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Full Length Research Paper

Relationship between phenotypic and sperm traits of South African indigenous cockerels

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Abstract

The aim of the present study was to investigate the relationship between body measurements such as body weight, shank, comb and wattle length and semen traits. A total of 33 Indigenous cockerels (Naked Neck, Ovambo and Potchefstroom Koekoek) were used in this experiment. Semen was collected by abdominal massage technique from each individual cockerel. Semen was analysed macroscopically (colour, volume, and pH) and microscopically (sperm concentration and sperm motility) by Computer Aided Sperm Analysis. There were no significant differences on the shank length (SL), comb length (CL) and wattle length (WL) of Naked Neck, Ovambo and Potchefstroom Koekoek cockerels. However, the body weight of Ovambo was significantly ($P < 0.05$) higher compared to the Naked Neck and Potchefstroom Koekoek cockerels. Semen volume of the Naked Neck was significantly higher ($P < 0.05$) compared to Ovambo but similar to Potchefstroom Koekoek cockerels. Shank and wattle length were positively correlated with semen volume, sperm concentration and semen pH but negatively correlated with total motility. Furthermore, comb length was positively correlated with semen volume, sperm concentration, semen pH and total motility. In conclusion, South African indigenous cockerel's body weight and comb length of all three indigenous breeds were positively correlated with total sperm motility, sperm concentration, semen pH and percentage of live normal sperm. However, shank and wattle length were negatively correlated with total sperm motility.

Key words: Ovambo, naked neck, potchefstroom koekoek, phenotypic traits, sperm quality.

INTRODUCTION

Keeping poultry in households of South African villages is common and provides a vital source of protein and income to the poor communities. Moreover, one of the important aims to keep indigenous chicken (*Gallus domesticus*) is to conserve their genetic resources and maintain the genetic variation for future breeding. Indigenous chickens contribute greatly to human supply of eggs and meat in tropical and subtropical areas. It is therefore imperative to understand and improve their reproductive potentials in South Africa, to form a basis for rapid breeding programmes. It is also necessary that the breeding programme should always include the local

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poultry, since they possess some distinctive tolerance to certain local diseases and adaptability to current or future climatic conditions. However, in South African village communities, facilities for evaluating semen microscopically or by Computer Aided Sperm Analysis (CASA) are not available. Therefore, an indirect method (body measurements) of predicting semen traits of cocks is required by poultry keepers (Ifeanyichukwu, 2012).

The Naked Neck is found all around South Africa even in diverse climates (J. Grobbelaar, Tshwane University of Technology, South Africa, M:Tech dissertation). Male reaches sexual maturity at the age of 155 days with an average weight of 1.95 and 1.4 kg for the females at 20 weeks of age. However, the Ovambo is a dark coloured breed and smaller in size, which helps to camouflage and protect it from predators. Male weigh 2.16 kg at 20 weeks and 1.54 kg for females (JJ. Joubert, Agricultural Research Council, South Africa, Unpublished observations). Potchefstroom Koekoek is a South African composite breed. It was bred from crosses between the Black Australorp and the White Leghorn at the South African Potchefstroom Agricultural College. It is characterised by a relatively high egg production, and adaptability for household production (K. Ramsey, Farm Animal Conservation Trust, South Africa, Unpublished observations). It reaches sexual maturity at 130 days, much earlier than the Ovambo and Naked Neck cockerels.

Parker and McDaniel (2002) reported that there is no real breeding soundness evaluation in poultry. Roosters are selected based on physical characteristics that are associated with maturity in cockerels male for example comb, wattle size, colour, body size and shank length. However, it is imperative to evaluate the semen and sperm quality, as it can be used to predict the fertility of an individual male. Cock reproductive performance has a major impact on the reproductive efficiency of poultry operations (Bakst et al., 1994; Adenokun and Sonaiya, 2001). The assessment of semen characteristics of poultry birds may give an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility (Peters et al., 2004). Three sperm characteristics have been reported to be generally used to evaluate the male's fertility that is, sperm concentration, viability, and motility (Bakst and Cecil, 1997). Moreover, in poultry, fertility of cockerels changes with age, leading to a progressive decline in sperm quality (Bakst and Cecil, 1992; Kelso et al., 1996). The objective of the study was to investigate the relationship between phenotypic and sperm traits of the South African indigenous cockerels Naked Neck, Ovambo and Potchefstroom Koekoek.

MATERIALS AND METHODS

Experimental site

The study was conducted in the Republic of South Africa (RSA) at the Agricultural Research Council, Animal Production Institute, Germplasm Conservation and Reproductive Biotechnologies Unit.

Experimental chickens

33 indigenous cockerels Ovambo (n = 11), Naked Neck (n = 11) and Potchefstroom Koekoek (n = 11) were reared under standard industry conditions in floor pens until maturity. When the cocks were 25 weeks of age, they were transferred into individual battery cages where they were housed under light cycle of 16 h daylight and 8 h darkness. The cockerels were fed layer commercial diet and water *ad libitum*. The experimental cockerels were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (APIEC10/04).

Semen collection and analysis

The cockerels were trained for semen collection following 2 weeks of adaptation. Semen was collected 2 times per week by dorso-abdominal massage described by Burrows and Quinn (1937). Individual ejaculates were collected from each cockerel into a 15 ml tube (falcon® 352099, USA). Body temperature of adult cockerels was between 40.6 and 41.7° (Weaver Jr, 2002). During semen collection, the collection tube was maintained at 40°C in a thermo flask to avoid cold shock. After semen collection, the semen was transported to the laboratory within 10 min of collection. Care was taken during semen collection to minimize contamination by faeces or urine. Semen volume was measured using the graduated falcon tube, while semen pH was measured with a calibrated pH meter (H1 931401 microprocessor Hanna). Sperm concentration was measured using the spectrophotometer (Jenway 6310 spectrophotometer, Bibby Scientific, England) and was recorded in billions ($\times 10^9$/ml) according to Makhafola et al. (2012).

Collected semen was also evaluated using the swim-up (10 µl of semen added to 500 µl of swim-up medium) technique to analysed sperm motility rate using Sperm Class Analyser® (SCA, Microptic, Barcelona, Spain). Following sperm swim-up preparation, 5 µl of diluted semen sample were placed on a warm glass slide (~76 × 26 × 1 mm, Germany) and covered with warmed cover slip (22 × 22 mm, Germany) on the microscope-warm stage adjusted to 37°C. The sperm motility and velocity rate were evaluated by computer Aided sperm analysis (CASA) system known as Sperm Class Analyzer® (SCA) v.5.0, Microptic, Barcelona, Spain) at the magnification of 10× (Nikon, China). The kinematic values recorded for each sperm included, overall percentage of motile sperm, the velocity of movement, the width of the sperm head's trajectory and the frequency of the change in direction of the sperm head (Table 1).

Live performance traits

Measurements and observation

The cockerels were weighed weekly for six weeks. Shank, wattle and comb length were measured once a week for six consecutive weeks, starting when cockerels were 36 weeks old. Shank length was determined on live birds by measuring the length of the tibiotarsus (from the top of hock joint to the foot pad). Head appendages (comb and wattle length) were measured using a vernier calliper at a distance between the upper and the lower point of the organ.

Statistical analysis

Data were analysed using the SAS Institute, Inc. (1999), SAS/STAT

Table 1. Sperm Class Analyser® (V.5.0) settings used to analyse the sperm motility and velocity characteristics.

Parameter	Setting
Contrast	169
Brightness	470
Image/second	50
Optic	pH-
Chamber	Cover slide
Scale	10×
Particle area	5<190 µm^2
Slow	<10 µm/s
Medium	<50 µm/s
Rapid	<100 µm/s
Progressivity	>70% of straightness
Circular	<50% of linearity
Connectivity	18
Velocity on the average path line	7 µm/s
Number of images	30

User's Guide, Version 9, 1st printing, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513. A one way analysis of variance was performed. For a one-way, there is only one factor and the factor in our case was bred with 3 levels (NN, OV and PK) with 11 Cocks randomly selected from each breed (Shapiro and Wilk, 1965). A significance level of 0.05% was used ($P < 0.05$).

RESULTS AND DISCUSSION

There were no significant differences on the shank, comb and wattle length of all three breeds as indicated in Table 2. However, the body weight of Ovambo was significantly ($P < 0.05$) higher compared to the Naked Neck and Potchefstroom Koekoek cockerels. The results of macroscopic semen evaluations are set out in Table 3. Semen volume of the Naked Neck was significantly higher ($P < 0.05$) compared to Ovambo but similar to Potchefstroom Koekoek cockerels. The semen pH of Ovambo cockerels was significantly higher ($P < 0.05$) compared to Naked Neck and Potchefstroom Koekoek cockerels. The sperm concentration of the Potchefstroom Koekoek ($8.0 \pm 5.0 \times 10^9$/ml) was significantly higher ($P < 0.05$) compared to Ovambo and Naked Neck cockerels. The total sperm motility and live normal sperm were significantly different within the breed. Data presented in Table 4 shows Pearson Correlation Coefficients of semen characteristics and sperm live performance traits for Indigenous cock breeds. Body weight of all three indigenous breeds was positively correlated with sperm concentration, semen pH, and total sperm motility. Shank and wattle length of all three indigenous breeds were positively correlated with semen volume, sperm concentration, and semen pH but not with total sperm motility. However, comb length was positively correlated with semen volume and pH, sperm concentration, and total motility.

Semen evaluation is an essential aspect in the assessment of the breeding soundness of any male animal. Sperm motility becomes critical at the time of fertilization because it allows or at least facilitates passage of the sperm through the zona pellucida (Debby et al., 2012). Sperm must be able to traverse the vagina to enter the sperm storage tubules (SST) in the hen and ultimately reach the site of fertilization (Bakst et al., 1994). Highly mobile populations of sperm (motility) are most likely to reach and occupy storage sites than slower moving sperms. The present study demonstrated for the first time that South African indigenous cockerel's body weight and comb length of all three indigenous breeds were positively correlated with total sperm motility, sperm concentration, semen pH and percentage of live normal sperm. Shank and wattle length were negatively correlated with total sperm motility but positively correlated with semen volume, sperm concentration, semen pH and percentage of live normal sperm. Phenotypic traits such as comb and wattle length have been reported to be useful to predict the fertility and sperm quality in broiler cockerels (McGary et al., 2003). Similarly, it was reported that comb length in Norfa cocks had positive phenotypic correlations with semen volume, sperm concentration, percentage of live sperm and sperm motility. Whereas, the comb length had negative phenotypic correlations with semen pH however, similar phenotypic correlations were observed between wattle lengths in all semen physical characteristics (Galal, 2007).

Assessing the fraction of a sperm population that is motile is the most widely-used measure of semen quality in male animals. In artificial insemination programs, ejaculated semen volume and sperm concentration determines how many females can be inseminated by preparing semen doses (Fan et al., 2004). Donoghue and Wishart (2000) and Siudzinska and Lukaszewicz (2008b) reported several trials that indicate that chicken sperm can tolerate a pH range of 6.0 to 8.0. An abnormally high or low semen pH can kill sperm or affect their ability to move or to penetrate an egg. Moreover, there was no breed difference in phenotypic traits among cockerel Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK). The OV had the highest body weight and total sperm motility compared to NN and PK cockerels. It was reported that heavy body weight cockerels have higher sperm motility (Holcman et al., 1993) and higher normal live sperm (Holcman et al., 1993) compared to light body weight cockerels. Similarly, OV had the highest sperm motility and percentage of live normal sperm. However, NN had the lowest sperm motility and percentage of live normal sperm. Wilson et al. (1988) found that sperm concentration was positively correlated with body weight in agreement with the findings of this study. Inversely, Scogin et al. (1982) found a negative

Table 2. Live performance traits (Mean ± SD) of cockerels of NN, OV and PK.

Cockerel (n)	Live performance trait			
	Body weight (kg)	**Shank length (cm)**	**Comb length (cm)**	**Wattle length (cm)**
NN (11)	2.0±0.3^{c}	8.4±0.8	5.4±0.7	5.6±0.7
OV (11)	2.5±0.4^{a}	8.3±0.9	5.8±0.9	6.0±0.9
PK (11)	2.3±0.3^{b}	7.7±0.8	5.9±0.9	5.6±0.9

$^{a\text{-}b}$Values within each column with the same letter or letters do not differ significantly at a 5% level of significance. NN = Naked Neck; OV = Ovambo; PK = Potchefstroom Koekoek.

Table 3. Comparison of Indigenous cockerels breeds on macroscopic evaluation of cock semen characteristics (Mean ± SD).

Cockerel (n)	Ejaculate volume (ml)	Semen pH	Sperm concentration (× 10^9/ml)	Total motility (%)	Live normal sperm (%)
NN (11)	0.5±0.2^{a}	6.8±1.3^{b}	6.3±6.2^{a}	76.4±22.2^{c}	69.9±20.0^{b}
OV (11)	0.3±0.2^{b}	7.7±0.9^{a}	3.8±6.2^{b}	95.0±7.2^{a}	75.5±14.0^{ab}
PK (11)	0.4±0.2^{ab}	6.6±2.2^{b}	8.0±5.0^{a}	86.0±13.7^{b}	79.9±13.7^{a}

$^{a\text{-}b}$Values within each column with the same letter or letters do not differ significantly at a 5% level of significance. NN = Naked Neck; OV = Ovambo; PK = Potchefstroom Koekoek.

Table 4. Pearson correlation coefficients of semen characteristics and live performance traits for Indigenous cock breeds.

Variable	Weight	Shank length	Wattle length	Comb length	Volume (ml)	Concentration × 10^9	pH	Total motility (%)	Progressive (%)	Live normal (%)
Body weight	1	-0.141	-0.005	0.331	-0.318	0.235	0.119	**0.476**	0.235	0.090
Shank length	-0.141	**1**	**0.417**	0.218	0.295	0.238	0.202	-0.114	-0.294	**0.376**
Wattle length	-0.005	**0.417**	**1**	0.149	0.094	0.218	**0.368**	-0.087	-0.286	0.107
Comb length	0.331	0.218	0.149	**1**	0.166	0.044	0.116	0.255	0.015	0.196
Vol (ml)	-0.318	0.295	0.094	0.166	1	0.206	-0.268	-0.057	0.080	0.273
Concentration ×10^9	0.235	0.238	0.218	0.044	0.206	1	0.077	-0.134	-0.092	**0.404**
pH	0.119	0.202	**0.368**	0.116	-0.268	0.077	**1**	0.058	-0.188	0.340
Total motility (%)	**0.476**	-0.114	-0.087	0.255	-0.057	-0.134	0.058	1	**0.611**	0.059
Progressive (%)	0.235	-0.294	-0.286	0.015	0.080	-0.092	-0.188	**0.611**	1	-0.190
Live normal (%)	0.090	**0.376**	0.107	0.196	0.273	**0.404**	0.340	0.059	-0.190	1

Values in bold are different from 0 with a significance level alpha = 0.05.

correlation between body weight and semen volume.

Conclusion

Body weight of all three indigenous breeds was positively correlated with sperm concentration, semen pH, and total sperm motility. Comb length was positively correlated with total motility, semen volume and pH and sperm concentration. Shank and wattle length were negatively correlated with total sperm motility but positively correlated with semen volume, sperm concentration, and semen pH. These phenotypic traits are found to provide reliable indicator to facilitate the identification and removal of sub-fertile males from the breeder flock using body measurement. Further studies are required to conduct artificial insemination for quantification of better sperm fertility. Based on these results, it was suggested that at village communities of South Africa where there is no facility for microscopic evaluation of semen; village communities can use body weight, the length of shank, comb and wattle to predict the fertility of their breeding cockerels.

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