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

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Abstract: Twenty-five cocks consisting of 14 red and 11 white Shikabrown 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 intraocularly. Twenty-five cocks consisting of 14 red and 11 white Shikabrown cocks served as controls. Cloacal temperatures, live weights and semen samples of both control and infected cocks were taken weekly for six weeks. Semen was collected by abdominal massage and evaluated for volume, colour, motility, concentration, percent live spermatozoa and percent total spermatozoa abnormalities. The semen volume of infected red cocks showed a general increase over that of control red cocks. The semen volume of the control white Shikabrown cocks was significantly ($p < 0.05$) higher than that of the infected white cocks. The white Shikabrown cocks had higher semen volume than the red Shikabrown cocks. The red Shikabrown cocks had slightly better semen colour than the white Shikabrown cocks. The control white cocks had better ($p < 0.05$) spermatozoa motility than the infected white cocks, while the infected red cocks had significantly ($p < 0.05$) spermatozoa motility than the control red cocks. Generally, the white Shikabrown cocks 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 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 higher spermatozoa concentration than the red cocks. The control white Shikabrown cocks had significantly ($p < 0.05$) higher percent live spermatozoa than the infected white cocks. The infected red Shikabrown cocks had significantly ($p < 0.05$) higher percent live spermatozoa than the control red cocks. The control white cocks had significantly ($p < 0.05$) higher percent live spermatozoa than the control red cocks. The infected red and white Shikabrown cocks had higher percentage total spermatozoa abnormalities than the control red and white cocks. It can be concluded from this study that the white Shikabrown cocks had better semen quality than the red Shikabrown cocks. It is recommended that breeder cocks be routinely vaccinated against Newcastle disease to ensure that the level of antibodies is high enough to prevent adverse effects on semen quality.

Key words: Newcastle disease, semen production, semen quality

INTRODUCTION

Several studies have been conducted on semen production and quality in breeds of poultry. It has been reported that semen production is strongly correlated with age, testis size, body weight and breed (Burrows and Titus, 1939; Boone and Hughes, 1969; Weil *et al.*, 1999 and Togun *et al.*, 2006). Seasonal variations in semen production have also been reported in poultry (Saeid and Al-Soudi, 1975; Onuora, 1982; Obidi *et al.*, 2008). Reproductive inefficiency is recognized as the costliest and one of the most limiting factors to efficient animal production. It is also well known that both qualitative and quantitative characteristics of semen have a marked effect on egg fertility (McDaniel *et al.*, 1996).

Guy *et al.* (1995) experimentally induced a decrease in egg production in turkey hens by infecting the breeder hens with Eastern Equine Encephalitis Virus (EEEV) or

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

Nutritional deficiencies may impair reproduction by producing their primary effect on a variety of tissues and organs, resulting in non-specific decreased reproductive performance (Gerioff, 1986). Such nutrient imbalances may primarily affect the anterior pituitary or hypothalamus, thus interfering with normal luteinizing hormone and follicle stimulating hormone production (Gerioff, 1986).

Apart from the general debilitating effects of disease on the health of an animal, 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. Newcastle disease is known to cause a decrease in egg production. There is a dearth of information in the literature on the effects of ND on the semen characteristics in breeder cocks in the study location. Therefore the objective of the present study was to determine the effect of a velogenic Newcastle disease virus on the semen characteristics of vaccinated Shikabrown cocks.

MATERIALS AND METHODS

Location: The research was conducted at the Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru, Zaria. Samaru is situated in the Northern Guinea savannah zone between latitudes 11° and 12°N and between longitudes 7° and 8°E at an elevation of 650 meters above sea level. Samaru has two seasons: dry (October to April) and rainy (May-October) with an annual rainfall of 1107 mm (Rekwot, 2000).

Experimental cocks: Fifty 20 week-old Shikabrown cocks consisting of 22 white and 28 red strains were purchased from the National Animal Production Research Institute Shika and used for this study. The cocks had been routinely vaccinated against ND, using the Vom produced vaccine before purchase.

Management of cocks: The cocks were kept in pairs in cages and fed layers mash containing 18% crude protein *ad libitum*. Water was also provided *ad libitum*. All the necessary veterinary health care were given when due. For a period of six weeks, the cocks had their cloacal temperatures taken using a digital thermometer and weekly live weights were taken.

Infection of cocks with velogenic Kudu 113 strain of Newcastle disease virus: Twenty-five cocks consisting of 8 red Shikabrown and 17 white Shikabrown were infected with a velogenic Kudu 113 strain (National Veterinary Research Institute, Vom, Nigeria) of Newcastle disease virus. The cocks were infected with 2 ml of $10^{6.0}$ EID₅₀ of a velogenic 113 strain Kudu of Newcastle disease virus intranasally and intraocularly after screening.

The virus was described by Echeonwu *et al.* (1993) as having 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 and embryo infective dose of 50 %. Other characteristics are: endpoint per ml 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 and evaluation: 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.

Immediately after collection, all semen samples were immersed in a flask containing water at 40°C and carried to the laboratory for immediate evaluation. The semen samples were evaluated for volume, color, motility, concentration, percent live spermatozoa and percent total sperm abnormalities as described by Zemjanis (1970). 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 x100 magnification. The concentration of the spermatozoa was determined using the red blood cell counting chamber of a haemocytometer as described by Coles (1980). Semen smears were stained with eosin-nigrosin for the determination of percent live sperm. Live sperm cells repel the stain and were colorless, while dead cells absorbed the stain and appeared reddish (Coles, 1980). At least 400 spermatozoa per slide were counted using the phase contrast microscope at x40 magnification with oil immersion. Fresh raw semen samples were also fixed in buffered formol saline for 2-3 days to determine sperm abnormalities. About 400 spermatozoa per slide were counted using the phase contrast microscope at x40 magnification with oil immersion (Coles, 1980).

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

RESULTS

Cloacal temperatures and live weight: The results of this study showed that the live weights of the control red Shikabrown cocks were not significantly ($p > 0.05$) different. Similarly there was no significant ($p > 0.05$) difference in the cloacal temperatures of the control and infected white Shikabrown cocks. The infected red and

white cocks had slightly higher cloacal temperatures (Table 1).

The live weights of the control and infected red Shikabrown cocks were not significantly ($p>0.05$) different; the same was true for the control and infected white Shikabrown cocks. The infected red and white cocks were slightly lighter in weight than the control cocks (Table 1).

Semen volume: The semen volume of the control and infected red Shikabrown cocks was not significantly different in week 1, 2, 3, 4, 5 and 6. However, the semen volume of the infected red cocks showed a slight decrease over that of the control red cocks in weeks 2 and 4. The semen volume of the control white cocks was significantly ($p<0.05$) higher than that of the infected white cocks in week 2. There was no significant difference between the semen volume of the control and infected white cocks in week 3, 4, 5 and 6. However, the semen volume of the infected white cocks showed a slight decrease in weeks 1, 4 and 5 (Table 1). Generally, the white Shikabrown cocks had higher semen volume than the red cocks. The semen colour of the control and infected red cocks and control and infected white cocks did not show any significant ($p>0.05$) difference.

Spermatozoa motility: Spermatozoa motility of the control and infected red Shikabrown cocks was not significantly different ($p>0.05$) in weeks 1 and 6 post infection. The motility of the control red cocks was significantly higher ($p<0.05$) than that of the infected red cocks in weeks 2, 3, 4 and 5 the control red cocks had a significantly ($p<0.05$) higher spermatozoa motility than the infected red cocks (Table 1).

The control white Shikabrown cocks had significantly higher spermatozoa motility than the infected white cocks in weeks 1, 2, 3, 4, 5 and 6. The infected red cocks had lower spermatozoa motility than the control red cocks while the control white cocks had significantly ($p<0.05$) higher spermatozoa motility than the infected white cocks. The white Shikabrown cocks generally had better spermatozoa motility than the red Shikabrown cocks (Table 1).

Spermatozoa concentration: The spermatozoa concentration of the control red Shikabrown cocks was significantly ($p<0.05$) higher in weeks 1, 2, 3, 4 and 5 (Table 1). The infected red cocks had significantly lower spermatozoa concentration than the infected white cocks. The spermatozoa concentration of the control white Shikabrown cocks was significantly higher than that of the infected white cocks in week 1, 2, 3 and 5. There was no significant difference ($p>0.05$) in the spermatozoa concentration of the control and infected white cocks at week 4 and 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 study. The white cocks had generally better spermatozoa concentration than the red cocks (Table 1).

Percent live spermatozoa: The control red Shikabrown cocks had significantly higher percent live spermatozoa than the infected red cocks in weeks 2, 3, 4 and 6 post infections (Table 1). In week 5 there was no significant difference between the infected and control cocks ($p > 0.05$).

The control white Shikabrown cocks had significantly higher percentage live spermatozoa ($p<0.05$) than the infected white cocks in weeks 1, 2, 3, 4, 5 and 6. The percent live spermatozoa of the control white Shikabrown cocks were significantly higher ($p<0.05$) than that of the control red Shikabrown cocks.

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

DISCUSSION

Results indicate that the cloacal temperatures of the control red and white Shikabrown cocks were not significantly different ($p>0.05$). Similarly the cloacal temperatures of the infected red and white cocks were not significantly different ($p>0.05$). However, 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. It is noteworthy that cloacal temperature has been established as an important tool in poultry health management, such as during environmental stress (Ayo *et al.*, 2005) and disease condition (Oladele, 2004). The slight increase in cloacal temperatures of infected subjects in the present study may be due to a physiologic response to the administered vaccine; this corroborates the reports of Oladele (2004), who documented such an increase in rectal temperatures of Shaver brown chicks that were vaccinated with Newcastle disease virus.

In the present study the infected red cocks were slightly lesser in weight than the white. This is in contrast to the findings of Nwagu *et al.* (1996) who reported that the control red Shikabrown cocks were heavier than the

Table 1: Effects of Newcastle disease virus on live weight, cloacal temperature and semen characteristics of red and white Shikabrown 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 temp. (°C)	RSB	41.7± 0.2	41.8± 0.7	41.6± 0.1	41.8± 0.1	41.6± 0.9	41.1± 0.4	41.5± 0.3	41.8± 0.1	41.7± 0.1	42.1± 0.1	41.4± 0.1	41.7± 0.9
	WSB	41.0±	41.8±	41.4±	41.7±	41.5±	41.7±	41.5±	42.3±	41.6±	41.9±	41.7±	41.5±
Live weight (kg)	RSB	2.2± 0.2	2.1± 0.2	2.2± 0.1	2.1± 0.2	2.3± 0.1	2.1± 0.1	2.2± 0.1	2.1± 0.1	2.2± 0.0	2.2± 0.1	2.2± 0.1	2.2± 0.1
	WSB	2.2± 0.2	2.2± 0.2	2.2± 0.1	2.2± 0.0	2.1± 0.1	2.0± 0.1	2.2± 0.1	2.2± 0.1	2.3± 0.1	2.2± 1.0	2.3± 0.1	2.2± 0.4
Semen volume (ml)	RSB	0.3± 0.1	0.3± 0.1	0.3± 0.3	0.2± 0.4	0.3± 0.1	0.3± 0.1	0.3± 0.1	0.2± 0.1	0.3± 0.3	0.3± 0.1	0.3± 0.1	0.3± 0.1
	WSB	0.3± 0.1	0.2± 0.1	0.8± 0.3	0.5± 0.3	0.5± 0.1	0.5± 0.1	0.4± 0.1	0.3± 0.1	0.4± 0.1	0.3± 0.1	0.5± 0.1	0.5± 0.1
Color (1-3)	RSB	1.6± 0.4	1.3± 0.3	1.6± 0.4	1.4± 0.3	1.9± 0.2	1.3± 0.2	2.0± 0.4	1.6± 0.3	2.1± 0.3	1.3± 0.4	1.8± 0.3	1.6± 0.4
	WSB	2.1± 0.3	1.2± 0.3	1.9± 0.3	2.0± 0.3	2.4± 0.3	1.7± 0.2	1.9± 0.3	2.5± 0.3	2.1± 0.3	1.9± 0.3	2.8± 0.4	2.4± 0.3
Sperm motility (%)	RSB	38.0± 9.9 ^a	35.7± 8.6 ^a	*53.1± 0.9 ^a	*41.8± 6.8 ^b	*73.8± 0.8 ^b	*42.9± 0.6 ^b	*60.6± 9.8 ^a	*47.1± 7.5 ^b	*55.7± 7.7 ^a	*32.5± 0.1 ^b	50.7± 0.8 ^b	50.0± 0.1 ^a
	WSB	70.5± 9.7	43.8± 0.7	73.6± 7.7	55.6± 6.2	66.8± 0.7	60.3± 5.7	61.2± 6.8	55.9± 8.4	60.0± 8.7	55.0± 0.7	69.5± 9.8	57.2± 7.5
Sperm conc. (x10 ⁹ /ml)	RSB	2.3± 0.6 ^a	1.7± 0.5 ^b	3.2± 0.8 ^a	2.3± 0.6 ^b	4.1± 0.7 ^a	1.9± 0.5 ^b	3.6± 1.5 ^a	2.5± 1.2 ^b	3.8± 0.3 ^a	1.5± 0.5 ^b	2.6± 0.6	2.4± 0.8
	WSB	2.7± 0.6 ^a	1.8± 0.5 ^b	*5.0± 0.7 ^a	3.6± 0.5 ^b	3.8± 0.6	2.6± 0.5 ^b	4.9± 1.3 ^a	4.1± 1.1 ^a	3.6± 0.3	2.8± 0.7 ^a	5.0± 0.7 ^a	4.6± 0.6 ^a
Live sperm. (%)	RSB	38.2± 0.9 ^a	38.2± 0.9	64.4± 0.1 ^a	53.9± 0.1 ^b	69.4± 0.9 ^a	40.0± 0.7 ^b	63.1± 0.1	58.2± 0.8	56.8± 0.8 ^a	54.9± 0.1 ^a	54.7± 0.1 ^a	47.5± 0.1 ^b
	WSB	69.5± 0.1 ^a	45.0± 0.8 ^b	*75.9± 0.9 ^a	*66.2± 0.7 ^b	*76.8± 0.8 ^a	*66.2± 0.6 ^b	63.8± 0.7	57.7± 0.9	72.3± 0.9 ^a	40.1± 0.7 ^b	*80.0± 0.1 ^a	*66.2± 0.8 ^b
Total abnormalities (%)	RSB	3.1± 1.3	3.3± 0.1	3.6± 0.2	7.4± 2.6	6.4± 1.3	6.6± 1.7	3.6± 4.8	6.2± 3.6	2.4± 0.7	7.0± 0.9	2.7± 0.9	5.1± 1.4
	WSB	3.9± 0.9 ^a	6.4± 1.2 ^b	3.9± 2.2 ^a	9.8± 1.8 ^b	5.8± 1.4	6.1± 1.2	5.0± 4.1	6.3± 3.3	3.0± 0.8 ^a	7.6± 0.7 ^b	3.8± 1.2 ^a	6.6± 0.9 ^b

^a = Mean with different letter superscripts within treatments (rows) and asterisks within strains (columns) are significantly (p<0.05) different.

control white Shikabrown cocks. This observed difference in the reports of Nwagu *et al.* (1996) may be attributed to the relatively shorter duration (four weeks) in their study. The causes of the changes in live weights observed between the two strains of birds in the present study can only be speculated upon, because the birds were maintained on identical diet throughout the study. In view of this, differences due to diet are unlikely. One possible explanation is likely due to a transient loss of appetite in the experimental subjects; again this may be a physiologic response to the viral (active) immunization. This finding corroborates that of Okoye *et al.* (2000), who found that Newcastle disease infected chickens were significantly lighter in weight than the control birds at 2 and 3 weeks post infection.

The control and infected white Shikabrown cocks had relatively higher semen volume throughout the period of the study than the red Shikabrown counterparts. Similarly, the control red cocks had higher semen volume than the infected cocks. In this study, it is imperative to note the apparent strain difference in an important semen parameter as seminal volume, which showed the white Shikabrown cocks as having a highly consistent semen volume than the red Shikabrown

cocks. This observation is consistent with the findings of Obidi *et al.* (2008), which may be an important advantage in artificial insemination in this breed. However, the findings of Nwagu *et al.* (1996) is contrary to these two reports in that the Shikabrown red had a higher semen volume in their study. Again, this observed difference in the reports of Nwagu *et al.* (1996) may be attributed to the relatively shorter duration (four weeks) in their study.

Generally, the results from the present study showed a significant (p<0.05) drop in sperm motility. This is in contrast to the normal range reported by Lake and Stewart (1978), for poultry species and Obidi *et al.* (2008) for Shikabrown breeder cocks. It may be reasonable to say that this significant drop in motility is partly due to a subjective assessment of sperm motility by the scorer. It is also reasonable to observe that the control subjects in both strains had a significantly (p<0.05) higher sperm motility than the infected cocks. In this respect, it may be assumed that the Newcastle disease vaccine had a temporary or transient inhibition on sperm motility. The mechanism of this physiological inhibition is subject to further investigation. The study showed that the white cocks had a better spermatozoa

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

The result of the sperm cell concentration for both strains in the present study is within the range reported by Lake and Stewart (1978), Nwagu *et al.* (1996) and Obidi *et al.* (2008). Within strain sperm concentration, the white Shikabrown cocks had a relative advantage, which corroborates earlier report of Obidi *et al.* (2008). It is pertinent to emphasize this as another advantageous characteristic for artificial insemination in this breed of cock. Further studies are required to determine the effects of NDV on total sperm abnormalities. In conclusion, this study has supported a high reproductive potential of Shikabrown breeder cocks as highlighted by Obidi *et al.* (2008a) and a better potential for the white strain in particular, it is suggested that routine and timely vaccination of this breeders be observed for adequate protection from Newcastle disease which may be said to be endemic to Zaria.

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