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RESEARCH ARTICLE

Comparison of semen characteristics between indigenous and Amo breeds cockerel of Gombe State, Nigeria

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Abstract

The experiment was carried out to compare the semen characteristics of indigenous and Amo strains of cockerel at poultry unit of teaching and research farm of Federal University of Kashere, Gombe State, Nigeria. Semen samples were collected from nine indigenous and nine Amo breeds of cockerel at three days interval for two weeks using abdominal massage technique. Semen samples were examined macroscopically for semen colour, pH and ejaculation volume. Then, microscopic observation was carried for sperm concentration, mass motility, progressive motility, live and dead sperms percentage, normal and abnormal sperm, all for semen characteristics. The results showed a significant difference ($P \leq 0.05$) between mass motility, progressive motility, sperm concentration and head defects of 4.85 ± 0.27 to 4.37 ± 0.19, 95.13 ± 0.43 to 81.63 ± 1.15%, 4.93 ± 1.84 to 3.40 ± 1.07×10^{9}/ml and 2.96 ± 0.17 to 3.44 ± 0.12% for indigenous and Amo breeds of cockerel, respectively. There were no significant differences observed as semen colour, ejaculate volume, semen pH, live / dead normal sperm neck (mid-piece), tail defects and sperm total abnormalities were found to be 2.85 ± 0.07 to 2.00 ± 0.090.21 ± 0.17 to 0.20 ± 0.02 /ml, 88.85 ± 0.58 to 72.70 ± 0.54% /ml, 11.14 ± 0.58 to 27.29 ± 0.54%, 81.00 ± 0.78 to 66.22 ± 0.61%,9.03 ± 0.42 to 13.96 ± 0.47%, 9.70 ± to 13.00 ± 0.30 and 21.70 ± 0.59 to 30.40 ± 0.53% for the indigenous and Amo breed groups of cockerel, respectively. It was concluded that semen quality characteristics could be differed between genetically improved (Amo strain) and indigenous breed of cockerels.

Keywords: Cockerels, semen, sperm, indigenous, Amo breed

INTRODUCTION

Poultry production can potentially be increased with the help of assisted reproductive technologies (ART's) such as artificial insemination (AI), since it allows optimum use of genetically superior cockerels with high reproductive performance. The increased application of AI techniques in the poultry industry emphasizes the need for the distribution of good quality sperm (Dumpala *et al*., 2006). Therefore, proper semen processing, storage and evaluation are generally important for successful application of AI techniques. The importance of semen evaluation in poultry breeding is for selecting breeding males and for routinely monitoring their reproductive performance (Cheng *et al*., 2002). The fertilizing ability of semen can be accessed by its motility, live or dead sperm and morphological evaluation (Alkan *et al*., 2002). Semen collection is the first critical stage of AI and successful collection results in high quality of semen obtained, with the maximum number of sperms is being collected per ejaculation (Tijjani, *et al*., 2014).

This emphasizes that semen collection cannot be performed by anyone and proper procedures have to be followed in order to achieve maximum acceptable quality semen. These procedures include proper handling of the cockerel as well as the semen, as improper handling may lead to lower quality of semen and subsequently poor conception rates (Hafez & Hafez, 2000). The semen collector can also improve the semen quality by ensuring non-contamination with faces, urine, and/or blood during collection at ejaculation (Alkan *et al*., 2002). It is important to know the proportion of abnormal spermatozoa in semen sample in order to determine the semen characteristics for optimum fertility (Alkan *et al*., 2002). Therefore, the aim of this study was to assess the semen characteristics of indigenous and Amo breeds of cockerel in Gombe, Nigerian, by characterizing their semen quantity and quality. Results obtained from this study would assist in the development of the application of AI for indigenous and Amo breeds of chicken.

EXPERIMENTAL

Materials

The study was conducted at Poultry Unit of Teaching and Research Farm, Federal University of Kashere, Gombe State, Nigeria. Kashere is located in Akko Local Government Area, Gombe State, Nigeria. It is located at an elevation of 431 meters above sea level and its population is 77,015 according to the 2006 Population Census. Its coordinates are 9°46'0" N and 10°57'0" E or 9.76667 and 10.95 (in decimal degrees). The annual rainfall of Kashere ranges between 800mm-900mm per annum and is characterized by distinct dry (October - May) and rainy (June - September) seasons. The annual temperature ranges from 30-32^{0} C, and it experiences a relative humidity of 17-90% Gombe state archive, (2013).

Experimental birds and management

Nine Amo breeds of cockerel were purchased from a reputable farm from Sharada Phase 1, Kano Municipal, Kano State and nine Nigerian Indigenous cockerels were purchased from Kumo, Gombe State. The cockerels were managed intensively. The birds were fed on grower mash and water *ad libitum*.

Semen collection

The cockerels were rested for a period of two weeks, which served as an adaptation period in order to make the cocks to be familiar with the semen collector and to improve the effectiveness of collection; likewise they were trained to respond to the abdominal massage technique prior to the onset of semen collection. Single ejaculate of semen was collected from each two strains of cockerels three times within ten days between 10am and 11am by an abdominal massage method described by Burrows & Quinn (1937). The technique involved was restraining and gently stroking the back of the male from behind the wings toward the tail with firm rapid strokes. The male was responded with tumescence (erection) of the phallus at time the handler would gently squeeze the cloaca by expressing semen through external papillae of the ductus deferens and collecting the semen into a container.

After the collection, the semen was macroscopically evaluated for colour, volume and pH value. Then, it was microscopically evaluated for sperm concentration, sperm morphology, semen mass motility sperm individual progressive motility and spermatozoa defects (of head, neck and tail).

Semen volume, colour and pH

The ejaculate volume from each two strains of cockerel 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) (Peters *et al*., 2008). Semen pH was measured using pH test strip.

Semen mass motily

To evaluate mass activity, a drop of undiluted semen from each two strains of cockerel was placed on a microscope slide and covered with a glass cover slip to spread the semen in order to obtain a uniform thickness and to prevent 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 and covered with glass cover slip. 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 of each strains of cockerel was measured using haemocytometer with the direct cell count method. Haemocytometer is a specially designed slide that contained two counting chambers and two dilution pipettes. The counting chambers are 0.1mm in depth and have a ruled area on the bottom of the chambers that is 1.0mm^2 of width. The square is sub-divided into 25 smaller squares. In this study, 10 µl of semen was 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 then placed on the microscope at 400x magnification. The sperm's head that fell within the sub-divided smaller squares at the four edges and centre of the haemocytometer was counted and the average per cockerel was considered and based on the judgment of the individuals in 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, 5 g of nigrosin and 3% of sodium citrate in 100 mL of distilled water (Lake *et al*., 1978), which was later pre-warmed to body temperature for about 30 minutes and filtered before usage. Briefly, 10µl of fresh semen was mixed with 20µl of Ringer's solution. 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 then used to swipe quickly and formed a thin layer and then air dried. The sperm was examined at 1000x magnification under light microscope.

Sperm morphology

The slides of live and dead stains were used to check abnormalities (defects) of the sperm in terms of 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 200 sperms were examined for each sample under microscope at 1000× magnification.

Statistical analysis

The data regarding the percentage of sperm progressive motility, live/dead sperm, morphological normal intact sperm, total abnormal head, mid-piece/neck, tail and overall abnormalities were subjected to statistical program (SPSS, Version 16.0) using student t-test to find the differences in both the quality and quantity of the above semen parameters.

RESULTS AND DISCUSSION

Microscopic evaluation

The mean values and standard errors of the ejaculate colour, pH, volume, mass motility, sperm concentration, progressive motility, proportion of live and dead sperm and proportion of sperm with normal morphology were shown in Table 1. There were significantly higher ($P \leq 0.05$) values in the percentage of sperm concentration, mass motility and sperm progressive motility in indigenous cockerel compared to Amo cockerel. However, no significant differences were observed in semen colour, semen volume, live sperm, dead sperm and morphological normal sperm between the groups of cockerels.

The percentage of morphologically abnormal sperm head, neck and tail, and proportion of total abnormalities in the findings indicated that there were no significant differences between the groups of cockerels in morphologically sperm abnormality.

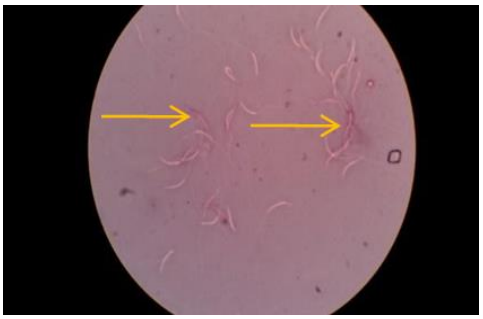

Fig. 1 Photomicrography of cockerel sperm (1000xmagnification), pink color (stained with eosin: showed by yellow arrows) regarded as dead and without any color (no penetration of eosin stain) regarded as live.

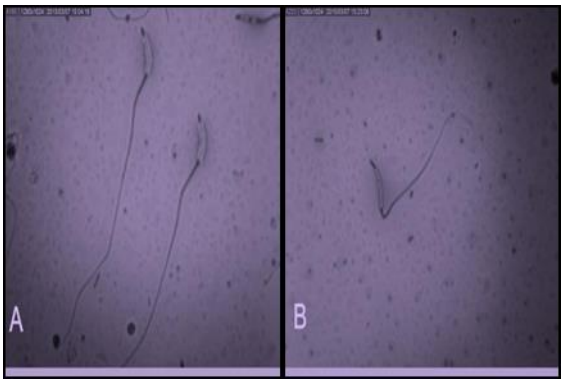

Fig. 2 Photomicrography of normal and abnormal morphologies of cock spermatozoa (1000× magnification). (A) normal; (B) simple bent at the neck region.

Table 1 (Mean±SE) semen characteristics of Nigerian indigenous and Amo cockerels.

Semen parameter	Indigenous cockerels	Amo cockerels
Macroscopic parameters		
Semen colour (scale 1-4)	2.85 ± 0.07	2.00 ± 0.9
Semen pH	7.00 ± 0.0	7.00 ± 0.0
Semen volume (ml^{-1})	0.21 ± 0.17	0.20 ± 0.2
Microscopic parameters		
Sperm mass motility (score 0-9)	4.85 ± 0.27*	4.37 ± 0.19
Sperm concentration (10^9 ml^{-1})	4.93 ± 1.84*	3.40 ± 1.07
Progressive motility (%)	95.13 ± 0.43*	81.63 ± 1.15
Live sperm (%)	88.85 ± 0.58	72.70 ± 0.4
Dead sperm (%)	11.14 ± 0.58	27.29 ± 0.4
Normal sperm	81.00 ± 0.78	66.22 ± 0.1
Morphological defects		
Head defects (%)	2.96 ± 0.17*	3.44 ± 0.12
Neck defects (%)	9.03 ± 0.42	13.96 ± 0.47
Tail defects (%)	9.70 ± 0.30	13.00 ± 0.30
Total defects (%)	21.70 ± 0.59	30.40 ± 0.53

*Indicates a significant difference at ($p \geq 0.05$)

Macroscopic evaluation

Evaluations on semen quality characteristics and assessment prior to artificial insemination were important for the AI techniques in the poultry industry (Donoghue *et al*., 2000). Poultry breeders must ensure the highest quality of collected semen for successful AI (Alkan *et al.,* 2002). Fresh semen assessment was also important to determine males of different fertilizing abilities for animal selection (Wishart, 2009).

Semen colour was commonly used to evaluate the quality of semen and was varied from a dense opaque suspension to a watery fluid with a relative high density (Peters *et al*., 2008). In the present study, no significant difference was obtained in the semen colour. On the other hand, the mean value was consistent to that of ayam kampong (Tijjani *et al.*, 2014a) and in rhode Island Redcockerels (Churchil *et al.,* 2014), but higher than brown leghorn cockerel (Hrn *et al*., 2013). The colour of semen was generally an indication of the ejaculate density where milky was usually contained highest sperm concentration while declining sperm number was indicated to creamy, slightly creamy and watery fluids, respectively (Tijjani *et al.,* 2014a).

Both the strains showed a constant semen pH and were consistent to the result reported on indigenous cockerel semen (Hafiz & Hafiz, 2000). The results were similar to the result obtained from Peters et al., (2008) which revealed a pH range of 7.01 ± 0.01 to 7.04 ± 0.02 for Nigerian indigenous and exotic strains of cockerel semen characteristics. Semen pH was essentially used to regulate the osmolarity in the semen fluids thereby enhancing fertilizing ability of the sperm.

The mean values obtained for semen volume in the present study were consistent as reported by Gordon (2005) and similar to that of white leghorn cockerel as reported by Ajayi *et al*., (2011). The overall reported average ejaculate volume of cockerels for different poultry breeds was also consistent with present work (Hafiz & Hafiz, 2000). These variations might be as a result of the differences in cockerels' body weight. Previous study shown that the cockerels with a higher body weight could produce greater ejaculate volume but with a lower sperm concentration (Adeyemo *et al*., 2007). However, low in semen volume with high sperm concentration was an indication of the superior genetic tendencies of indigenous cockerels in reproductive ability and higher fertility.

Microscopic evaluation

Semen mass motility assessment was an indicative of the viability of sperm and the quality of the semen sample. In the present study, the observed mean values were significantly different ($p \leq 0.05$) between the two groups. The result was in agreement with the previous study that revealed the average mass motility was recorded on white leghorn cocks (Hafiz & Hafiz, 2000). The difference obtained in this study might be due to the frequency of collection and difference in body weight.

Sperm concentration was significantly higher in indigenous cockerel ($p \leq 0.05$) compared to Amo breed of cockerel which was similar to the findings by (Ajayi *et al*., 2011). This significant difference in the sperm concentration of the ejaculates might be attributed as a result of breed differences, frequency of semen collection and fertilizing capacity of individual's cockerels. However, high sperm concentration was not a good indicator for fertility if a large number of those sperm was found to be either dead or immobile, thus it was unable to either reach or penetrate the egg yolk (Tijjani *et al.,*2014a).

Individual progressive motility was the most often used criterion for semen evaluation, both before and after preservation. In the present study, the values obtained for individual sperm progressive motility were within the acceptable range for AI (Hafiz & Hafiz, 2000; Ezekwe & Machebe, 2004). However, high value obtained on indigenous cockerel semen might be as a result of breed differences as reported that local cocks could provide in high motility percentage compared to exotic breed (Tijjani *et al.,* 2014b).

In the present study, no significant difference was observed in live sperm percentage between the groups of cockerel. However, the proportion of live spermatozoa in the present study was in contrast to the previous finding on white leghorn as reported by (Lukaszewicz *et al*., 2008b). Moreover, lower proportion of live spermatozoa (72 to 82%) in cock semen has been reported by Siudzinska and Lukaszewicz, 2008. The low number of dead sperms recorded in this study might be attributed to season (rainy) as the study was done during rainy season and has been shown to favour the rate of spermatogenesis. The percentage dead sperm recorded in the present study was high and more consistent with the 13 to 30% in previous study (Suidzinska and Lukazsewicz, 2008a) and 30% as revealed by Ameen *et al.* (2014). The higher number of dead sperms recorded in this study could be attributed to season (cold weather and high relative humidity) light intensity and temperature changes.

Sperm morphology evaluation was essential qualitative parameters of ejaculates and could provide the clinical information about the potential fertility of semen sample. It could be used as an

important qualitative parameter for predicting the fertilizing ability of sperm (Tijjani *et al*., 2014a). In the present study, morphologically normal spermatozoa did not differ significantly between the two breeds of cocks. However, the proportion of morphologically normal was consistent to the result reported by Tabatabaei *et al*. (2009) but in contrast to the findings by (Abu *et al*., 2013). This difference might be attributed to the breed of chicken used, live-weight, age, season, environmental temperature and humidity, and semen collection techniques.

Percentage head defects obtained in the present study were significantly higher in indigenous breed of cocks at ($p \leq 0.05$). The result was in agreement with the work by Tabatabaei (2009) which revealed a higher percentage of head defects on Indigenous cockerel sperm compared to Ross broiler breeders and was also consistent to the finding by Ajayi *et al*. (2011) on normal, naked neck and frizzled feather semen quality characteristics. However, sperm defects could indicate disturbances of spermatogenesis and this could be attributed to age, nutrition and pollution (Bah *et al*., 2001).

In the present study, no significant differences were recorded between the two strains of cockerel. However, the outcome was higher compared to the result obtained by (Ameen *et al*., 2014) and (Ajayi *et al*., 2011) but the result was consistent as reported by (Tabatabaei *et al*., 2009) in Indigenous cockerel semen.

The outcomes revealed no significant difference in sperm tail abnormality between the breeds at ($p \leq 0.05$). However, the result in the present work was in agreement with the report on local strains of cockerel (Ajayi *et al*., 2011) but lower to the recent finding according to Ameen *et al*., 2014.

The most frequent sperm abnormality recorded was in the sperm neck (mid-piece), followed by the sperm tail, and neck defects in the indigenous and exotic breeds of cockerel. Blesbois (2007) reported the number of live sperms without any defects in cockerel semen was varied from 91 to 94%, which was contrary to the results of this study, but lower compared to the result revealed by (Tabatabaei *et al*., 2009) on Ross and Indigenous cockerel's semen quality. In generally, variation in sperm morphologically defects might be attributed as a result of inadequate ejaculate handling and processing during microscopic examination which could be described as factors that were responsible to cause sperm abnormalities in poultry (Tijjani *et al.*, 2014b).

CONCLUSION

Semen characterization prior to AI is an important initial starting point in poultry breeding program, because it is essential to select cockerels with acceptable semen quality for successful AI. However, semen parameters of both the Indigenous and Amo cockerels recorded were comparable with those reported in previous studies. Moreover, Indigenous cockerel's seminal viability characteristics were higher compared to Amo cockerels. Therefore, indigenous cockerels should be selected for AI in order to obtain higher fertility and hatchability of eggs. The percentage of hatchability in both the strains of cockerel should be examined for future study.

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