

## INFLUENCE OF BREED TYPE AND AGE ON SPERMATOLOGICAL TRAITS OF NIGERIAN LOCAL CHICKENS

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### ABSTRACT

*This study evaluated semen quality traits of 36 roosters, comprising 18 roosters each of normal feather and naked neck Nigerian local chicken (NLC) breeds at different ages. The abdominal massage technique was used to collect semen samples from individual roosters at 28, 32, 36 and 40 weeks of age. Individual ejaculates were assessed for semen volume, sperm concentration, total sperm count/ejaculate, motility, morphology and viability. Results of the semen evaluation indicated that except for semen volume, breed of rooster did not statistically influence other spermatological traits studied. The normal feather had higher semen volume (0.13 mL) than the naked neck roosters. Semen volume, sperm concentration and total sperm count/ejaculate were influenced by age ( $p < 0.05$ ). There was a 44.16% increase ( $p < 0.05$ ) in semen volume between 28 and 36 weeks of age and 24.67% decrease by 40 weeks. Sperm concentration was highest at 28 weeks with a decrease of up to  $1.32 \times 10^9 \text{ mL}^{-1}$  at 40 weeks whereas total sperm count/ejaculate was highest at 36 weeks of age. Sperm viability ranged from 70.60-80.00% and did not vary between the two breeds at different ages. It can be concluded that though there exist marginal variations in spermatological traits of the normal feather and naked neck roosters due to their genetic constitution and age, both breeds could still be used efficiently in planning artificial insemination/breeding programmes for genetic improvement of the Nigerian local chicken since the results obtained were within the accepted reference range for chickens.*

**Key words:** artificial insemination, naked neck, Nigerian local chicken, normal feather, semen quality

### INTRODUCTION

Local chickens are important in overall food production systems in developing economies mostly because of their adaptation to the tropical environment. They may appear to produce less than highly specialized commercial breeds; however, they possess attributes that make their sustainability on available local resources more ecological and economical in the long term (Oleforuh-Okoleh, 2013; Mkpughe and Bratte, 2015). Incorporation of the Nigerian local chicken as a parent breed stock in the nation's breeding programme is important to meet the increase in poultry product demand by the citizenry. Jie and Liu (2011) reiterated that rapid development of breeding technologies has resulted in enormous progress in the global poultry industry. Such technologies include the use of artificial insemination (AI) which has been considered as a valuable technique in the poultry industry by ensuring effective selection of males and better management of the breeding stock (Benoff *et al.*, 1981; Gebriel *et al.*, 2009). The first successful AI in poultry was in 1899; this was achieved by using semen recovered

from the ductus deferens after killing a cock to inseminate hens to produce fertile chicken eggs (Lunak, 2010). Noteworthy, is the fact that AI is more profitable and beneficial when the semen quality is at optimum level. This necessitates that breeding management practices which are beneficial and effective towards proper identification, selection, management and preservation of breeding stocks be undertaken (Lake, 1989). To optimize reproduction output therefore, there is need for periodic evaluation of semen in a breeding stock since only fewer cocks usually run with the hens. Semen evaluation involves quantitative (macroscopic) and qualitative (microscopic) measures of features of the semen and sperm. These features include the appearance and volume of the semen, concentration, motility and morphology of the sperm (Galal, 2007). There have been numerous reports that the semen quality of cocks is influenced by several factors which include nutrition (Tadondjou *et al.*, 2014), season (Ayo *et al.*, 2007; Santiago-Moreno *et al.*, 2011; Elagib *et al.*, 2012), frequency of ejaculation (McDaniel and Sexton, 1977), endocrine disrupting

chemicals (Rengaraj *et al.*, 2015), photo/lighting period (Bajpai, 1963; Almahdi *et al.*, 2014), physiology (Omeje and Marire, 1990), breed or strain (Molekwa and Umesiobi, 2009; Oke and Ihemeson, 2010; Tarif *et al.*, 2013) and age (Cerolini *et al.*, 1997; Shanmugam *et al.*, 2012). The present study was aimed at investigating the influence of breed type and age on spermatological traits of two Nigerian local chicken breeds (normal feather and naked neck).

## MATERIALS AND METHODS

### Site of Study and Management of

#### Experimental Birds

The study was done in the Poultry Unit of Department of Animal Science Farm, Rivers State University of Science, Port Harcourt, Nigeria between September and December, 2016. A total of 36 Nigerian local chicken roosters, consisting of 18 roosters each of the normal feather and naked neck breeds, were randomly selected from a heterogeneous local chicken population maintained at the Unit. This population as described in Oleforuh-Okoleh *et al.* (2016) originated from a parent population of purebred Nigerian local (indigenous) chicken developed at Federal University of Agriculture's PEARL Chicken Research Farm at Abeokuta, Ogun State, Nigeria. The birds were raised on deep litter and transferred to individual battery cages at 20 weeks of age. They were served daily throughout the study period with growers diet (17.5% crude protein and 2769 kcal kg<sup>-1</sup> metabolizable energy) based on 10% of the mean weekly body weight of the population. Clean water was given *ad libitum*. Vaccination against Newcastle disease, fowl pox, fowl typhoid, infectious bronchitis and gumboro diseases were done during their early growth phase. Newcastle disease booster vaccine was administered subsequently every 6 weeks. Vitamins supplement were given once a month via drinking water.

#### Semen Evaluation

Prior to actual semen collection, the roosters were primed twice weekly from 24 weeks of age, using the abdominal massage techniques stipulated by Hafez (1987). Spermogram of the two breeds was done monthly from 28-40 weeks of age, thus a total of 144 semen samples (72 per breed) were evaluated. To achieve this, individual ejaculates were collected into sterile labeled calibrated collection vial of 2-mL capacity. Semen volume was assessed by drawing the ejaculate from the collection vial using calibrated syringe with 0.02 mL accuracy. The sperm concentration was evaluated using an improved Neubauer haemocytometer as indicated in Hafez (1987). A drop of semen was thoroughly mixed with half-normal saline using a dilution factor of 1:20. Ten µL of the diluted semen was dropped at each side

of the haemocytometer using a micropipette and allowed to settle for 5 min. and then placed under the light microscope under ×400 magnification. Sperm count was done by counting any sperm head that fell within the sub-divided smaller squares at the four edges and centre of the haemocytometer and recording the average. Sperm concentration was obtained by multiplying the number of sperm counted by dilution factor/volume and the multiplying factor of the chambers counted (Bah, 2001). Semen motility was done using a light microscope under ×400 magnification on the basis of the proportion of sperm showing progressive forward movement (Ax *et al.*, 2000). The eosin-negros in differential staining techniques described by Burke (1984) was used to estimate the percentage of live or dead sperms and sperm morphology. Sperm morphology was categorized as either normal form or abnormal form depending on the characteristics of the head, mid-piece and tail region, and also on stage of maturity of the germ cells. The result of active motile sperm and the proportion of live sperm were used to predict sperm viability for individual ejaculate sample.

#### Statistical Analysis

Data obtained from the spermogram were subjected to analysis of variance using multivariate analysis of the general linear model (GLM), with breed and age as fixed factors. Significant means were separated using Duncan (at  $p < 0.05$ ). All analyses were done using IBM SPSS (2013).

## RESULTS

### Main Effects of Breed and Age on Spermatological Traits

The main effects of breed and age on semen volume, sperm concentration, total sperm count/ejaculate, sperm motility, sperm morphology, proportion of live and dead sperm and sperm viability are presented in Table 1. Mean semen volume of 0.52±0.05 mL was obtained from the NLC population investigated. Semen volume reported in our study was relatively higher in normal feather roosters (0.61±0.05 mL) compared to their naked neck counterpart (0.48±0.05 mL). The result further indicates that there was a remarkable increase (44.16%) in semen volume between 28 and 36 weeks of age and a reduction (24.67%) at 40 weeks of age. Mean sperm concentration and sperm count obtained from both breeds ( $6.42 \times 10^9$  mL<sup>-1</sup> and  $3.49 \times 10^9$  sperms ejaculate<sup>-1</sup> respectively) were not influenced by breed type. Aging in roosters was associated with a corresponding significant decrease ( $p < 0.05$ ) in sperm concentration but with an increase in total sperm count/ejaculate up till 36 weeks of age. Sperm concentration was highest at 28 weeks of age with a decreased of up to  $1.32 \times 10^9$  mL<sup>-1</sup> by 40 weeks of age. Neither the breed nor the age of

**Table 1:** Main effects of breed and age on spermatological traits (mean±SEM) of NLC roosters

Factor/ Effect	Semen volume (ml)	Sperm concentration (×10 <sup>9</sup> mL <sup>-1</sup> )	Sperm Count (×10 <sup>9</sup> ejaculate <sup>-1</sup> )	Sperm motility (%)	Normal morphology (%)	Dead (%)	Live (%)	Viability (%)
Total mean	0.52±0.05	6.42±0.36	3.49±0.29	70.76±2.53	76.46 ±1.91	18.78±2.37	81.89±1.90	75.51±1.93
Breed								
Normal feather	0.61±0.05 <sup>a</sup>	6.22±0.42	3.96±0.41	73.33±2.68	77.36±2.11	17.45±2.66	82.55±2.67	75.27±2.31
Naked neck	0.48±0.05 <sup>b</sup>	6.52±0.42	3.02±0.42	69.35±2.71	76.94±2.13	18.77±2.69	81.23±2.69	76.50±2.28
Age (weeks)								
28	0.34±0.06 <sup>c</sup>	7.01±0.10 <sup>a</sup>	2.46±0.53 <sup>b</sup>	69.36±3.43	73.97±1.20	19.61±3.41	80.39±3.41	73.75±2.92
32	0.49±0.06 <sup>c</sup>	6.57±0.25 <sup>b</sup>	3.44±0.54 <sup>b</sup>	72.38±3.52	76.37±1.07	18.21±3.55	81.79±3.50	76.79±2.99
36	0.77±0.07 <sup>a</sup>	6.20±0.13 <sup>b</sup>	5.11±0.60 <sup>a</sup>	73.00±3.93	77.00±1.09	19.00±3.59	81.00±3.91	75.50±3.35
40	0.58±0.08 <sup>b</sup>	5.69±0.27 <sup>c</sup>	2.93±0.66 <sup>b</sup>	70.63±4.92	81.25±1.39	15.63±4.28	84.38±4.27	77.50±3.66
		value		P-				
Breed	0.046	0.476	0.091	0.287	0.911	0.706	0.706	0.695
Age	0.000	0.026	0.010	0.888	0.280	0.789	0.789	0.750

<sup>abc</sup>Means on the same column, for individual effect, not sharing a common superscript are significantly different (p < 0.05).

**Table 2:** Breed × age interaction effect (B×A) on semen volume and sperm concentration, count/ejaculate and motility<sup>1</sup>

Breed	Semen volume (mL)		Sperm concentration (×10 <sup>9</sup> mL <sup>-1</sup> )		Sperm count (×10 <sup>9</sup> ejaculate <sup>-1</sup> )		Sperm motility (%)	
	na	Na	Na	Na	na	Na	na	Na
Age (weeks)								
28	0.33±0.06	0.35±0.05	6.50±0.64	7.52±0.46	2.31±0.68	2.61±0.77	61.22±4.40 <sup>b</sup>	77.50±1.48 <sup>a</sup>
32	0.58±0.09	0.41±0.08	7.50±0.36	5.64±0.69	4.32±0.80	2.57±0.93	83.33±2.12 <sup>a</sup>	61.42±3.15 <sup>b</sup>
36	0.84±0.08	0.70±0.07	7.20±0.52	5.20±0.32	6.37±0.84	3.84±0.84	80.00±2.98 <sup>a</sup>	66.00±2.29 <sup>b</sup>
40	0.68±0.10	0.48±0.09	4.88±0.48	6.50±0.26	2.76±0.84	2.88±0.84	68.75±1.28 <sup>b</sup>	72.50±1.32 <sup>a</sup>
				p-value				
B×A	0.640		0.087		0.214		0.001	

<sup>ab</sup>Means on the same row not sharing a common superscript, for each trait, are significantly different (p < 0.05).

<sup>1</sup>Mean±SEM; na - Normal feather; Na - Naked neck

cocks influenced (p > 0.05) the sperm motility. The proportion of live sperm cells in the normal feather and naked feather were 82.55 and 81.23% respectively, whereas sperm viability ranged from 70.60 to 80.00%. Although, there appeared to be numerical improvement in sperm morphology, proportion of live and dead sperm and sperm viability as the roosters aged, there were no evidences that the breed and age of roosters influenced (p > 0.05) these sperm quality traits.

### Interaction Effect of Breed × Age (B×A) on Spermatological Traits

The result of breed × age (B×A) interaction effects on semen volume, sperm concentration, total sperm count/ejaculate and sperm motility are presented in Table 2. Although normal feather roosters had numerically higher semen concentration at 32 and 36 weeks of age than the naked neck, the variations due to B×A interaction was not significant. Sperm motility ranged from 61.22 to 83.33% and from 61.42 to 77.50% in the normal feather and naked neck breeds, respectively. Best sperm motility result was obtained at 32 and 36 weeks of age for the normal feather roosters whereas the naked neck roosters sperm had highest motility rate at 28 and 40 weeks of age (p < 0.05).

A significant (p < 0.05) interaction relationship existed between breed and age for sperm morphology and proportion of live and dead sperm (Table 3). The naked neck roosters had better sperm morphology at 28 and 40 weeks of age than

normal feather. Much of the variance in the interaction of B×A on proportion of live and dead sperm cells was found between the 28<sup>th</sup> and 32<sup>nd</sup> weeks of age. The naked neck appeared to have more live sperm at 28 weeks than the normal feather. The B×A interaction was not observed in sperm viability.

### DISCUSSION

Poultry semen volume compared to other livestock is relatively low because birds lack accessory glands which are well developed in mammals (Almahdi *et al.*, 2014). Although semen volume does not necessarily equate to fertilizing ability or viability of the sperm, the volume cannot be neglected in semen evaluation for the semen serves as the transportation system/medium for the sperm. Our findings refute the report of Galal (2007) which indicated that the presence of the naked neck allele (Na) resulted in significantly higher semen volume compared to its normal feather allele (na) in a population of Fayoumi and Dandarawi Egyptian chickens. Hammad *et al.* (1987) also found that naked neck males produced significantly higher semen volume than their normal counterparts. However, our result, affirms the findings of Peters *et al.* (2008) and Ajayi *et al.* (2011) who reported that the semen volume of the normal feather was higher (0.58-0.83 mL) than those of the naked neck (0.18-0.37 mL) Nigerian local chicken. The mean semen volume of 0.48 and 0.61 mL obtained from the naked neck and normal

**Table 3:** Breed × age interaction (B×A) effect on sperm morphology, proportion of dead and live sperm, and sperm viability<sup>1</sup>

Breed	Normal morphology (%)		Dead sperm (%)		Live sperm (%)		Sperm viability (%)	
	na	Na	na	Na	na	Na	na	Na
Age (weeks)								
28	68.77±3.71 <sup>b</sup>	79.17±2.09 <sup>a</sup>	27.56±4.79 <sup>a</sup>	11.67±0.88 <sup>b</sup>	72.447±4.51 <sup>b</sup>	88.33±5.11 <sup>a</sup>	70.60±3.86	77.50±2.01
32	79.67±0.81	73.57±4.02	10.00±1.36 <sup>b</sup>	26.43±5.41 <sup>a</sup>	90.007±5.11 <sup>a</sup>	73.57±4.78 <sup>b</sup>	80.00±1.80	73.57±4.07
36	84.00±1.37 <sup>a</sup>	70.00±1.29 <sup>b</sup>	16.00±1.89	22.00±2.31	84.007±5.52	78.01±5.52	81.00±1.89	70.00±1.29
40	77.50±1.32 <sup>b</sup>	85.00±1.18 <sup>a</sup>	16.25±0.98	15.00±1.18	83.757±6.05	85.07±6.05	75.00±2.04	80.00±1.86
				p-value				
B×A	0.013		0.013		0.013		0.117	

<sup>ab</sup>Means on the same row, for individual traits, not sharing a common superscript are significantly different ( $p < 0.05$ ).

<sup>1</sup>Mean±SEM; na - normal feather; Na - Naked neck

feather roosters respectively was higher than the mean volume of 0.28 mL reported by Bah *et al.* (2001) for some Nigerian local breeder roosters and 0.2 mL by Molekwa and Umesiobi (2009) for South African naked neck indigenous chicken. The semen volume of 0.34 mL obtained at 28 weeks of age was higher than 0.26 mL obtained by Shanmugam *et al.* (2012) from naked neck broiler breeders, of the same age raised in Hyderabad, with significant increase in volume from 24 to 48 weeks of age. Similar volume was obtained by Hu *et al.* (2013) in a population of Beijing-You chickens at 43 weeks of age. The variations between our results and some other authors could be attributed to genetic and environmental factors.

Irrespective of the influence of breed or age factor in our study, the mean semen volume (0.52±0.05 mL) obtained was above the reference range of 0.1-0.3 mL given by Long (2006) but within some other accepted range of 0.5-0.8 mL (Smyth, 1968), 0.4-1.0 mL (Froman *et al.*, 1995) and 0.2-0.5 mL (Getachew, 2016). Low semen volume (hypospermia) is often associated with several factors like blockage of vas deferens or seminal vesicle and hormonal imbalance. Conversely, high semen volume (hyperspermia) is an indication of hormonal imbalance due to the presence of certain steroids (Rengaraj *et al.*, 2015).

The number of sperm found per unit volume (mL) of semen describes the sperm concentration (Malejane *et al.*, 2014). According to Cole and Cupps (1997), this can be used when planning artificial insemination in a flock to predict the number of breeding hens to be inseminated. Mean sperm concentration of  $6.42 \times 10^9 \text{ mL}^{-1}$  obtained from both breeds was similar to the sperm concentration reported by Udeh *et al.* (2011) in a population of Nigerian local chicken ( $6.99 \times 10^6 \text{ mL}^{-1}$ ) and by Makhafola *et al.* (2012) in South African indigenous naked neck cockerels ( $6.3 \times 10^9 \text{ mL}^{-1}$ ). Our result which showed that concentration of sperm cells and sperm count per ejaculate was not influenced by genotype affirms the report of Zhang *et al.* (1999). Previous studies by Machebe and Ezekwe (2002) indicated that the naked neck cocks had higher sperm concentration than the normal feather cocks. Strain differences were also observed with respect to sperm concentration in Denizil cocks by Tuncer *et al.* (2006). Our finding

is higher than the values obtained by Malik *et al.* (2013) but lower than those reported by Tarif *et al.* (2013) among four chicken lines (Sasso, Synthetic, Assel RIR and White Rock). Lake (1983) attributed inherent variations in sperm production to differences between individuals within strains and breeds. The decrease in sperm concentration with age as seen in the present study is in line with Cerolini *et al.* (1997) but contrary to Hermiz *et al.* (2016) who did not observe any influence of age in semen traits of Iranian roosters belonging to different local genetic groups and their crosses with the commercial strain ISA brown. Our observation that sperm count per ejaculate increased with age agrees with Onuora (1987) and Gebriel *et al.* (2009).

Sperm morphology is of great value in poultry breeding because it is mostly used in estimating the fertilizing ability of the sperm cells (Lukaszewicz, 1988). Baskt and Brillard (1994) pointed out that only sperms with normal morphology can ascend through the vagina of the hen to the sperm storage tubules. Most often abnormal spermatozoa are found in normal semen analysis but it becomes challenging when there is high proportion of these sperms in the sample, as they possess defects which could affect their ability to fertilize an egg or result in poor hatchability if the egg is fertilized. Our findings on sperm morphology attest to Shanmugam *et al.* (2012), Almahdi *et al.* (2014) and Ameen *et al.* (2014) who reported non-significant variation in the sperm morphology in different breeds/strains of cockerels. Tabatabaei *et al.* (2010) observed an increase in rate of sperm morphological defect in Iranian indigenous broiler breeder chickens with aging of roosters. Tanemura *et al.* (1993) attributed such defects to impaired spermatids and spermatocytes resulting in interrupted spermatogenesis.

The total motile sperm suggests the total number of sperms assumed to be capable of progressing from the site where semen is deposited in the course of insemination to the sperm storage tubules in the hen. It is a good indicator of a sire's reproductive potential and is often used in predicting fertility (Donoghue *et al.*, 1998). Decreased sperm motility has been associated with abnormal spermatogenesis and epididymal sperm maturation problems (Rengaraj *et al.*, 2015). Sperm motility of the two breeds studied was within the reference range of 60-80% reported for cockerels in

Getachew (2016). The non-significant variation observed in sperm motility in the present study disputes the report of Ajayi *et al.* (2014) who reported differences in the normal feather and naked neck Nigerian local cocks, with the naked neck cocks having better sperm motility. Molekwa and Umesiobi (2009) also reported breed variations in sperm motility in South African indigenous chicken breeds. Gebriel *et al.* (2009) found higher values for sperm motility in Norfa cocks at 46 weeks of age compared to cocks at 30 and 38 weeks of age. Sperm viability is one of the major components of routine semen quality assessment. Our observations concur with Sonseeda (2014) as well as Mothibedi *et al.* (2016) who did not observe any difference in sperm viability in between different strains/breeds of chicken.

## CONCLUSION

In poultry breeding, semen evaluation is critical in achieving maximum fertility and hatchability. Our results show that there existed marginal variations in the spermatological traits of the normal feather and naked neck roosters. Much of these variations were due to age. Semen volume was the only trait affected by the breed type. However, since the values obtained from the spermogram of both breeds at different ages were within the reference range documented for chickens, we infer that any of the two breeds can be used effectively in breeding programmes/schemes aimed at propagating the Nigerian local chicken gene resource.

## ACKNOWLEDGEMENT

We wish to thank the Poultry Unit of Department of Animal Science farm of the University for granting us permission to use its facilities. We also appreciate the assistance of Dr. B. O. Agaviezor and F. Ologboso, of University of Port Harcourt, for training us on semen collection and artificial insemination in chicken. The unrelenting and tireless effort of the laboratory technician, Mr. U. Anusionwu, is worthy of commendation.

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