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Semen characteristics and fertility response of crosses between white and black plumage indigenous turkeys under artificial insemination

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ABSTRACT

Semen quality and fertility in crossbreeding between white plumage and black plumage indigenous turkey hens using artificial insemination were investigated. Eighty-two healthy indigenous turkeys, comprising 72 hens (Thirty-two black and forty white) and 10 toms (five black and five white) were used. The turkeys were grouped into four treatments based on the breeding plans: T1 (White toms X White hens), T2 (Black toms X Black hens), T3 (White toms X black hens) and T4 (Black toms X White hens). Semen was harvested from white toms, pooled, and inseminated at 0.02ml into hens in T1 and T3. Semen harvested from black toms were also pooled and inseminated at same dose into hens in T2 and T4. White tom semen quality parameters were 97%, 91%, 96%, and $532x10^6$ cells/mL for mass activity, motility, livability, and sperm concentration, respectively. Black tom semen quality parameters were 91%, 93%, 95%, and $293x10^6$ cells/mL for mass activity, motility, livability, and sperm concentration, respectively. Egg fertility were 99.63% in T1, 99.81% (T2), 99.84%(T3), and 99.27% (T4). Hatchability in T1, T2, T3, and T4 were 57.67%, 72.54%, 67.65%, and 64.82%, respectively. Besides sperm concentration, semen quality parameters and fertility in indigenous turkey were not affected by plumage colour

Keywords: Semen quality, egg fertility, plumage colour, artificial insemination

INTRODUCTION

Semen quality is an essential component of male reproduction. Evaluation of semen quality characteristics of domesticated birds provides an excellent

indicator of their reproductive potential and has been reported to be a critical determinant of fertility and subsequently hatchability of eggs (Peters et al., 2008). Some semen quality parameters are characterized in terms of volume, colour, concentration, motility, mass activity, viability, and morphology. The parameters vary with the age of males in all poultry species leading to a reduction in fertility as the birds grows older (Kotlowska et al., 2005), but evaluation of semen quality parameters based on plumage colour has not been properly documented. Fertility is a complex interaction of traits involving two individuals (male and female) of different genetic composition and their ability to mate and produce viable offspring (Foote, 2010). Fertility is a crucial factor in the success of any breeding programme. Success of any breeding programme commences with a male animal that is free of disease, healthy, and that produces enough high-quality semen. However, equally important is the fertility potential of the female animal as well as the environmental influences. Each of these components must be maintained at a high standard to achieve maximum reproductive efficiency. Low fertility and hatchability have been reported to be major problems for Turkey breeding enterprises (Ozcelik et al., 2009). There is a paucity of information on whether fertility and hatchability are affected by turkey plumage colour.

Therefore, the objectives of this study were to evaluate the semen quality parameters of white and black indigenous turkey toms as well as assess the fertility potential of crossbreeding between white and black plumage indigenous turkey hens using artificial insemination.

MATERIALS AND METHODS

The experiment was carried out at the poultry unit of the Teaching and Research Farm and the Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Nigeria. A total of 82 white and black indigenous turkeys (*Meleagris gallopavo*) aged approximately 8 months old comprising 10 toms (5-black toms and 5-white toms) and 72 turkey hens (40-white hens and 32-black hens) were sourced locally from a turkey breeder in Ibadan, Oyo State, Nigeria. The birds were randomly allotted to treatment based on crosses between white and black indigenous turkeys. The feeding and management of the birds were the same.

The turkeys of different colours and sexes were randomly selected and grouped into 2 by plumage and sex. Individuals in each group were tagged for proper identification. The first group contained 5-white and 5-black indigenous turkey toms. The second group comprised 40-white and 32-black indigenous turkey hens. The birds were randomly placed into 4 treatments using plumage colour as an identification factor. Treatment 1 (T1 - cross between white toms and white hens), T2 (cross between black toms and black

hens), T3 (cross between white toms and black hens), and T4 (cross between black toms and white hens).

Semen harvested from the 5 white toms were pooled and inseminated into 20 white hens and 16 black hens. In the same manner, semen harvested from the 5 black toms were pooled and inseminated into 16 black hens and 20 white hens. Before the commencement of the study, each tom was trained for semen collection as described by Adebisi and Ewuola (2019).

Semen samples were collected from each tom twice a week in week one and once weekly subsequently by the Dorso-lumber-abdominal massage method as described by Burrows and Quinn (1937) and modified by Adebisi and Ewuola (2019). The semen was pooled to eliminate the effect of individual variability of gamete donors. Care was taken to avoid contamination of semen with cloaca fluids. Collection of semen was done by stimulating the copulatory organ to protrude by massaging the abdomen and back over the testes. This was followed by pushing the tail forward and upward with one hand and at the same time using the thumb and forefingers of the same hand to milk semen from the duct of this organ. The semen was collected in a collection tube. The pooled semen was gently stirred for uniformity. The collection tube was wrapped with dry cotton wool to minimise the influence of external temperature on the semen. A portion of the pooled semen was evaluated before insemination for progressive sperm motility (subjective scoring method), sperm concentration (Neubauer haemacytometer method), and sperm livability (Eosin–nigrosin staining method), as described by Ewuola and Egbunike (2010).

All hens were inseminated weekly according to the method described by Adebisi and Ewuola (2019). The hen chest was placed on a bench and little pressure was applied to the right side of the abdomen until the vent everted. An insulin syringe loaded with semen was coupled to an insemination tubular glass rod which was inserted to a depth of about 1.5-2.5 cm into the genital tract through the cloaca, and the known volume of semen was deposited in the vagina on a treatment basis.

All inseminations were carried out on farm immediately after collection and each hen received a dose of 0.02ml as recommended by Adebisi and Ewuola (2019) and the process of insemination was also done as described by Adebisi and Ewuola (2019). The oviduct of each hen was everted, and semen was deposited into it at a depth of about 2.5 cm using a graduated tuberculin syringe with a glass rod attached to it. As soon as the required dose of semen was dispensed, the pressure on the abdomen was released to suck in the semen and return the oviduct everted to its position. The process of semen collection and insemination of all hens did not exceed 60 minutes. The insemination was done for two successive days only in the first week of the experimental trial and once weekly subsequently. Insemination was done after 5-pm to minimise the presence of an egg in the oviduct. The oviductal

sperm age was measured as the period the sperm cells had stayed in the oviduct post insemination according to Adebisi and Ewuola (2019).

The colour and consistency of the raw semen were determined as described by (Bearden, 2004). Only slightly watery milky samples were analysed while soiled and coloured or contaminated semen were discarded. The volume of ejaculate of each tom was determined from the calibrated glass tubes immediately after collection and was recorded in ml. Microscopic examination for wave pattern (gross sperm motility) was determined by placing a drop of raw undiluted semen on a prewarmed slide and covered with a coverslip; it was viewed under a microscope using x10 objective lens and motility was estimated by subjectively by scoring on a scale of 0-100. The values were recorded in percentage (Bearden, 2004; Ewuola and Egbunike, 2010).

A raw semen sample was pipetted with a rubber pipette and a drop was added to one drop of sodium citrate on a clean prewarmed microscope glass slide; it was clipped under a light microscope and viewed at x400 magnification. The percentage of live and dead spermatozoa were determined by differential staining technique as described by Bearden et al. (2004). A drop of diluted semen sample was placed on a clean dry slide with a stirring rod and two drops of Eosin-negrosin were added to it with a dropper. A smear was made from the mixture of the two on a glass slide and clipped to a light microscope, viewed at x 400 magnification for the number of live and dead sperm cells. Spermatozoa, which picked up the stain were considered dead while those that exuded the stain were considered alive. 100 sperm cells were counted in each slide and classified as alive or dead at the time of staining. Live and dead sperm were reported in per cent. The temperature was measured using a digital thermometer. The thermometer was gently inserted into a collection tube containing raw semen immediately after collection. The values were recorded in degree centigrade (°C).

Sperm concentration was determined with a haemocytometer as described by Bearden et al. (2004). The haemocytometer was wiped dry with tissue paper before use. 1200µL of 0.9% physiological saline was measured into a clean test tube with a 10ml pipette. 4µL of raw semen was aspirated into a tuberculin syringe and dropped into the test tube containing 1200µL formal saline (1%) to dilute for ease of enumeration and to immobilize the sperm cells. Using a capillary pipette, both chambers of the haemocytometer were charged with sample of diluted semen and and allowed to settle for two minutes. Using a light microscope, the number of spermatozoa in the five diagonal large squares of the haemocytometer was determined at x400 magnification. Three counts were taken for each sample and the average was recorded. The number of spermatozoa per sample of semen was calculated with the formula:

Concentration (sperm cells/ml) = Number of sperm cells counted in the haemocytometer chamber x dilution factor x 50 000

The day after the second insemination marked the day of the first egg collection. Eggs were collected daily from each treatment group, marked, and stored in egg crates at a temperature of 24°C to 26°C and a relative humidity of 70% to 85%. Setting of eggs into incubating machine was done weekly for 10 weeks following hatchery protocols. Egg candling was done on day 25 of incubation for each batch and all candling clears were removed. Eggs with evidence of developing embryos were transferred into the hatching unit. Hatching occured on day 28 and all unhatched eggs and candling clears were broken out, classified, and recorded according to Adebisi and Ewuola (2019a) as follows:

I. Fertile eggs (eggs that had a form of embryonic mass upon break-out were categorized as fertile eggs with embryo mortality.)

II. Infertile eggs (egg-containing milky white albumen, no embryo, or

brownish albumen/ infertile eggs were those eggs which upon break-out, were devoid of any form of embryonic mass.)

III. Early dead embryos (embryos without visible formation of eyes/ occurring at the 1st week of incubation)

IV. Mid embryonic mortality at the 2nd and 3rd week of incubation

V. Late dead embryos (embryos with large black eyes, but lacking

feather formation/ late embryonic mortality was at the 4th and last week of incubation (Fairchild et al., 2002). Also, Eggs with embryo mortality at piping were classified as late embryo death.

VI. Hatched eggs

All dead embryos were considered fertile. The fertility levels of each treatment flock were calculated as outlined by (Sotirov et al., 2002) and Adebisi and Ewuola (2019) as follows:

$$\text{Fertility (\%)} = \frac{\text{Number of fertile eggs}}{\text{Number of set eggs}} \times 100$$

The hatchability levels per treatment were calculated as outlined by Adebisi and Ewuola (2019) and recorded in percentage.

$$\text{Hatchability (\%)} = \frac{\text{Number of poults hatched}}{\text{Number of fertile eggs at Candling}} \times 100$$

Data were collected were weekly egg fertility, embryo mortality, poult plumage classification. The data were subjected to descriptive statistics and analysed for significance at $P<0.05$ using the General Linear Model of SAS (Statistical Analytical System, 2003) and means were separated using Duncan's multiple range test.

RESULTS

The means spermatozoa characteristics of pooled semen inseminated are presented in Table 1. For white tom, semen parameters like semen volume, mass activity, spermatozoa motility, livability, semen temperature, colour, and sperm concentration were 0.42±<0.001ml, 80%, 91.50±9.19%, 96.50±2.12%, 33.50±2.12°C, creamy, and 532.50±371.12x10^6 cells/mL, respectively. On the other hand, the black tom semen had 0.53±0.01ml, 80%, 93.40±7.60%, 95.77±1.37%, 33.07±0.12°C, creamy and 293.20±93.78 cells/mL for volume, mass activities, motility, livability, temperature, colour and concentration, respectively. Plumage colour had no significant (P>0.05) effect on the macroscopic and microscopic semen parameters of both white and black indigenous toms except spermatozoa concentration. Compared with the semen of black tom, spermatozoa concentration of semen from white toms were significantly higher than the black toms. The temperature of semen of white tom was similar to the black tom. The spermatozoa livability and sperm motility in the black tom was similar to that of the white tom.

The percentage livability for white turkey tom semen was higher than that of black turkey tom semen. The mass activity and motility of white turkey tom semen were higher than the black turkey tom semen.

Table 1. Characteristics of pooled indigenous turkey semen

Parameters	**White tom Mean values (±Standard Deviation)**	**Black tom Mean values (±Standard Deviation)**	**P Value**
Semen Volume(mL)	0.42±<0.001	0.53±0.17	0.50
Mass Activity (%)	80.00	80.00	0.61
Spermatozoa Motility (%)	91.50±9.19	93.40±7.60	0.82
Sperm cell Livability (%)	96.50±2.12	95.77±1.37	0.66
Semen Temperature (°C)	33.50±2.12	33.07±0.12	0.34
Semen Colour	Creamy	Creamy	
Spermatozoa Concentration (x10^6 cells/mL)	532.50±371.12^a	293.20±93.78^b	0.03

The effects of crosses between white and black indigenous turkeys on embryonic mortality are shown in Table 2. Comparing the four treatments, it was shown that total embryo mortality, late embryo mortality, Mid embryo mortality and Early embryo mortality in treatments 1, 2, 3 and 4 were similar among the treatments.

Table 2. Embryo mortality (%) from crosses of turkey with different plumage colour (Mean±SD)

Treatments	Number of Set Eggs (n)	EEM	MEM	LEM	TEM
T1	286	2.18±2.77	1.40±1.81	0.46±0.78	4.49±5.06
T2	331	1.17±1.56	0.79±0.96	0.60±0.95	2.55±3.13
T3	269	2.13±3.38	1.09±1.48	0.12±0.39	3.35±4.86
T4	295	1.93±3.10	1.01±1.68	0.65±1.43	3.59±5.22
P value		0.56	0.84	0.46	0.72

T1: White Toms versus White Hens, T2: Black Toms versus Black Hens, T3: White Toms versus Black Hens. T4: Black Toms versus White Hens, SD: Standard Deviation; EE: Early Embryo Mortality MEM: Mid Embryo Mortality, LEM: Late Embryo Mortality; TEM: Total Embryo Mortality.

Egg fertility and hatchability of crosses between white and black indigenous turkeys are presented in Table 3.

Table 3. Fertility and hatchability responses of white and black indigenous turkeys

Treatment	Number of Set Eggs (n)	Fertility (%) (Mean±SD)	Hatchability (%) (Mean±SD)
T1	286	99.63±1.17	57.67±16.41
T2	331	99.81±0.61	72.54±13.37
T3	269	99.84±0.51	67.65±12.52
T4	295	99.27±1.53	64.82±17.15
P value		0.61	0.18

T1: White Toms versus White Hens, T2: Black Toms versus Black Hens, T3: White Toms versus Black Hens, T4: Black Toms versus White Hens, SD: Standard Deviation;

Egg fertility was similar among the treatments, while percentage hatchability ranged from 57.67% (T1) to 72.54% (T3) treatment 4 had the least fertility. Treatment 2 had the highest percentage of hatchability among the four treatments. Treatment 1 had the least percentage hatchability when compared with the other three treatments.

DISCUSSION

Semen characteristics are to ascertain the true semen quality of each tom before using them for insemination trials so that unproductive males would not be used. Semen colour, volume, and temperature were macroscopic parameters while mass activity, progressive sperm motility, livability, and sperm concentration were microscopic parameters. The average semen volume obtained from the black toms was similar to the one obtained for the white tom. The semen volume obtained in this study was found to be in the range of 0.42ml (white) - 0.7ml (black). The range was lower than the range of

semen reported for indigenous chickens (Bilcik et al., 2005; Peters et al., 2008). The volume of semen obtained were higher than those reported for exotic breeds of Turkeys with a range of 0.35-0.40ml (Yahaya et al., 2013). However, Ezike (2016) reported an average volume of 0.17±0.02ml for the local breeds of Turkey, which was lower than the observed result in the present study. The apparent reasons could be due to differences in the genetic background of the turkeys used and the age of the toms. The authors may have used aged or old toms, while in this study young toms exhibiting tremendous vitality were used, which was reflected in the quality of the ejaculates they produced during the study. The result of this study suggests that more breeding units can be prepared with the large semen volumes obtained from the toms under an intensive system of management for inseminating more females in an artificial insemination programme.

Progressive sperm motility was similar between black toms and white toms. None variation in sperm motility may be attributed to the fact that the indigenous breeds used were probably genetically related even though there are of different plumage colours, since they were raised on same diet and environment. This suggests that plumage colour do not affects the production traits in black and white indigenous turkey toms. However, the sperm motility values recorded in this study were higher than 84.25±2.23% and 83.47±2.36%, which were the highest values recorded by Ezike (2016) in local and exotic turkeys, respectively. (Holsberger et al., 2002) reported a mean motility value of 90.8±1.3% for high mobility phenotype exotic turkeys. In another study, a mean sperm cell motility value of 89.69±0.33% for British United Turkeys was reported (Noirault and Brillard, 1999). The values obtained in this study were slightly higher than those reported by these authors. This may be due more to the age of the toms used than to genetic differences. The toms used in the present study were pubertal toms exhibiting considerable vigour and vitality. They were apparently younger than toms used by Ezike (2016) and Noirault and Brillard (1999). Bearden (2004) reported that the age of the toms may affect sperm motility of ejaculates in turkeys.

Mass motility assessment of the semen is indicative of the viability of sperm cells and the quality of the semen sample (Ewuola and Egbunike, 2010). The non-difference obtained in the mass activity of semen from white and black plumage turkeys in this study indicates that they are comparably similar without influence due to the difference in plumage. 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 relatively 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 did not agree with that of Ayam Kampong (Tijjani et al., 2014), Rhode Island Redcockerels (Churchil et al., 2014) and Brown Leghorn cockerel (Hrnčár et al., 2013) reported an

approximate mean value of 2-3ml. Tijjani et al. (2014) reported that the colour of semen was generally an indication of the ejaculate density where milky was usually contained the highest sperm concentration while declining sperm number was indicated by creamy, slightly creamy, and watery fluids, respectively. Variation in semen colour among these breeds can be attributed to different genetic lines, possibly the feeds and expertise of semen collectors.

Sperm concentration was significantly higher in white tom compared to black tom. The significant difference in the sperm concentration of the ejaculates might be attributed to plumage differences and fertilizing capacity of individual toms since low sperm count is associated with infertility or reduced fertilizing potential. Possibly black plumage encouraged heat load and induce heat stress which may have depressed spermatogenesis in the black toms as against the reflective ability of white feather to heat load or radiant energy. In this study, the high sperm concentration recorded in the toms appears to suggest that high fertility could be achieved with toms when used in artificial insemination programmes. This is because ejaculates with low sperm concentration have been associated with low fertility (Bearden, 2004). However, high sperm concentration was not a good indicator for fertility if a large number of sperm cells were found to be either dead or immobile, thus it would be unable to either reach or penetrate the egg yolk (Tijjani et al., 2014).

In the present study, no significant difference was observed in the percentage of live sperm cells and semen temperature between the white and black toms. This result, however, appears to show that higher fertility could be achieved with active use of local toms in artificial insemination breeding programmes. Possibly because Donoghue and Wishart (2000) reported that a high correlation exists between sperm viability and fertility.

There was no significant difference between total embryo mortality of white and black indigenous turkey toms. This suggests that plumage colour does not have a significant effect on the embryos of indigenous turkeys. Although the total embryo mortality in each of the treatments was less than 5% as a result of the breeding method adopted for the study. This further confirms that in poultry species, better fertility can be obtained through AI with good quality semen than natural mating (Mohan et al., 2018).

The fertility and hatchability of crosses between white and black plumage indigenous turkeys obtained were favourably compared with those reported by Kotlowska et al. (2005) who reported an average percentage fertility value of 94.51% for Hybrid Large White Nicholas. The percentage hatchability recorded in this study was not affected by plumage colour. Percentage fertility was higher than percentage hatchability in all treatments. This result agreed with the report of McDaniel et al. (1993) who reported that hatchability can never be better than fertility. It was revealed that fertility and hatchability are directly proportional. This result corroborates with the report

of Cooper (1972) who reported that a decline in the hatchability of fertile eggs is usually accompanied by a decline in fertility. Thus, the result of this study appears to indicate that acceptable fertility and hatchability could be achieved in indigenous turkeys using artificial insemination.

CONCLUSION

Based on the results obtained from this study, semen quality parameters in indigenous turkeys were not influenced by their feather colour except for spermatozoa concentration which was higher in white toms than the black toms which are more susceptible to heat stress that depresses spermatogenesis due to accumulation of heat load by black colour feather as against white feathers. In addition, fertility, embryo mortality and hatchability were not plumage colour dependent.

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