 1: Effect of Newcastle disease virus on body weight, cloacal temperature and semen characteristics of red Shikabrown and white cocks

Parameters	Breed	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
		Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected
Cloacal Temperature ($^{\circ}\text{C}$)	RSB	41.7 $\pm$ 0.20	41.8 $\pm$ 0.68	41.6 $\pm$ 0.1 ^a	41.8 $\pm$ 0.1 ^b	41.6 $\pm$ 0.90	41.13 $\pm$ 0.4	41.5 $\pm$ 0.3	41.8 $\pm$ 0.1	41.7 $\pm$ 0.1	42.1 $\pm$ 0.1	41.40 $\pm$ 0.1	41.7 $\pm$ 0.9
	WSB	41.0 $\pm$ 0.30	41.8 $\pm$ 0.78	41.4 $\pm$ 0.1 ^b	41.7 $\pm$ 0.1 ^b	41.5 $\pm$ 0.70	41.70 $\pm$ 0.7	41.5 $\pm$ 0.6	42.3 $\pm$ 0.1	41.6 $\pm$ 0.1	41.9 $\pm$ 0.1	41.66 $\pm$ 0.0	41.5 $\pm$ 0.9
Body weight (kg)	RSB	2.2 $\pm$ 0.20 ^a	2.1 $\pm$ 0.20 ^b	2.2 $\pm$ 0.1	2.1 $\pm$ 0.2	2.3 $\pm$ 0.10 ^a	2.10 $\pm$ 0.1 ^b	2.2 $\pm$ 0.1	2.1 $\pm$ 0.1	2.2 $\pm$ 0.0 ^a	2.2 $\pm$ 0.1 ^a	2.20 $\pm$ 0.1 ^a	2.2 $\pm$ 0.1 ^b
	WSB	2.2 $\pm$ 0.20 ^a	2.2 $\pm$ 0.17 ^b	2.2 $\pm$ 0.1	2.2 $\pm$ 0.0	2.1 $\pm$ 0.07 ^a	2.00 $\pm$ 0.1 ^b	2.2 $\pm$ 0.1	2.2 $\pm$ 0.1	2.3 $\pm$ 0.1 ^a	2.2 $\pm$ 1.0 ^b	2.30 $\pm$ 0.1 ^a	2.2 $\pm$ 0.4 ^b
Semen volume (mL)	RSB	0.3 $\pm$ 0.10	0.3 $\pm$ 0.08	0.3 $\pm$ 0.3	0.2 $\pm$ 0.4	0.3 $\pm$ 0.10	0.30 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.3	0.3 $\pm$ 0.1	0.30 $\pm$ 0.1	0.3 $\pm$ 0.1
	WSB	0.3 $\pm$ 0.10	0.2 $\pm$ 0.05	0.8 $\pm$ 0.3	0.5 $\pm$ 0.3	0.5 $\pm$ 0.10	0.50 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.50 $\pm$ 0.1	0.5 $\pm$ 0.1
Color (1-3)	RSB	1.6 $\pm$ 0.40	1.3 $\pm$ 0.30	1.6 $\pm$ 0.4	1.4 $\pm$ 0.3	1.9 $\pm$ 0.20	1.30 $\pm$ 0.2	2.0 $\pm$ 0.4	1.6 $\pm$ 0.3	2.1 $\pm$ 0.3	1.3 $\pm$ 0.4	1.80 $\pm$ 0.3	1.6 $\pm$ 0.4
	WSB	2.1 $\pm$ 0.30	1.2 $\pm$ 0.27	1.9 $\pm$ 0.3	2.0 $\pm$ 0.3	2.4 $\pm$ 0.30	1.70 $\pm$ 0.2	1.9 $\pm$ 0.3	2.5 $\pm$ 0.3	2.1 $\pm$ 0.3	1.9 $\pm$ 0.3	2.80 $\pm$ 0.4	2.4 $\pm$ 0.3
Sperm motility (%)	RSB	38.0 $\pm$ 11.4	35.7 $\pm$ 8.60	53.1 $\pm$ 0.9	41.8 $\pm$ 6.8	73.8 $\pm$ 0.80	42.90 $\pm$ 0.6	60.6 $\pm$ 9.8	47.1 $\pm$ 7.5	55.7 $\pm$ 7.7	32.5 $\pm$ 0.1	50.70 $\pm$ 0.8	50.0 $\pm$ 0.1
	WSB	70.5 $\pm$ 9.70	43.8 $\pm$ 0.70	73.6 $\pm$ 7.7	55.6 $\pm$ 6.2	66.8 $\pm$ 0.70	60.30 $\pm$ 5.7	61.2 $\pm$ 6.8	55.9 $\pm$ 8.4	60.0 $\pm$ 8.7	55.0 $\pm$ 0.7	69.50 $\pm$ 9.8	57.2 $\pm$ 7.5
Sperm concentration ($\times 10^9$ mL ⁻¹)	RSB	2.3 $\pm$ 0.60	1.7 $\pm$ 0.50	3.2 $\pm$ 0.8	2.3 $\pm$ 0.6	4.1 $\pm$ 0.70	1.90 $\pm$ 0.5	3.6 $\pm$ 1.5	2.5 $\pm$ 1.2	3.8 $\pm$ 0.3	1.5 $\pm$ 0.5	2.60 $\pm$ 0.6	2.4 $\pm$ 0.8
	WSB	2.7 $\pm$ 0.60	1.8 $\pm$ 0.50	5.0 $\pm$ 0.7	3.6 $\pm$ 0.5	3.8 $\pm$ 0.60	2.60 $\pm$ 0.5	4.9 $\pm$ 1.3	4.1 $\pm$ 1.1	3.6 $\pm$ 0.3	2.8 $\pm$ 0.7	5.00 $\pm$ 0.7	4.6 $\pm$ 0.6
Percent live Spermatozoa (%)	RSB	38.2 $\pm$ 0.90 ^a	38.2 $\pm$ 0.90 ^a	64.4 $\pm$ 0.1 ^a	53.9 $\pm$ 0.1 ^b	69.4 $\pm$ 0.90 ^a	40.00 $\pm$ 0.7 ^b	63.1 $\pm$ 0.1 ^a	58.2 $\pm$ 0.8 ^b	56.8 $\pm$ 0.8	54.9 $\pm$ 0.1	54.70 $\pm$ 0.1 ^a	47.5 $\pm$ 0.1 ^b
	WSB	69.5 $\pm$ 0.10 ^a	45.0 $\pm$ 0.80 ^a	75.9 $\pm$ 0.9 ^a	66.2 $\pm$ 0.7 ^b	76.8 $\pm$ 0.80 ^a	66.20 $\pm$ 0.6 ^b	63.8 $\pm$ 0.7 ^a	57.7 $\pm$ 0.9 ^b	72.3 $\pm$ 0.9 ^a	40.1 $\pm$ 0.7 ^b	80.00 $\pm$ 0.1 ^a	66.2 $\pm$ 0.8 ^b
Total abnormalities (%)	RSB	3.1 $\pm$ 1.30	3.3 $\pm$ 0.10	3.6 $\pm$ 0.2 ^a	7.4 $\pm$ 2.6 ^b	6.4 $\pm$ 1.30	6.60 $\pm$ 1.7	3.6 $\pm$ 4.8 ^a	6.2 $\pm$ 3.6 ^b	2.4 $\pm$ 0.7 ^a	7.0 $\pm$ 0.9 ^b	2.70 $\pm$ 0.9 ^a	5.1 $\pm$ 1.4 ^b
	WSB	3.9 $\pm$ 0.90	6.4 $\pm$ 1.20	3.9 $\pm$ 2.2	9.8 $\pm$ 1.8	5.8 $\pm$ 1.40	6.10 $\pm$ 1.2	5.0 $\pm$ 4.1	6.3 $\pm$ 3.3	3.0 $\pm$ 0.8	7.6 $\pm$ 0.7	3.80 $\pm$ 1.2	6.6 $\pm$ 0.9

^aMeans with different letter superscripts within treatments and breeds are significantly ($p < 0.05$) different within rows

($p>0.05$) in the spermatozoa concentration of the control and infected white cocks at week 4-6. The spermatozoa concentration of the control red and white cocks was persistently higher than that of the infected red and white cocks throughout the period of the research. The white cocks had generally better spermatozoa concentration than the red cocks (Table 1).

Percent live spermatozoa: The control red SB cocks had significantly higher percent live spermatozoa than the infected red cocks at week 1-6 post-infection (Table 1). At weeks 5, there was no significant difference between the infected and control cocks ($p>0.05$). The control white SB cocks had significantly higher percentage live spermatozoa ($p<0.05$) than the infected white cocks at weeks 1-6 (Table 1). The percent live spermatozoa of the control white SB cocks was significantly higher ($p<0.05$) than that of the control red SB cocks (Table 1).

Percentage of total spermatozoa abnormalities: There was no significant difference ($p>0.05$) in the percentage total spermatozoa abnormalities of the control and infected red SB cocks at week 1 and 3 (Table 1). The infected red cocks had a significantly higher percentage total spermatozoa abnormalities at week 2, 4, 5 and 6 (Table 1). The infected white SB cocks had significantly higher percentage total spermatozoa abnormalities than the control at week 1-6. There was no significant difference ($p>0.05$) in the percentage total spermatozoa abnormalities between the control and infected white cocks at week 3 and 4.

The cloacal temperatures of the control red and white SB cocks were not significantly different ($p>0.05$). Similarly the cloacal temperatures of the infected red and white cocks were not significantly different ($p>0.05$). The red control cocks had slightly higher cloacal temperatures than the white control cocks. The cloacal temperatures of the infected red and white cocks were slightly higher than those of the control red and white cocks. This corroborates with the finding of Oladele (2004) who found an increase in cloacal temperatures of SB chicks vaccinated with Newcastle disease virus.

There was no significant difference ($p>0.05$) in the body weights of the control red SB and white SB cocks. Similarly, there was no significant difference ($p>0.05$) in the body weight of infected red and white cocks, though the infected red cocks were slightly less heavy. This does not agree with the finding of Nwagu *et al.* (1996) who reported that the control red SB cocks were heavier than the control white SB cocks.

The infected red and white cocks were slightly less heavy in weight. This finding could be explained by the fact that the infected cocks eat less than the control cocks. This finding agrees with that of Okoye *et al.* (2000) who

found that Newcastle disease infected chickens were significantly lighter in weight than the non-infected chickens at 2 and 3 weeks post infection. The chickens used by Okoye *et al.* (2000) were vaccinated against Newcastle disease using the VGF-1 strain of the virus.

The control and infected white Shikabrown cocks had higher semen volume throughout the period of the study than the red Shikabrown cocks. The control red cocks had higher semen volume than the infected cocks. The red Shikabrown cocks had better semen colour than the white Shikabrown cocks.

The white cocks had better spermatozoa motility than the red cocks thus agreeing with the finding of Nwagu *et al.* (1996) who found out that the white cocks had better spermatozoa motility than the red cocks.

The control white Shikabrown cocks had better spermatozoa concentration than the red cocks. The red cocks had higher antibody titres than the white cocks hence more protection from the Newcastle disease virus and its effects. The high spermatozoa concentration of the white cocks obtained in this study agrees with the finding of Nwagu *et al.* (1996) who found a similar result working with the same strains of cocks.

The white Shikabrown cocks had higher percent live spermatozoa than the red cocks. This shows that the white cocks had better semen quality than the red cocks. Infection reduced the percent live spermatozoa of both the white and red cocks. The red cocks had higher antibody titres which gave them more protection from the Newcastle disease virus.

The control white cocks had higher percentage total spermatozoa abnormalities than the control red cocks. The infected red and white cocks had higher percentage total spermatozoa abnormalities than the control red and white cocks. The percentage total spermatozoa abnormalities in this study ranged from 4.1-9.9% for the red Shikabrown cocks and 4.1-7.8% for the white Shikabrown cocks. These results differed from the findings of Omeje and Marire (1990) who reported the percentage total spermatozoa abnormalities of Rhode Island Red and Rhode Island White cocks to be 48 and 42%, respectively.

CONCLUSION

It can be concluded from this study that the control white cocks had better semen quality with respect to volume, motility, concentration and percent live spermatozoa than the control red cocks. The infected white cocks had lower semen concentration and total abnormalities than the infected red cocks. In a Newcastle disease endemic environment like Zaria the cocks should be adequately protected from Newcastle disease through

adequate and routine vaccination to prevent negative effects on semen quality. To ascertain adequately whether the abnormalities observed in infected cocks may be translated into infertility in breeder hens requires further breeding studies.

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