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Assessment of semen characteristics among three phenotypes of chicken raised in Akko, Gombe State of Nigeria

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ABSTRACT

In poultry breeding program the success of artificial insemination (AI) is highly influenced by the quality of semen. The aim of the present research was to evaluate the variations in semen characteristics of three phenotypes of chicken of the same specie raised in Akko, Nigeria. A total of 9 cocks from threedifferent phenotypes; Red feathered (n = 3), White feathered (n = 3) and Black feathered (n =3). The semen was evaluated for macroscopic (i.e. semen volume and colour) and microscopic (i.e. sperm concentration, motility and morphology) criteria after being collected by abdominal massage method. There were phenotypes variation ($P > 0.05$)effects on semen motility, number of live/dead sperm and sperm abnormalities. No differences($P>0.05$) were observed on volume, colour, concentration, mass motility and pH of semen. The observed sperm progressive motility was ranged from 90.5 ±1.21% to 95.09±0.82%. The White feathered strain had the highest sperm progressive motility ($P<0.05$)and highest value for live and normal sperm (93.5±0.63% and 87.90±0.25%), while the black feathered had the least and (92.4±0.73% and 85.5±0.50%) respectively. The Red feathered strain had the highest value for both dead and sperm head defect (9.2±0.33%and 7.0±0.52%). Whilst, the Black feathered strain scored the highest percentage of both sperm tail and neck defects with values ranged from 11.4±0.43%to 14.2±1.08% and 19.4±0.53% to 23.6±0.69%, respectively. This study suggests that there are large variations present in semen characteristics of different phenotypes of cocks; White feathered strain is likely have better semen characteristics compared to Red and Black feathered strains. Therefore, White feathered strain can potentially be used in artificial insemination (AI) for chicken production and improvement.

Keywords: Semen characteristics, cock, White feathered cock, Red feathered cock, Black feathered cock,

INTRODUCTION

The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters *et al*., 2004). Fertility and hatchability on the other hand are the major determinant of profitability in the hatchery enterprise. The semen of the domestic fowl according Tijjani *et al*, (2014), varies from milky, creamy and slightly creamy to a watery fluid with a relative high density. The differences in volumes and sperm concentration of the domestic fowl semen depends largely on the relative contribution of the various reproductive glands, the number of sperm that could be obtained from a breed/strain and the extent to which the genetic potentials can be exploited (Peters *et al*., 2004). Previous findings on cock's semen characteristics have shown that significant genotype differences affect body size and semen characteristics of cocks, except the pH value (Makhafola *et al.,* 2012). Although, there are studies on semen characteristics of the domestic fowls in the literature have been published, but little or none has been reported on the Nigeria local chicken with particular emphasis on the influence of strains or genetic on semen characteristics. The information of semen characteristics of these Nigerian local chicken strains is very important as the local chickens constitute between 80 and 90 percent of the total population of chickens in Nigeria. This study therefore focuses on the comparative evaluation of semen quality traits of three chicken predominant phenotypes.

MATERIALS AND METHODS

Experimental site

The study was conducted at Poultry Unit of Teaching and Research Farm, Federal University of Kashere, Gombe State, Nigeria.

Experimental chickens and management

Nine (n = 9) phenotypic Nigerian indigenous breed of cockerels (Black, Red and White; n = 3 each) age ranged from 44 to 51 weeks with an average weight of 2.1kg were obtained from a reputable farm in Kumo, Gombe State. The cockerels were managed under intensive management system. The birds were fed on grower mash and water *ad libitum*.

Semen collection

The cockerels rested for a period of two weeks, which served as an adaptation period in order to make the cocks familiar with the semen collector and to improve the effectiveness of collection; likewise, they were trained and responded to the abdominal massage technique prior to the onset of semen collection. Single ejaculate of semen was collected from each phenotypic Nigerian indigenous breed of cockerels (Black, Red and White strains) for three times within ten days in the morning between 10am to 11am by an abdominal massage method described by Bakst and Dymond (2013).
After collection, the semen was macroscopically evaluated for; colour, volume and pH value. Then, microscopically evaluated for; sperm concentration, semen mass motility, sperm individual progressive motility and sperm morphology (abnormality of sperm head, mid-piece and tail)(Peters, *et al*., 2008).

Semen volume, colour and pH

The ejaculate volume from each group of three phenotypic Nigerian indigenous breed of cockerels (Black, Red and White) was measured with the use of 1ml syringe.

Semen colour was visually evaluated immediately after collection and graded on a scale of 1– 4 (where, 1 = watery, 2 = slightly creamy, 3 = creamy, 4= milky) (Tijjani*et al*, 2014). Semen pH was measured using pH test strip.

Semen mass motility

To evaluate mass activity, a drop of undiluted semen from each group of three phenotypic Nigerian indigenous breed of cockerels was placed on a microscope slide, covered with a glass cover slide to spread the semen in order to have a uniform thickness and to prevent it from drying. It was then placed on a microscope for examination at 100× magnification and given scores of 0–9 according to Blesbois *et al.* (2008).

Individual progressive motility

To evaluate progressive motility, semen was diluted with ratio of 1:100 (semen extender) using modified Ringer's solution (sodium chloride: 9.0 g, potassium chloride: 0.4 g, calcium chloride: 0.3 g, dextrose: 1.3 g, sodium bicarbonate: 0.2 g, into 1000 mL of distilled water). The individual cell motility was estimated by placing a drop of the diluted semen on the slide covered with glass cover slide. Sperm motility was assessed by microscopic observation at 100x magnification. Motility was expressed as the percentage of motile cell with moderate to rapid progressive movement. At least 15 microscopic fields were examined and 150 sperms were counted for each sample.

Sperm concentration

The semen concentration for each group of three phenotypic Nigerian indigenous breed of cockerels were measured using haemocytometer with the direct cell count method. Haemocytometer is a specially designed slide that contains two counting chambers and two dilution pipettes. The counting chambers were1mm in depth and have a ruled area on the bottom of the chambers that is 1.0mm^2 of width. The square was sub-divided into 25 smaller squares. In this study, 10 µl of semen were mixed with 990 µl of distilled water at the dilution rate of 1:100. One drop of the diluted semen was dropped on one end of the haemocytometer and also on the other end and this was done in order to allow the diluted semen to settle. The loaded haemocytometer was placed on the microscope at 400x magnification. The sperm's head that falls within the sub-divided smaller squares at the four edges and centre of the haemocytometer were counted and the average per cockerel was considered based on the judgment of the individuals making the determination. The concentration of sperm/semen was calculated using the formula as below:

Concentration =Sperm Counted x Dilution Rate x Depth of Haemocytometer.

Live/dead sperm ratio

The eosin-nigrosin stain was used to determine the percentage of live and dead sperm. The stain was prepared by dissolving 1 g of eosin, 5g nigrosin and 3% sodium citrate in 100 mL of distilled water (Peters *et al.,* 2008; Kondracki *et al.,* 2017), which was pre-warmed to body temperature for about 30 minutes and filtered before use. Briefly 10µl of fresh semen was mixed with 10µl of Ringer's solution (Peters *et al.,* 2005). The 10µl of eosin-nigrosin stain was dropped onto a clean glass slide and mixed with 10µl of diluted semen. The second glass slide was used to swipe quickly and formed a thin layer and air dried. The sperm was examined at 1000x magnification under light microscope (Fig.1).

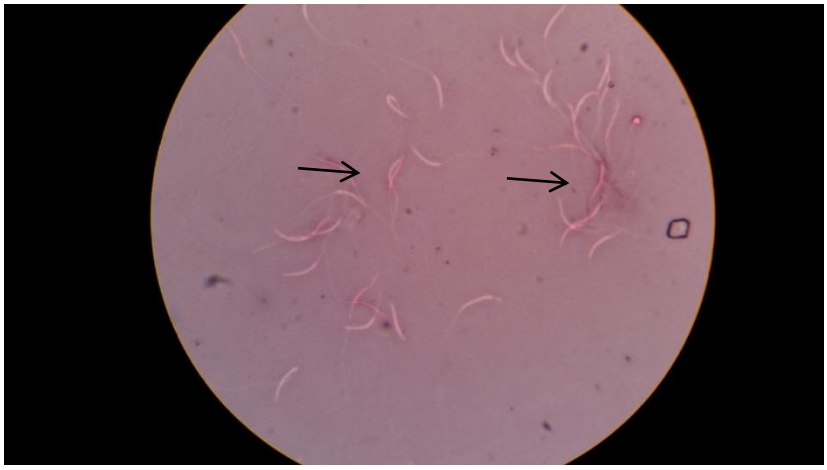

Fig.1 Photomicrography of cockerel sperm (1000xmagnification), pink color (stained with eosin: showed by black arrows) regarded as dead and without any color (no penetration of eosin stain) regarded as live.

Sperm morphology

The slides of live and dead stains were also used to check the morphology of the sperm in terms of abnormalities in sperm head (pear head, double head, elongated head, detached head), mid-piece (swollen mid-piece, coiled mid-piece), and tail (coiled tail, double tail, bent tail). About 300 sperms were examined for each sample under microscope at 1000× magnification.

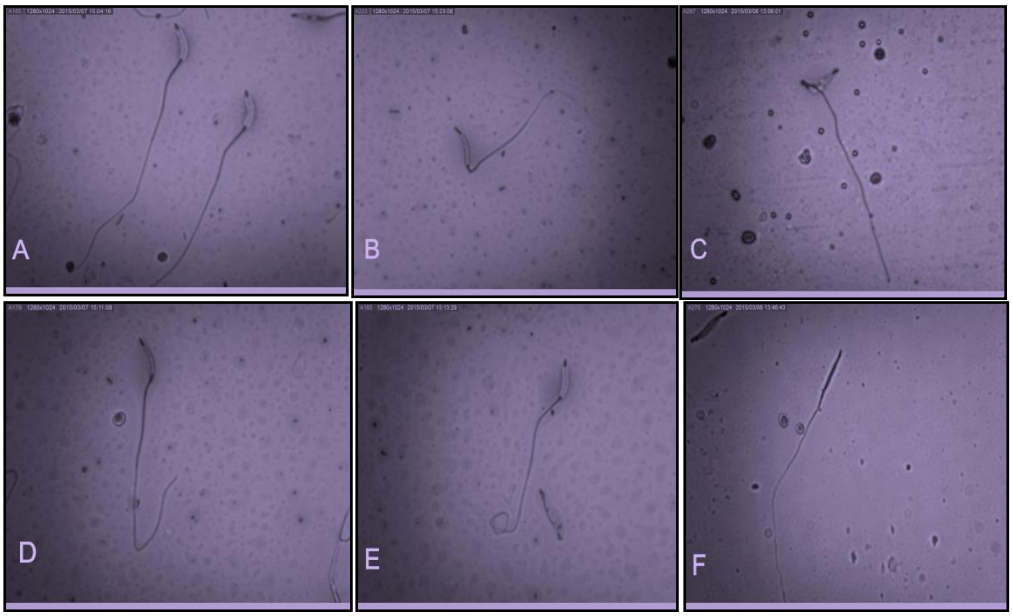

Fig.2 Photomicrography of normal and abnormal morphology of cock spermatozoa (1000x magnification) (A) Normal, (B) Simple bent at the neck region (C) Coiled head, (D) Bent tail (E) Coiled tail (F) Loose head.

Statistical analysis

The data regarding the percentage of sperm progressive motility, live/dead sperm, morphological normal intact sperm, total abnormal head, mid-piece/neck and tail abnormalities were subjected to analysis of variance (ANOVA) using statistical Package for Social Sciences (SPSS). A significance level of 0.05% was used

(P<0.05) to test differences on the different chicken phenotypes for both the quantity and quality semen parameters.

RESULTS AND DISCUSSION

Second level heading (Heading 2) with Sentence case

This study was carried out to evaluate the variation in semen characteristics(i.e. semen volume, semen pH, semen colour, mass motility, sperm concentration, sperm progressive motility, live/dead sperm and sperm morphology (i.e. normal, abnormal head, neck and tail)) of three different strains of chicken (i.e. White, Red and Black feathered cocks).

The mean values and standard error means (SEM) of the three strains of chicken's results on semen characteristics based on macroscopic and microscopic evaluations were presented in Table 1, Table 2, Table 3 and Table 4.

Table 1: The mean ± SEM of semen volume (million/mL), semen colour (scale 1 to 3) and semen pH (1 to 14) of three different strains of local Nigerian chicken (i.e. Red, White and Black feathered).

Strain	Semen Volume (mL)	Semen Colour (Scale 1-3)	Semen pH
Red Feathered	0.5 ± 0.04	2.2 ± 0.32	7.2 ± 0.05
White Feathered	0.4 ± 0.05	2.4 ± 0.28	7.3 ± 0.03
Black Feathered	0.5 ±0.06	2.3 ± 0.63	7.2 ± 0.04

Table 2: The mean ± SEM of mass motility (%), sperm motility (%) and sperm concentration (1 to 14) of three different strains of local Nigerian chicken (i.e. Red, White and Black feathered).

Strain	Mass Motility (%)	Sperm Motility (%)	Sperm Concentration ($x10^9$/mL)
Red Feathered	5.4 ± 0.31	92.8 ± 0.74^{ab}	3.2 ± 3.79
White Feathered	5.7 ± 0.27	95.1 ± 0.82^{a}	3.3 ± 2.57
Black Feathered	6.3 ± 0.30	90.5 ±1.21^{b}	3.3 ± 3.83

Means in the same column with different letters are significantly different (P < 0.05).

Table 3: The mean ± SEM of live sperm (%) and dead sperm (%) of three different strains of local Nigerian chicken (i.e. Red, White and Black feathered).

Strain	Live Sperm (%)	Dead Sperm (%)
Red Feathered	90.8±0.33^{b}	9.2±0.33^{a}
White Feathered	93.5±0.64^{a}	6.5±0.64^{b}
Black Feathered	92.4±0.73^{ab}	7.6 ±0.74^{ab}

Means in the same column with different letters are significantly different (P < 0.05)

Table 4: The mean ± SEM of sperm abnormalities (head (%), mid-piece (%), tail defects (%), and normal sperm (%)) of three different strains of local Nigerian chicken (i.e. Red, White and Black feathered).

Strain	Head Defects (%)	Mid-piece Defects (%)	Tail Defects (%)	Normal Sperm (%)
Red Feathered	7.0±0.52^{a}	19.6±0.22^{b}	13.6±0.58^{ab}	86.6±0.37^{b}
White Feathered	5.6 ±0.34^{b}	19.4 ±0.53^{b}	11.4 ±0.43^{b}	87.9±0.25^{a}
Black Feathered	5.8±0.29^{b}	23.6 ±0.69^{a}	14.2 ±1.08^{a}	85.5 ±0.50^{b}

Means in the same column with different letters are significantly different (P < 0.05)

The summary of the analysis of variance indicated that cockerel phenotypes had significant effect (P<0.05) on sperm individual progressive motility, sperm concentration, semen volume, sperm motility, semen colour, and active sperm. There were significant differences (P<0.05) in live and dead sperm, and sperm morphologically among those three different strains of cockerels.

The least square means as presented in Table 3 revealed that the White feathered cockerel had the highest live sperm followed by Black feathered cockerel and Red feathered cockerel with corresponding mean values of 93.5±0.64%, 92.4±0.73% and 92.4±0.73%, respectively. Similarly, Red feathered cockerel had the highest percentage of dead sperm and head defects compared to White and Black feathered cockerels. The highest sperm tail and sperm neck defects were recorded on the Black feathered cockerel followed by Red and White feathered cockerels. White feathered cockerel had the highest percentage of normal sperm over the Red and Black feathered cockerels.

DISCUSSION

The results showed that there were no significant differences in cockerel phenotypes with respect to semen volume, semen colour, semen pH and sperm mass motility. These observations were lower than those previously reported by Peters *et al.* (2008), but consistent with recent study as reported by Tijjani *etal.*(2014). These variations might be as a result of different of frequency of semen collection, different in cockerel body weight and environment. The values obtained for all the cocks in semen volume were within the acceptable range for artificial insemination (Peters *et al.,* 2008).

The most obvious evaluation of semen quality parameter is colour. The results of semen colour as affected by cockerel phenotypes in this study and indicated no differences existed among the three phenotypes cockerels.

The values obtained for semen pH in this study was within the normal range and consistent with previous studies conducted by Ajayi*et al.* (2014) and Hafez and Hafez (2000) in normal feathered cockerels. The concentration of sperm in the indigenous cockerels of the present study was higher when compared with that reported by Keskin *et al.*, (1995), and Tuncer *et al.*, (2008) but lower than that of Moya *et al.,* (1996) in broiler cockerels. This variation might be as a result of the differences in age, breed, nutrition, season, frequency of semen collection and fertilizing capacity of individual cockerels. The values obtained for semen pH for all the cockerel phenotypes were within the range reported for poultry semen (Tijjani *et al.,* 2014).

Sperm motility is one of the most often used parameters for semen evaluation (King *et al.,* 2000). The value obtained for sperm progressive motility in this study was similar to the result reported by State *et al.*, 2014)in dominant brown breed cockerel, but differed to that of indigenous broiler breeder (Tabatabaei *et al.*2009) and naked-neck breed (Machebe and Ezekwe, 2007), which were reported to have a low and higher sperm progressive motility respectively when compared with the result of the present study. However, the values obtained for semen motility for all the cockerel phenotypes were within the range reported for normal cock semen (Tijjani *et al.,* 2014).

Semen concentration in all the cockerel strain are similar but was lower compared with red jungle fowl and bantam chicken as reported by Abu *et al.* (2013). The difference in semen concentration is likely due to many factors such as intake of feed as level of nutrition is well known factor that affect semen parameters and the body size that could be attributed to their different genetic makeup and body weight.

Sperm morphology was recommended to be one of the most essential qualitative characteristics of poultry semen. It could be used as an essential parameter for predicting the fertilizing ability of sperm. The results of this study showed that the mean abnormalities of all ejaculates were similar compared to those of previous studies in frizzle feathered breed (Ajayi *et al.*, 2011) and white leghorn breed (Mosenene, 2009), but higher than in necked-neck breed (Machebe and Ezekwe, 2007). These differences may be attributed to the breed of chicken used, live-weight, age, season, environmental temperature and humidity, and semen collection techniques.

CONCLUSION

It was concluded that the semen characteristics of three cockerel phenotypes raised in Akko, Gombe state were within the acceptable range for artificial insemination (AI). In addition, low semen volume with high sperm concentration shows an indication of the superior genetic potential of the strain of cockerel for reproductive ability and higher fertility. This study suggests that the White feathered cockerelas a potential strain to be selected for breeding purpose because it showed better sperm characteristics than the other two strains of cockerels especially on progressive motility which is the most often parameter that determine the success of AI in animal breeding program.
However, it is recommended to have more samples from each phenotype in the future study for a more rigorous conclusion.

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