SEMEN CHARACTERISTICS OF PUREBRED NAKED NECK TSWANA AND BLACK AUSTRALORP X NAKED NECK TSWANA CROSSBRED CHICKENS UNDER AN INTENSIVE MANAGEMENT SYSTEM IN BOTSWANA

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ABSTRACT

Evaluation of semen quality is very important before selection of breeding cocks used for artificial insemination. The aim of this study was to characterize the semen parameters of Black Australorp x naked neck Tswana chickens and purebred indigenous naked neck Tswana chickens raised under intensive management system in Botswana. Semen was collected from sixty four (64) purebred naked neck Tswana and sixty four (64) crossbred (Black Australorp X naked neck Tswana) cocks at 20 weeks of age for semen evaluation using the abdominal massage technique. Semen parameters evaluated included ejaculate volume, semen pH, sperm motility, sperm concentration and sperm viability. Crossbred cocks had significantly higher ($p<0.05$) ejaculate volume (0.41 ± 0.005 vs. 0.37 ± 0.005ml), sperm motility (81.79 ± 0.66 vs. 75.02 ± 0.63%) and ejaculate concentration (4.78 ± 0.03 vs. 3.17 ± 0.03 x 10^9 sperms/ml) than their purebred naked neck counterparts. However, the degree of semen pH, semen color and the percentage of live and dead sperms showed no significant breed differences ($P>0.05$). Both purebred naked neck Tswana and Black Australorp x naked neck Tswana crossbred chickens produced semen of acceptable quality. Crossbred cocks however produced better quality semen than their purebred counterparts. Crossbreeding can therefore be used as a strategy to improve semen characteristics of indigenous Tswana chickens under an intensive management system.

Key words: Semen characteristics crossbred Tswana chickens, purebred Tswana chickens, intensive system, Botswana

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INTRODUCTION

The reproductive potential of poultry birds (cocks) is determined to large extent by the quality of the semen they produce (Islam *et al.*, 2002**).** 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). According to Bratte and Ibe (1989), sperm concentration of $50x10^6$ is adequate for good fertility in chickens and turkeys. The genetic effects of breeds, varieties and individuals within breeds on fertility and hatchability have been identified (Islam *et al.*, 2002). Several tests to evaluate semen quality have been described by Umesiobi (2004), but they have rarely been applied in on-farm settings. The industry previously relied on the evaluation of semen using colour and volume parameters which gave estimates of sperm quantity (Okereke *et al*., 2008).

Semen volume and color are also evaluated to determine the teasing of male and presence of any lesion or infection in genital tract (Tarif *et al.*, 2013). The quality of semen may vary with breed and strain, age, body weight of cocks, collection technique and diluents used (Mosenene, 2009). There are reports that breed and seasonal differences may also affect semen production of cocks (Tuncer, 2008).There are very limited studies on evaluation of semen quality of chicken breeds/strains in Botswana. The objective of this study was therefore to evaluate semen parameters of Indigenous naked neck Tswana chicken and that of Black Austaralorp x Naked neck Tswana crossbred chicken raised under intensive management system in Botswana.

MATERIALS AND METHODS

Semen collection

Semen collection from sixty four (64) purebred naked neck Tswana and sixty four (64) crossbred (Black Australorp X naked neck Tswana) sires at 20 weeks of age was accomplished by the abdominal massage technique according to Hafez (1978). The birds were trained for collection of semen for two weeks. Each bird responded to massage by partial aversion of the cloaca, and semen was collected from the ventral lip of the vent in a tube maintained at 38-40ºC.

The collected semen was subjected to microscopic examinations and physical evaluations according to Zemjanis (1970). Observations were made and the variation between breeds with respect to semen characteristic were examined using the following parameters to characterize each cockerel's semen; ejaculate volume, semen quality, semen colour, sperm motility, sperm concentration, sperm viability (% live vs % dead sperms) and semen pH.

Semen volume: The ejaculate volume of the sample was macroscopically evaluated immediately after collection and recorded directly from the semen collection tube. Semen volume from each of the sire strain was measured with the use of a collection tube graduated in ml.

Sperm motility: Sperm mass motility scored on a scale of 0-5, was evaluated subjectively under a light microscope (X 40 magnification) giving a general indication of the type and intensity of sperm movement and the impact of movement on the number and size of the sperm agglutinates (Blesbois *et. al.,* 2008). Motility of semen sample was expressed as the percentage of sperm cells that were motile under their own power.

A drop of semen was placed on a warm microscope slide with the aid of a micropipette and then covered with a glass cover slip to spread the semen in order to have a uniform thickness and to prevent drying. It was then placed under a microscope for examination at x40 magnification.

Semen concentration: The sperm concentration of an ejaculate was determined by using a Neubauer hemocytometer and the sperm count performed as described by Hafez and Hafez (2000). Briefly, a volume of 10µl semen was diluted with 990µl Sabax water in a flask and

stored in a refrigerator before counting to immobilize the sperms. To determine the percentage live sperm, an eosin/nigrosin stain was used for microscopic morphologic observations. A 10µl drop of fresh semen was mixed with 200µl of eosin-nigrosin stain and a smear made from the mixture was placed on a slide and examined under X1000 magnification. Approximately 100 sperms were counted to determine the percentage dead-live sperm (Lukaszewicz *et. al.,* 2008).

Sperm concentration was calculated using the formula: Nunmber of sperms/ml = Number of sperms in $0.1mm^3$ x 10 x dilution rate x 1000 (ml = cubic centimeter or ml) (Bearden *et. al.,* 2004).

Semen pH: The pH of a fresh semen sample of each cockerel was determined with the aid of a digital pH meter**.**

Statistical analysis

Data collected on semen characteristics were analyzed by General Linear Models procedures of Statistical Analysis System (SAS, 2009) version 9.2.1 and the model included the fixed effects of breed (Purebred naked neck Tswana and Crossbred Australorp x naked neck Tswana). The results are presented as least square means ± standard error and means separation were by paired t-test .The differences between means were declared significantly different at $p<0.05$.

RESULTS AND DISCUSSION

The semen volume of crossbred cockerels was significantly higher ($p<0.05$) than that of their indigenous counterparts (Table 1). The reported average ejaculate volume of a cockerel has been estimated at 0.7 ml for different poultry breeds (Tuncer *et al.,* 2008). All the breed/strain ejaculate volumes recorded in the current study were less than this 0.7 ± 0.12 ml and factors which might have contributed to the lower semen volumes may include breed, age, body weight, excessive stimulation, season and environmental factors including management of the cocks. The ejaculate volumes obtained in this study are similar to those obtained by other researchers and are within the acceptable range for poultry artificial insemination (Hafez, 1978). Other researchers obtained mean ejaculate volume of 0.28 ± 0.14 ml which is lower than the results

obtained in the current study (Bah *et al.,* 2001; Galal, 2007; Tuncer *et al.,* 2008). The mean ejaculate volumes obtained in this study were within the range of 0.34 -0.59 ml reported by Bilcik *et al.* (2005) on broiler cocks and 0.40-0.73 ml obtained by Peters *et al.* (2008) on seven different indigenous chickens of Nigeria.

There was no significant ($P > 0.05$) difference in semen pH between the breeds/strains. The semen pH of the two breeds/strains was slightly alkaline and averaged 7.05 ± 0.03 for purebred naked neck Tswana and 7.06 ± 0.03 for crossbred Black Australorp x naked neck Tswana sire strains, respectively. These results are all within the range generally reported for poultry semen (Etches, 1998). The pH of cockerel semen recorded by other researchers was 7.02 ± 0.01, 7.4 ± 0.2 and 7.68 ± 0.01 (Bah et *al.,* 2001; Peters *et al.,* 2008; Tuncer *et al.,* 2008). A factor that could play a role in semen pH is the technique of semen collection and stimulation of the accessory sex glands. The accessory sex gland fluid is generally alkaline (Bah *et al.,* 2001).

The colour of the cockerel ejaculates did not differ significantly between the two sire breeds/strains under investigation and were creamy-white indicating that the massage technique used may be acceptable for cockerel semen collection to obtain good quality semen for artificial insemination. Creamy-white ejaculates found in this study were consistent with Peters *et al.* (2008) who also reported creamy-white ejaculates (1.00 ± 0.03) in Nigerian indigenous naked neck cockerels. Machebe and Ezekwe (2005) revealed that variations in semen colour may arise in part due to the presence of contaminates or as a result of low sperm concentrations.

Sperm motility of crossbred cockerels was significantly higher ($p<0.05$) than that of their purebred indigenous naked neck counterparts. High sperm motility is regarded as a good indicator of high semen quality with acceptable fertilizing ability and good fertility (Malejane *et al.,* 2014). In agreement with the results of this study, Bah *et al.* (2001) reported sperm motility in fresh semen samples of New Hampshire males of 73.9±0.2% and 83.2±0.6% in White leghorn. Peters *et al.* (2008) also reported sperm motility of 82.50±10.00% for improved indigenous crossbred (Alpha) cocks. Contrary to our results, Mosenene (2009) reported lower sperm motility of 59.6 ±14.5%, 61.6 ±14.1%, 58.8 ±12.5% and 63.8 ±13.6% in Rhode Island Red, Potchefstroom Koekoek, New Hampshire and White Leghorn, respectively. The disparities in sperm motility between the two studies could be attributed to the season of semen collection. The current study was carried out in the summer whereas that of Mosenene (2009) was carried

out in winter. Season affects semen production and the rainy season has been shown to favour high rate of spermatogenesis. The rainy season has also been associated with high ejaculate volume, sperm concentration and high fertility in poultry (Machebe and Ezekwe, 2005). According to Obidi *et al.* (2008) cockerels are seasonal breeders and generally produce more semen at the onset of the breeding season (long daylength) and lower volumes towards the end. Light intensity also affects semen characteristics during the warm and cold season, resulting in lower ejaculate volumes relative to the rainy season, which may be attributed to reduced spermatogenesis and a higher sperm mortality rate. High relative humidity also causes a temporary decrease in sperm production and hence lower ejaculate volumes and sperm concentration that could affect sperm motility and fertility (Obidi *et. al.*, 2008).

Table 1: Semen characteristics of purebred naked neck Tswana and Black Australorp x naked neck Tswana crossbred cocks raised under an intensive management system in Botswana

	Breed	
Parameters	F1 Cross	Naked neck Tswana
Ejaculate volume (ml)	$0.41^{a} \pm 0.005$	$0.37^{b} \pm 0.005$
Semen pH	7.05 ± 0.03	7.06 ± 0.03
Semen colour	1.00 ± 0.09	1.0 ± 0.08
Sperm motility (%)	$81.79^{a} \pm 0.66$	$75.02^{b} \pm 0.63$
Concentration ($x10^{9}$ sperm/ ml)	$4.78^{a} \pm 0.03$	$3.17^{b} \pm 0.03$
Live sperm (%)	76.8 ± 29.4	75.2 ± 33.3
Dead sperm (%)	23.2 ± 29.1	24.8 ± 29.5

Means with different superscripts in a row were significantly different from each other ($P<0.05$).

The ejaculate concentration of crossbred Black Australorp x naked neck Tswana cockerels was significantly higher ($p<0.05$) than that of their purebred indigenous naked neck Tswana counterparts. The sperm cell concentration or sperm density is usually an indication of the number of sperm cells per unit volume (ml) of seminal plasma (Malejane *et al.*, 2014). The concentration of spermatozoa in crossbred cocks and indigenous cocks was higher than 1.2 billion/ml semen (Nwagu et al., 1996), 2.0 billion sperms/ml semen (Keskin *et al.*, 1995 and Sarka *et al.*, 1996) but lower than 7.0 billion sperm/ml semen (Hafez, 1978). The differences in sperm concentration can only be attributed to the fact that strains are from different genetic backgrounds. Higher sperm concentration in crossbred cocks than their indigenous counterparts could be attributed to the favourable effect of increased heterozygosity or heterosis effect on fitness (survival and reproduction) traits. This is consistent with Peters *et al*. (2008) who observed strain differences in semen concentration in Nigerian indigenous cocks.

There was no significant difference in sperm viability (dead and live sperm) between the two cock breeds/strains under investigation. The percentage of live sperm recorded in this study was high, averaging 75.2 ± 33.3% and 76.8 ± 29.4% for purebred naked neck Tswana and crossbred cockerels, respectively. Tselutin *et al.* (1999) reported the number of live sperm without any abnormalities in cockerel semen to vary from 91 to 94%, which is higher than the results obtained in this study. However, Siudzinska and Lukaszewicz (2008) recorded 58 to 70% live, morphologically normal sperm and Lukaszewicz *et al*. (2008) reported 70 to 80% live normal sperms, which are consistent with the results obtained in this study. Mosenene *et al*. (2009) also reported the number of live sperm to be 75.9 ± 33.3% and 80.7 ± 27.9% in South African indigenous cockerels. The percentage dead sperm recorded during semen collection in the two sire breeds/strains ranged between 23 and 24%, which is consistent with the 18 to 24% semen mortality recorded by Mosenene *et al.* (2009) and 27% recorded by Siudzinska & Lukaszewicz (2008).

CONCLUSIONS

Both purebred naked neck Tswana and Black Australorp x naked neck Tswana crossbred chickens produced semen of acceptable quality. Crossbred cocks however produced better

quality semen than their purebred counterparts. Crossbreeding can therefore be used as a strategy to improve semen characteristics of indigenous Tswana chickens under an intensive management system.

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