

**CORRELATIONS BETWEEN LIVE PERFORMANCE AND REPRODUCTIVE
TRAITS OF SOUTH AFRICAN INDIGENOUS MALE CHICKEN BREEDS**

MAKHAFOLA MAILA BERVLY

**CORRELATIONS BETWEEN LIVE PERFORMANCE AND REPRODUCTIVE
TRAITS OF SOUTH AFRICAN INDIGENOUS MALE CHICKEN BREEDS**

by

Makhafola Maila Bervly

Dissertation submitted in partial fulfillment of the requirements
for
the degree

MAGISTER TECHNOLOGIAE: AGRICULTURE

in the

Faculty of Health and Environmental Sciences
Department of Agriculture

at the

Central University of Technology, Free State

Supervisor: Prof D.O Umesiobi [Ph.D, FUTO; MAHES (UoFS)]

Co-Supervisor: Prof T.L Nedambale [Ph.D (UCONN, USA)]

March 2013

ACKNOWLEDGEMENT OF FINANCIAL SUPPORT

I wish to express my sincere gratitude and appreciation to the Central University of Technology, Free State, the Agricultural Research Council (ARC) Animal Production Institute at Irene, and Department of Agriculture Forestry & Fisheries (DAFF), Professional Development Program (PDP) for their financial contribution to the successful completion of this study.

DECLARATION AND COPYRIGHT

I, Maila Beverly Makhafola, identity number: 8512020657082 and student number 209082100 declare that the dissertation : **Correlations between live performance and reproductive traits of South African indigenous male chicken breeds** submitted to the Central University of Technology, Free State for the MAGISTER TECHNOLOGIAE: AGRICULTURE is my own independent work and that all the sources used and quoted have been acknowledged by means of complete references; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulation of the Central University of Technology; and has not been submitted before to any institution by myself or any other person in fulfillment (or partial fulfillment) of the requirements for the attainment of any qualification. I also disclaim the copyright of this dissertation in favour of the Central University of Technology, Free State.



MAKHAFOLA MAILA BERVLY

24.01.2013

DATE

DEDICATION

I dedicate this to my loving family, my father Jacob, my dearest mother Rose for their guidance, moral support, understanding, encouragement, prayers and to my one and only beloved sister Mamolefe "Maze" Makhafola for always being there for me, she is the best sister in the whole wide world.

And lastly to the memory of my late grandparents (Petrus and Elizabeth Lebelo) (David and Maria Makhafola) I know that you would have been very proud of me.

ACKNOWLEDGEMENTS

The author wishes to express her gratitude and appreciation to the following persons and Institute.

- First and foremost, I give glory to Almighty God for giving me strength and seeing me through this task successfully
- I would like to express my heartfelt thanks to my supervisor Prof D.O Umesiobi (Central University of Technology, Free State) and co-supervisor Prof T.L Nedambale (Programme Manager: Germplasm Conservation & Reproductive Biotechnologies) for their guidance, enormous encouragement, constructive criticism, planning and implementation of this study
- To Mr F Calitz (ARC-Statistician) for his patience and assistance in data analysis
- To the School of Agriculture and Environmental Science (CUT) for providing financial support
- To the Department of Agriculture Forestry & Fisheries(DAFF), Professional Development Program (PDP) and National Research Foundation (NRF) for providing financial support in making this study possible
- I wish to express my sincere gratitude and appreciation to the Agricultural Research Council (ARC), Poultry Breeding Unit staff, Mr Swartjas Molekwa and Mr Sillas Molalakgotla for helping me collect data
- Germplasm Conservation & Reproductive Biotechnologies (GCRB) staff for their assistance in collecting data, moral support and their friendship

TABLE OF CONTENTS

CONTENTS	PAGES
List of tables	XI
List of figures	XIII
List of acronyms and abbreviations	XIX
General abstract of the study	XVI
Chapter 1: Background of the study	
1.1 Introduction	1
1.2 Motivation of the study	4
1.3 Problem statement	5
1.4 Project rational	6
1.5 Objectives	6
1.5.1 Main objective	6
1.5.2 Specific objectives	7
1.6 Hypothesis	7
1.7 References	7
Chapter 2: Literature review	
2.1 Introduction	11
2.2 Characteristics of South African indigenous chicken breeds	13
2.2.1 Naked Neck breed	14
2.2.2 Ovambo breed	16
2.2.3 Potchefstroom Koekoek breed	17
2.3 Live performance traits of South African indigenous chicken breeds	19
2.3.1 Live performance traits of cocks	19
2.3.1.1 Body weight	20
2.3.1.2 Shank length	20
2.3.1.3 Wattle length	21

2.3.1.4 Comb length	21
2.4 Semen evaluation	22
2.4.1 Cock Semen	22
2.4.2 Semen Collection	22
2.5 Macroscopic semen evaluation	24
2.5.1 Semen volume	24
2.5.2 Semen pH	24
2.6 Microscopic sperm cells evaluation	24
2.6.1 Sperm concentration	25
2.6.2 Sperm motility	25
2.6.3 Preparation of sperm cell swim up	26
2.6.4 Assessment of kinetics and motility of sperm	29
2.7 Sperm morphology	32
2.7.1 Morphology analysis	33
2.7.2 Smears preparation for sperm morphology	34
2.8 Artificial insemination	35
2.8.1 History of artificial insemination in chickens	36
2.8.2 Methods of artificial insemination	36
2.8.3 Fertility rate following artificial insemination	37
2.9 Hatchability	38
2.9.1 Hatchability traits	38
2.9.2 Management of day old chickens	39
2.11 References	40

Chapter 3

Relationship between live performance and testis traits of South African indigenous cockerels

3.1 Abstract	53
3.2 Introduction	54
3.3 Materials and Methods	55

3.4 Statistical analysis	57
3.5 Results	57
3.6 Discussion	61
3.7 Conclusion	62
3.8 Acknowledgements	62
3.9 References	62

Chapter 4

Characterization of sperm motility rate of South African indigenous cocks following analysis by Sperm Class Analyser (SCA)

4.1 Abstract	65
4.2 Introduction	67
4.3 Materials and Methods	69
4.4 Statistical analysis	73
4.5 Results	74
4.6 Discussion	80
4.7 Conclusion	81
4.8 Acknowledgements	82
4.9 References	82

Chapter 5

Relationship between secondary sexual characteristics and sperm traits of South African indigenous cockerels

5.1 Abstract	86
5.2 Introduction	87
5.3 Materials and Methods	89
5.4 Statistical analysis	93
5.5 Results	93
5.6 Discussion	98
5.7 Conclusion	99
5.8 Acknowledgements	100
5.9 References	100

Chapter 6

Quantification of indigenous cockerels sperm traits measured by computer assisted sperm analysis (casa) technology and the relationship to fertility

6.1 Abstract	103
6.2 Introduction	104
6.3 Materials and Methods	106
6.4 Statistical analysis	110
6.5 Results	112
6.6 Discussion	115
6.7 Conclusion	116
6.8 Acknowledgements	116
6.9 References	117

Chapter 7

General conclusion and recommendation

7.1 General conclusion	122
7.2 Recommendation	123

LIST OF TABLES

Tables	Pages
Table 2.1 Body weight of Naked Neck chickens	15
Table 2.2 Percentage of eggs for Naked Neck breed	15
Table 2.3 Body weight of Ovambo chickens	16
Table 2.4 Percentage of eggs for Ovambo breed	17
Table 2.5 Body weight of Potchefstroom Koekoek chickens	18
Table 2.6 Percentage of eggs for Potchefstroom Koekoek breed	19
Table 2.7 Preparation of Brackett and Oliphant Solution (A)	27
Table 2.8 Preparation of Brackett and Oliphant Solution (B)	28
Table 2.9 Preparation of Sperm Washing Solution	28
Table 2.10 Sperm Class Analyser [®] (V.5.0) settings used to analyse the sperm motility and velocity characteristics	31
Table 3.2 Live performance traits (Mean $\pm$ SD) of cockerels of NN, OV and PK	59
Table 3.3 Testis traits (Mean $\pm$ SD) of cockerels of NN, OV and PK	60
Table 4.2 Sperm Class Analyser [®] (V.5.0) settings used to analyse the sperm cell motility and velocity characteristics	73
Table 4.3 The means ($\pm$ SD) macroscopic evaluation of cockerel semen characteristics	76
Table 4.4 Comparison of sperm morphological defects (Mean $\pm$ SD) of different cockerel breeds	77
Table 4.5 Characterization (Mean $\pm$ SD) of sperm motility rate of cockerels of different breeds	78
Table 4.6 Pearson Correlation Coefficients between body weight and semen characteristics of Indigenous cockerel breeds	79
Table 5.2 Sperm Class Analyser [®] (V.5.0) settings used to analyse the sperm cell motility and velocity characteristics	92
Table 5.3 Live performance traits (Mean $\pm$ SD) of cockerels of different breeds	95
Table 5.4 Comparison of Indigenous Breed means of macroscopic evaluation	

of cock semen characteristics (Mean±SD)	96
Table 5.5 Pearson Correlation Coefficients of semen characteristics and live performance traits for Indigenous cock breeds	97
Table 6.2 Sperm Class Analyser [®] (V.5.0) settings used to analyse the sperm motility and velocity characteristics	111
Table 6.3 Means of macroscopic evaluation of indigenous cockerels semen characteristics (Mean±SD)	113
Table 6.4 Least square means ($\pm$ s.e) for fertility traits of South African Indigenous hens following artificial insemination	114

LIST OF FIGURES

Figures	Pages
Figure 2.1 Naked Neck chickens	15
Figure 2.2 Ovambo chickens	16
Figure 2.3 Potchefstroom Koekoek chickens	18
Figure 2.4 Spectrophotometer	20
Figure 2.5 Sperm Class Analyzer	25
Figure 2.6 Fluorescence microscope	27
Figure 2.7 Vernier Calliper	34
Figure 3.1 (A) Indigenous chickens used in this study, a) Ovambo, b) Naked Neck and c) Potchefstroom Koekoek.	75
Figure 3.1 (B) Stained cockerel spermatozoa (a, d and f) live normal sperm, (b & c) live abnormal sperm and (e) dead sperm.	76

LIST OF ACRONYMS AND ABBREVIATIONS

ABBREVIATIONS	DESCRIPTION
AI	Artificial insemination
bn	Billion
CASA	Computer Assisted Sperm Analyzer
DAFF	Department of Agriculture, Forestry & Fisheries
FAO	Food and Agricultural Organisation
LIN	Linearity
MEDIUM	Percentage of sperm that are moving at < 50 µm/sec
MOTILE	Percentage of motile sperm
mL	Mille litter
µm	Micro meter
®	Trademark
NN	Naked Neck
Nm	Nano meters
NPM	Non progressive motility
OV	Ovambo
P	Probability
PK	Potchefstroom Koekoek
PM	Progressive Motility
RAPID	Percentage of sperm that are moving at < 100 µm/sec
RH	Relative Humidity
SAS	Statistical Analysis Software
±s.e	Least square mean
SE	Standard Error
SD	Standard deviation
SLOW	Percentage of sperm that are moving at < 10 µm/sec
STATIC	Percentage of immotile sperm

STR	Straightness
VAP	Velocity Average Pathway
VCL	Velocity Curvilinear
VSL	Velocity Straight Line

GENERAL ABSTRACT OF THE STUDY

The objectives of the study were to find the relationship between live performance and testis traits of South African indigenous cockerels on Naked Neck, Ovambo and Potchefstroom Koekoek (Trial 1). Characterise Naked Neck, Ovambo and Potchefstroom Koekoek cockerels' semen macroscopically and microscopically (Trial 2). To investigate the relationship between phenotypic and sperm traits of the South African indigenous cockerels Naked Neck, Ovambo and Potchefstroom Koekoek. (Trial 3). Quantification of indigenous cockerels sperm traits measured by Computer Assisted Sperm Analysis (CASA) technology and the relationship to fertility (Trial 4). The cockerels shank, comb and wattle length were recorded with a vernier calliper. A total of 198 ejaculates were collected from 33 cockerels; Ovambo (n=11), Potchefstroom Koekoek (n=11) and Naked Neck (n=11) by means of an abdominal massage technique. Following analysis, 0.25 ml undiluted semen dose from Naked Neck (NN, 11), Ovambo (OV, 11) and Potchefstroom Koekoek (PK, 11) cockerels were inseminated NN (30), OV (30) and PK (30) hens once per week for 6 weeks. Semen was analysed macroscopically and microscopically. The sperm motility rate was evaluated with the aid of the Computer Assisted Sperm Analysis (CASA) called Sperm Class Analyser (SCA) system. Breed significantly ($P < 0.05$) affected the body weight of Ovambo (2.5 ± 0.4 kg), Naked Neck (2.0 ± 0.3 kg) and Potchefstroom Koekoek (2.3 ± 0.3 kg) cockerels, OV was statistically heavier than NN and PK. The semen volume of Naked Neck (0.5 ± 0.2 ml) breed was significantly higher, when compared to Ovambo (0.4 ± 0.2 ml), but similar to Potchefstroom Koekoek (0.3 ± 0.2 ml) breed. The overall sperm motility rate of Ovambo ($95.0 \pm 7.2\%$), Potchefstroom Koekoek ($86.0 \pm 13.7\%$) Naked Neck ($76.4 \pm 22.2\%$) group was significantly different. The normal sperm morphology in the Naked Neck ($76.4 \pm 22.2\%$) was significantly lower when compared to Potchefstroom Koekoek ($86.0 \pm 13.7\%$) and Ovambo ($95.0 \pm 7.2\%$) cockerels. A positive correlation existed between body weight and semen volume for Potchefstroom Koekoek ($r = 0.1477$) cockerels only. Moreover, there was a positive correlation between the body weight and total sperm motility for Naked Neck ($r = 0.3848$), Ovambo ($r = 0.4871$) and Potchefstroom Koekoek ($r = 0.2230$) cockerels. However, a negative correlation existed between body weight and semen volume for Naked Neck ($r = -0.4502$) and Ovambo ($r = -0.1244$). There was no significant differences ($P > 0.05$) on the shank length (SL), comb length (CL) and wattle length

(WL) of Naked Neck (8.4, 5.4 and 5.6 cm), Ovambo (8.3, 5.8 and 6.0 cm) and Potchefstroom Koekoek (7.7, 5.9 and 5.6). There was a positive correlation between shank length and wattle length, comb length, semen volume, sperm concentration, semen pH and live normal sperm. Inversely, a negative correlation of body weight and total sperm motility was observed. Introspective a negative correlation existed between body weight and total motility. Ovambo (11.45 ± 0.9 g) had the highest live weight of testis, it was significantly different from Naked Neck (9.2 ± 0.8 g) and Potchefstroom Koekoek (8.9 ± 0.8 g). For average length of the testis there was a significant difference amongst all breeds, Ovambo (4.0 ± 0.5 cm), Naked Neck (3.4 ± 0.3 cm) and Potchefstroom Koekoek (3.7 ± 0.4 cm). Following AI, significant differences were recorded in Potchefstroom Koekoek and produced eggs that had highest average egg weight (55.5 g), fertility (68.2 %), hatchability (78.6 %), percentage of normal chicks (80.1%) and chick weight (37.9 g) compared to Ovambo (40.4 g, 52.1 % , 70.2 %, 71.5 %, 32.8 g and Naked Neck (37.1 g, 50.6 %, 71.1 %, 75.8 % and 23.5 g). In order to achieve the highest level of fertility and hatched of fertile eggs, an adequate number of active males that are producing high quality semen should be maintained in the breeder house at all times. In conclusion, body weight positively affected semen volume and sperm motility rate but this was breed dependent. The Sperm of Potchefstroom Koekoek and Ovambo resulted in a better motility and morphology rate. The Sperm Class Analyser or CASA provided more precise, repeatable and objective information. Evaluation of the sperm motility rate of South African indigenous cockerels holds potential for future use in semen evaluation. 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. In conclusion, there is no correlation between live performance and testis traits. Though the study suggests that body weight and testicular length are good indicators of sperm production. Simple and indirect method of estimation is still needed to predict the fertility of the breeding cockerels. In order to achieve the highest level of fertility and hatch of fertile eggs, an adequate number of active males that are producing high quality semen should be maintained in the breeder house at all times. The Potchefstroom Koekoek, Ovambo and Naked Neck donated quality

semen as well as in improving the fertility and hatchability traits of the indigenous chicken breeds. Chicken hatched from larger eggs had higher body weight, hatchability and fertility therefore breeders should select bigger egg sizes probably of 50-55g for breeding programmes. Consequently, the local cockerels have higher potential for use in natural mating and AI programmes since seminal traits could be a viable index for selection of breeding cockerels.

Key words: Semen, fertility, hatchability, South African Indigenous chicken breeds, live performance, testis

CHAPTER 1: BACKGROUND OF THE STUDY

1.1 INTRODUCTION

The Indigenous chickens of South Africa are more adapted to the local environmental conditions and diseases. Horst (1989) considered the indigenous chicken as genes reservoir, particularly, those genes that have adaptive values in the tropical conditions. In domestic chicken (*Gallus domesticus*), live performance and reproductive capacity are some of the most important economic traits for improved poultry production, together with egg hatchability. Live performance traits have reportedly been determined through body weight (BW) and length of shank (SL), comb (CL) and wattle (WL) (Nwosuet *al.*, 1984; Korver&Lawes, 2004). Male reproductive capacity is defined as the ability to produce an ejaculate with viable spermatozoa that can fertilize eggs (Umesiobi, 2006a, b) and are noted to enhance accomplishment of all steps of the fertilization process (Umesiobi, 2007), including sperm moving across the female reproductive tract and reaching the sperm storage tubule, binding and penetration into the perivitelline layer, and fertilization (Celeghiniet *al.*, 2007).

No research work has been conducted on correlations between live performance and reproductive traits of South African indigenous male chicken breeds. There are many methods of assessing spermatozoa quality for estimating their fertilizing potency. Some of them are regarded as highly subjective; others require special laboratory facilities (Hazary & Wishart, 2001). In order to standardize the manual semen analysis, the Computer Assisted Sperm Analysis (CASA) system has been

developed in the past few years. One of the advantages of CASA system is that, a series of videotaped microscope image is digitalized and computer software programs are used to evaluate sperm motility, velocity and morphology (Ferrara *et al.*, 1997). Semen analysis is essential for studying male fertility. Semen analyses include evaluation of sperm concentration, motility, morphology and semen volume (Ferrara *et al.*, 1997).

In basic semen analysis and many research-oriented investigations, it is important to distinguish between dead and live spermatozoa. A proportion of live spermatozoa can be identified by their motility, while the ability to distinguish live, immotile spermatozoa from dead spermatozoa requires other techniques. Several dye exclusion techniques have been developed to distinguish live and immotile spermatozoa from dead spermatozoa (Björndahl, *et al.*, 2003). The concept of using eosin to mark dead cells, which take up eosin, and nigrosin as a background stain, to increase the contrast between faintly stained cells and an otherwise bright background, is well known and widely used (World Health Organization 1992).

Number of spermatozoa per ejaculate and body weight were positively correlated (Umesiobi, 2006a). Sperm morphology can also serve as an indicator of some disorders in spermatogenesis depending on stain preparation techniques, stains type and staining methods (Bilgili *et al.*, 1985). Chickens have a shorter generation interval than most farm animals, thus genetic improvement could be attained at a faster rate.

Accurate and reliable prediction equations for estimating semen characteristics from *in vivo* measurements are required by poultry breeders. The most accurate method

for the evaluation of the semen measurements are macroscopically and microscopically examination. However, this method is stressful, time consuming and costly. Thus, simple, reliable and indirect methods for *in vivo* estimation of semen attributes based upon the correlation between phenotypic traits and sperm motility were needed. Behavioural studies in sexual selection of red jungle fowl show female preference for males with larger combs (Ligon and Zwartjes, 1995; Zuk *et al.*, 1995). Additionally, male comb area may indicate health status. Positive correlation between wattle length, comb length and fertility indicates that the development of secondary sexual characteristics may convey male reproductive quality in some genetic strains (McGary *et al.*, 2003). Number of spermatozoa per ejaculate and body weight were reported to be positively correlated (Wilson *et al.*, 1988). Local chickens constitute a significant contribution to human livelihood and contribute significantly to food security (Gondwe, 2004).

Thus the study was designed to (i) characterize Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) cockerels semen macroscopically and microscopically, (ii) study the relationship between South African indigenous cockerels fertility with phenotypical body measurements and (iii) Check the relation between cock sperm traits, egg traits, hatchability, fertility, chick hatch weight, and chick survivability of artificial inseminated South African indigenous hens.

1.2 MOTIVATION FOR THE STUDY

Indigenous chickens have potential to offset the problem of low reproductive performance compared to most other poultry species due to their short generation intervals (Guèye, 1998). They are also raised by the majority of the rural households

in South Africa. The genetic diversity within indigenous chicken breeds is shrinking due to their replacement by high-producing exotic commercial hybrids. They are one of the cheapest sources of meat and can be kept by anyone, even in backyards. In South Africa, poultry are kept by smaller farmers, as well as by households in backyards (RSA DoA, 2007). The local breeds of chickens provide meat and eggs which are a valuable yet affordable source of high quality protein and vitamins required for improved nutrition and health. It is speculated that with minimal technical and institutional support, the indigenous chicken breeds could contribute significantly to the rural economy and could curtail the vicious cycle of unemployment and poor human nutrition. Most important, they are known for their adaptation superiority in terms of their resistance to endemic diseases and other harsh environmental conditions. The purpose of this study therefore, was to evaluate Correlations between live performance and reproductive traits of South African indigenous male chicken breeds.

1.3 PROBLEM STATEMENT

No research has been done on correlations between live performance and reproductive traits of Naked Neck, Ovambo, and Potchefstroom Koekoek cockerels breeds. Fertility decline in local chickens is likely attributable to individual cockerel in the flock with extreme sub fertility. This differential fertility among males may reduce productivity for the poultry industry (Molekwa & Umesiobi, 2008). Male chicken breeders must be physiologically and behaviorally mature to successfully elicit the female sexual response and copulate. Fertility problems, particularly those associated with local chicken breeds may result from a physical inability to successfully copulate or from the absence of sexual behavior (Molekwa & Umesiobi,

2008). Furthermore, various programs between local chickens and exotic chickens had been initiated in an attempt to combine desirable features from these two diverse genetic groups (that is, high productivity from exotic genotypes and high adaptability from local chicken genotypes (Joubert, 1996). However, such programs became unsustainable due to unreliable supply and high costs of acquiring and maintaining exotic breeding cocks, reduced broodiness and ability to evade predation by the crossbred birds and incompatibility of genotypes with farmers' breeding objectives and production systems (Tadelle *et al.*, 2000; Udo *et al.*, 2001; Kosgey, 2004; Njega, 2005). In order to optimize selection response, adequate characterization of relationships between live performance and reproductive traits of selected South African indigenous male chicken breeds need urgent investigation.

1.4 PROJECT RATIONAL

Sexual selection theory (Tadelle *et al.*, 2000; Kosgey, 2004) predicts that males with larger combs may elicit a stronger behavioral response in females and, therefore, should successfully initiate and likely complete more mating. Males with higher copulation frequency should achieve higher fertility. Several studies (El- Sayiad *et al.*, 1994; Abdellatif, 1999) have recommended the use of lengths of comb and wattle as good indicators for selection of quantitative traits in chickens. Larger combs may reliably indicate roosters with greater semen production (Burrows & Titus, 1939). These reports need to be confirmed by evaluating correlations between live performance and reproductive traits of selected South African indigenous male chicken breeds.

1.5 OBJECTIVES OF THE STUDY

1.5.1 PRIMARY OBJECTIVE

The primary objective is to comparatively evaluate the correlations between live performance and sperm traits of South African indigenous male chicken breeds

1.5.2 SPECIFIC OBJECTIVES

- Determine the relationship between live performance and testis traits of South African indigenous cockerels on Naked Neck, Ovambo and Potchefstroom Koekoek
- Characterization of sperm motility rate of South African indigenous cockerel semen following analysis by Sperm Class Analyzer
- Determine the relationship between live performance traits (body weight, shank, wattle, and comb length) and semen viability of South African indigenous cockerels
- Evaluate the relation between cock semen viability and the fertility following artificial insemination of South African indigenous chicken hens

1.6 HYPOTHESIS

There will be positive correlations between semen traits and reproductive traits of selected South African indigenous male chicken breeds. Semen traits and

reproductive traits of cocks will positively affect the fertility rate of artificially inseminated South African indigenous layer hens.

REFERENCES

- Abdellatif, M.A., 1999. Inheritance of comb and wattle lengths and its as indicators to select cockerels in a population of Dandarawi chicken. *ASSIUT Journal Of Agricultural Sciences*. 30, 123-131.
- Bilgili, S.F., Renden, J.A. & Sexton, K.J., 1985. The influence of staining techniques and examiners on evaluation of the morphology of fowl spermatozoa. *Poultry Science*. 64, 2358–2361.
- Bjorndahl, L., Soderlund, I. & Kvist, U., 2003. Evaluation of the one-step eosin nigrosin staining technique for human sperm vitality assessment. *Human Reproduction*. 18, 813–816.
- Burrows, W.H. & Titus, H.W., 1939. Some observation on the semen production of the male fowl. *Poultry Science*. 18, 8-10.
- Celeghini, E.C.C., Arruda, R.P., Andrade, A.F.C., Nascimento, J. & Raphael, C.F., 2007. Practical techniques for bovine sperm simultaneous fluorimetric assessment of plasma, acrosomal and mitochondrial membranes. *Reproduction in Domestic Animal*. 42, 479-488.
- El-Sayiad, G.H., Hossari, M.A., Yamani, K.A., Sabri, H.M. & Abdel-Warith, A., 1994. A genetical study on Egyptian Fayoumi fowl. *Egyptian Poultry Science*. 14, 285-315.
- Ferrara, F., Daverio, R., Mazzini, G., Bonini, P. & Banfin, G., 1997. Automation of human sperm analysis by flow Cytometry. *Clinical Chemistry*. 43, 801-807.

- Gondwe, T.N.P., 2004. Characterization of local chicken in low input-low output production systems: is there scope for appropriate production and breeding strategies in Malawi? PhD thesis, Georg-August-Universität Göttingen, Germany,
- Guèye, E.F., 1998. Poultry plays an important role in African village life. *World Poultry*.14(10), 14-17.
- Hazary, R.C. & Wishart, G.J., 2001. Assay of sperm quality in the domestic fowl by MTT reduction. *British Poultry Science*. 42, 111–114.
- Horst, P., 1989. Native fowl as reservoir for genomes and major genes with direct and indirect effects on the adaptability and their potential for tropically oriented breeding plans. *Archives Animal Breeding*.53, 93–10.
- Joubert, J.J., 1996. The story of the indigenous domestic animals in South Africa. Agricultural Research Centre, Private Bag X2, Irene, 0062. South Africa. (Unpublished).
- Korver, D.R. & Lawes, K.R., 2004. Performance Characteristics and Economic Comparison of Broiler Chickens Fed Wheat- and Triticale-Based Diets. *Poultry Science*. 83(5), 210-216.
- Kosgey, I.S., 2004. Breeding objectives and breeding strategies for small ruminants in the tropics. PhD Dissertation, Wageningen University. The Netherlands: 272.
- Ligon, J.D. & Zwartjes, P.W., 1995. Ornate plumage of male red jungle fowl does not influence mate choice by females. *Animal Behaviour*. 49, 117-125.
- McGary, S., Estevez, I., Bakst, M.R. & Pollock, D.L., 2003. Phenotypic traits as reliable indicators of fertility in male broiler breeders. *Poultry Science*. 81(1), 102-111.

- Molekwa, J.T. & Umesiobi, D.O., 2008. Relationships between cock semen viability and the fertility of artificially inseminated South African indigenous chicken breeds. Published Proceedings of the 10th World Conference on Animal Production of the World Association for Animal Production, 23-28 November 2008, International Conference Centre, Cape Town.
- Njega, S.M., 2005. Productivity and socio-cultural aspects of local poultry phenotypes in Coastal Kenya. MSc. Dissertation. The Royal Veterinary and Agricultural University (RVAU), Copenhagen, Denmark. p. 79
- Nwosu, C.C., Obioha, F.C., Omeje, S.S., Gowen, F., Bellonwu, C.T. & Onuora, G.I., 1984. Growth performance of chickens in deep litter systems. *World Review of Animal Production*. 20 (2), 17-26.
- RSA DoA (Republic of South Africa, Department of Agriculture). 2007. Poultry diseases. www.nda.agric.za/publications. Accessed on 24 November 2007.
- Tadelle, D., Alemu, Y. & Peters, K.J., 2000. Indigenous chickens in Ethiopia: genetic potential and attempts at improvement. *World Poultry Science Journal*. 56, 45-54.
- Udo, H.M.J., Asgedom, A.H. & Viets, T.C., 2001. Modeling the impact of interventions in village poultry systems. In: Livestock Community and Environment. Proceedings of the 10th Conference of the Association of Institutions for Tropical Veterinary Medicine. 21-22 August 2001, Copenhagen, Denmark, [http://www.aitvm.kvl.dk/C_poultry/C13Udo.htm]. Accessed on 13 April 2009.
- Umesiobi, D.O., 2006a. Effect of oral administration of Clomiphene citrate on sperm viability and fertility of boar semen. *Journal of Applied Animal Research*. 30, 67-170.

- Umesiobi, D.O., 2006b. The effect of hemi-orchidectomy on reproductive traits of boars. *South African Journal of Animal Science*.36, 181-188.
- Umesiobi, D.O., 2007. Measures of libido and their relation to testicular hypertrophy and fertilizing competence in boars. *Journal of Animal Science*.85(1), 815.
- Wilson, J.L., Krista, L.M., McDaniel, G.R. & Sutton, C.D., 1988. Correlation of broiler breeder male semen production and testes morphology. *Poultry Science*. 67, 660-668.
- World Health Organization.,1992. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interactions.3rdedn.Cambridge University Press, Cambridge, UK.
- Zuk, M., Pompa, S.L. & Johsen, T.S., 1995. Courtship displays, ornaments, and female mate choice in captive red jungle fowl. *Behaviour*.132, 821-836.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Poultry farming has become especially popular, due to the quick cash return, low capital inputs and subsequent poverty alleviation and income generation in the rural poor communities of South Africa. Poultry production is widely spread in South Africa, comprising of large-scale, small-scale commercial and extensive poultry production units. Large-scale systems are characterized by huge investments, intensive management and a high degree of mechanization and specialization. These systems, because of the nature of the inputs required, are confined to a few wealthy companies or individuals (Aganga *et al.*, 2000). Small-scale systems are found in both peri-urban and communal areas. Exotic or improved breeds are used in commercial poultry production systems. Extensive systems are found mainly in the communal areas, with almost every household owning some chickens. Some of the local chickens seen in rural areas may have been crossed with exotic cocks in earlier years through the cockerel exchange program amongst others, but such gene may have been dispersed and lost in the population because of unplanned breeding programs and absence of selection (Njue *et al.*, 2002). One way of overcoming challenges posed by past strategies on improving sustainable productivity is through improving the performance and reproductive capacity of breeding stock.

The livestock sector forms an important component of the agricultural output in both developed and developing country. Around 20 percent of the world livestock breeds of cattle, goats, pigs, horses and poultry are currently at risk of extinction (FAO,

2006). Half of the domestic poultry breeds are currently considered to be endangered (Dohner, 2001; Blanco *et al.*, 2009). They are endangered for various reason, such as related to construction of rural areas, human agriculture and exposure to health risk (Blesbois *et al.*, 2007), replacement by high producing exotic breeds (Molekwa & Umesiobi, 2009; Woelders, *et al.*, 2006) and human selection (Long, 2008).

The South African indigenous chicken production has withstood the test of time and is often described as a low input-low output production system. This makes it a very sustainable system for the resource poor, communal area farmers. Needless to say, because of this attitude, very little attention has been paid to the sector in terms of research and development, training and marketing, resulting in the lack of information on the reproductive status of these local breeds. This lack of attention has led to many constraints in terms of improving productivity of local chickens, not only in South Africa, but also in other African and developing countries. Despite the lack of attention and constraints, there is potential to increase local chicken production. Local chickens are an important reservoir of germplasm that may be used in the future to improve hybrid for better adaptation to worse environmental conditions (Nwosu *et al.*, 1984). Local chickens are also relatively cheap to purchase, feed and house. Currently, local chicken breeds are kept under free range system without much emphasis on scientific breeding and management practices in remote rural areas. Minimal management of the flock has been practiced as the chicken can search for food, water and shelter, thus exposing themselves to the risks of theft and predation. In addition, they are an ideal source of high quality protein (both meat and eggs), income and manure fertilizer for economically

disadvantage people, the majority of whom live in the rural areas of South Africa (Molekwa & Umesiobi, 2008) and Africa (Nwosu *et al.*, 1984; Tadelles *et al.*, 2000). Correlations between live performance and reproductive traits of South African indigenous male chicken breeds will be experimented in this study in order to predict the fertility of cockerels without collection of semen and analysis. South African village communities do not have facilities such as microscope to evaluate semen; thus, it is vital to use body measurement for predicting cockerel's fertility.

2.2 CHARACTERISTIC OF SOUTH AFRICAN INDIGENOUS CHICKEN BREEDS

There are several indigenous chicken breeds in South Africa such as; Potchefstroom Koekoek, Venda, Ovambo, Naked Neck, Natal Game, Zulu and Nguni to name a few of the most popular breeds. Up to date there was not much detailed information available regarding the Reproduction potentials, neither under extensive or intensive conditions, regarding the Natal Game, Zulu or Nguni. The Natal game is a registered breed and is mainly used as a show bird. The Zulu and Nguni breeds are names that are regionally used which is typical of indigenous chicken breeds where the names are non-descriptive. Sonaiya (2003) described Indigenous chickens as chickens involving any genetic stock, improved or unimproved, that was raised extensively or semi-intensively in relatively small numbers. Naidoo (2003) stated that these chickens are left to free-range or scavenge for food, with little or no feed supplementation. According to Nhleko *et al.*, (2003), indigenous chickens are amongst the most adaptable domestic animals. They can survive cold and heat, wet and drought, sheltered in cages or unsheltered outside roosting in trees.

The Potchefstroom Koekoek, Venda, Ovambo, and Naked Neck are conserved at the Poultry Breeding Section of the Agricultural Research Council at Irene, in the province of Gauteng, South Africa. The production performance of the above mentioned indigenous chicken breeds was determined under an intensive system by Grobbelaar (2008).

2.2.1 Naked Neck

According to speculation by the archaeologists, the Naked Neck breed originated in Malaysia from where it spread globally. It is therefore possible that the Dutch East Indian Company introduced Naked Neck chickens in the 17th century to the Cape of Good Hope (Fourie & Grobbelaar, 2003). However, based on the speculation Naked Neck is recognized as an indigenous breed in South Africa. The Naked Neck is a very adaptable breed and can be found all around South Africa even in diverse climates (Grobbelaar, 2008). The Naked Neck of the chicken is caused by a single dominant gene (Na). When a dominant Naked Neck chicken is crossed with another dominant Naked Neck all the offspring will have Naked Necks. If a dominant Naked Neck is crossed with another chicken that is fully feathered the offspring will be 25% Naked Neck, 50% with some tassel of feathers on the front of the Neck and 25% will be fully feathered. Therefore if a person obtains a Naked Neck chicken and introduce it to other chickens the Naked Neck type of chickens will quickly multiply. Figure 2.1 Naked Neck chickens.



Figure 2.1: Naked Neck chickens

The body weight of NN at 16 and 20 weeks of age is depicted in Table 2.

Table 2.1: Body weight of the Naked Neck at 16 and 20 weeks of age

Age	Average Mass (Kg)	
	Cocks	Hens
16 weeks	1.50	1.10
20 weeks	1.95	1.40

Source: Ramsy, K., Harris, L. and Kotze (undated)

The egg production potential of an individual Naked Neck hen is 138 eggs per production cycle of 52 weeks. The days to the production of first egg are 129 days.

The percentage eggs per grade over a 52 week production cycle are depicted in Table 3.

Table 2.2: Percentage eggs per grade for the Naked Neck over a 52 week production cycle

Egg grades %					
Jumbo	X-large	Large	Medium	Small	Dirty or cracked
3.53	0.95	57.97	23.37	8.03	6.20

Source: Grobbelaar (2008)

2.2.2 Ovambo

A researcher at the Poultry Breeding Section at ARC-Irene visited the Ovamboland district of Namibia during 1975 and saw the Ovambo breed. He collected some of these chickens which were brought to the Agricultural Research Council (ARC) at Irene Poultry Breeding Unit to establish a stock colony to conserve the breed (Fourie & Grobbelaar, 2003). Figure 2.2 Ovambo chickens



Figure 2.2: Ovambo chicken

The body weight of OV at 16 and 20 weeks of age is depicted in Table 2.

Table 2.3: Body weight of the Ovambo at 16 and 20 weeks of age

Age	Average Mass (Kg)	
	Cocks	Hens
16 weeks	1.50	1.10
20 weeks	1.95	1.40

Source: Ramsy, K., Harris, L. and Kotze (undated)

The egg production potential of an individual Ovambo hen is 125 eggs per production cycle of 52 weeks (Fourie & Grobbelaar, 2003). The days to the production of first egg are 134, 5 days. The percentage eggs per grade over a 52 week production cycle are depicted in Table 4.

Table 2.4: Percentage eggs per grade for the Ovambo over a 52 week production cycle

Egg grades %					
Jumbo	X-large	Large	Medium	Small	Dirty or cracked
6.70	0.07	56.42	17.74	3.80	15.30

Source: Grobbelaar (2008)

2.2.3 Potchefstroom Koekoek

According to Fourie and Grobbelaar (2003) the Potchefstroom Koekoek was- bred at the Potchefstroom Agricultural College during the 1950's by a researcher known as Professor Chris Marais. This breed is a composite of the White Leghorn, Black Australorp and Bared Plymouth Rock. This breed can therefore be considered as a locally developed breed. The name "Koekoek" refers to the barred colour pattern of the birds. Most off the laying hens that were available during the developing period of the Potchefstroom Koekoek laid white shelled eggs. The consumer, however, preferred brown shelled eggs. Therefore the Potchefstroom Koekoek was developed for the following specific production traits: hens laying a brown shelled egg with an average weight of 55.7 g and the carcass should be attractive with a deep yellow coloured skin. The Potchefstroom Koekoek cocks and culled hens are used for meat production. Today the meat of this breed is still very popular amongst local communities and is preferred to that of the commercial broiler breeds. The Koekoek's colour pattern is a sex-linked gene that is very useful for colour sexing in

crossbreeding for egg producing type of hens used in medium input production systems. This breed is very popular amongst rural farmers both in South Africa and neighbouring countries for egg and meat production (Grobbelaar, 2008). Figure 2.3 Potchefstroom Koekoek chickens



Figure 2.3: Potchefstroom Koekoek

The body weight of PK at 16 and 20 weeks of age is depicted in Table 2.

Table 2.5: Body weight of the Potchefstroom Koekoek at 16 and 20 weeks of age

Age	Average Mass (Kg)	
	Cocks	Hens
16 weeks	1.50	1.10
20 weeks	1.95	1.40

Source: Ramsy, K., Harris, L. and Kotze (undated)

The egg production potential of an individual Potchefstroom Koekoek hen is 200 eggs per production cycle of 52 weeks (Grobbelaar, 2008). The days to the production of first egg are 138,5 days (Grobbelaar, 2008). The percentage eggs per grade over a 52 week production cycle are depicted in Table 6.

Table 2.6: Percentage eggs per grade for the Potchefstroom Koekoek over a 52 week production cycle

Egg grades %					
Jumbo	X-large	Large	Medium	Small	Dirty or cracked
3.49	2.05	61.14	22.01	4.2	7.06

Source: Grobbelaar (2008)

2.3 LIVE PERFORMANCE TRAITS OF SOUTH AFRICAN INDIGENOUS CHICKEN BREEDS

2.3.1 Live performance traits of cocks

Domestic chickens are particularly appropriate for studies testing the phenotype-linked fertility hypothesis because, while they are maintained in captivity, they retain many of the behavioral characteristics and social structure of their Asian ancestors, red jungle fowl (*Gallus gallus*). Differential male fertility in naturally mated chicken appears to be an inadvertent negative result of genetic selection for live performance traits. Identifying male performance traits that reliably indicate fertility could improve production while avoiding artificial insemination procedures. In many developing countries like South Africa, the local gene pool still provides the basis for the poultry sector. The genetic resource base of the indigenous chickens could form the basis for genetic improvement and diversification to produce breeds adapted to local conditions. Measurement of live performance traits in local male chicken using body weight (BW), length of shank (SL), comb length (CL), and wattle length (WL), appears to be beneficial in identifying and improving fertility traits of economic merit. Body weight and three (3) morphometric traits were measured on each animal. The anatomical reference points were as described by Gueye *et al.* (1998) and Tegui *et al.* (2008). The parts measured were, comb length (CL); horizontal distance from the

beginning to the end of the comb; shank length (SL), distance from the shank joint to the extremity of the Digitus pedis and wattle length (WL). All measurements were taken by the same person to avoid between-individual variations using a vernier calliper. Figure 2.4 vernier calliper.



Figure 2.4: Vernier Calliper

2.3.1.1 Body weight

A range of techniques is available to gain information about an animal's body mass and body composition. The most direct way to determine an animal's mass is to weigh it. An alternative is to measure a body part and relate the measurement to body weight. Information about body composition can be important in several ways. Those producing and selling animal products need information about body composition to track their efforts and produce leaner meat animals for production. Body weight is highly responsive to selection in chickens (Siegel1962a; Maloney *et al.*, 1963).

2.3.1.2 Shank length

Shank length is the body part that is commonly measured in poultry to relate to body weight. A linear relationship between body weight and shank length has been

reported (Lerner, 1937). Shank length is often considered as a parameter for monitoring growth and development of Leghorn pullets, because shank length is so closely correlated with body weight.

2.3.1.3 Wattle length

In *Gallus domesticus*, wattles are fleshy structures which hang from the lower jaw (Lucas and Stettenheim, 1972). Wattles are located under the chin. They are meaty, red growths. Chicken has two wattles. Wattles do not just hang there, they actually have an important function. They cool the chicken down, this happens when the blood circulates from the comb to the wattles. The circulating blood lowers the temperature of the chicken during the hot weather

2.3.1.4 Comb length

Comb size and color appear to be most important in mate choice decisions by hens (Von Schantz *et al.*, 1995). Hens may mate with several roosters and choose males based on a composite assessment of multiple secondary sexual characteristics, including bright red combs and wattles (Zuk, *et al.*, 1990). McGary *et al.* (2003) showed in one strain of broiler breeders that roosters with wider combs produced sperm that were better able to reach and hydrolyze the perivitelline membrane of the ovum. Previous studies show positive relationships between comb size and characteristics of sperm quality, however, these results are strain specific (Gebriel *et al.*, 2009; El Ghany *et al.*, 2011 & Udeh *et al.*, 2011). However in 3 studies, no relationship was found between comb size and measures of sperm quality, such as sperm concentration or mobility (Pizzari *et al.*, 2004; Bilcik and Estevez 2005; Baczynski 2008), and in one, comb size was negatively related to sperm movement

(Parker *et al.*, 2006). Thus, the nature of the relationship between comb size and sperm quality remains unclear in domestic chickens.

2.4 SEMEN EVALUATION

2.4.1 Cock semen

The sperm of the domestic birds are long, cylindrical, and tapered at both ends. The spermatozoa contain an acrozone, a head, a midpiece and a tail. Chicken semen is highly concentrated and is of low volume.

2.4.2 Semen collection

The abdominal massage technique as described by Burrows and Quinn (1937) was used to collect semen. This method entails firmly stroking down the middle of his back to the end of his tail with one hand. Once the bird is comfortable and relaxed, begin massaging the pubic bone between his legs. The copulatory (sexual) organ will become erect and extrude outside of the body, and semen will flow from the phallus of the bird (Donoghue & Wishart, 2000). During semen collection care must be taken to minimize any possible contamination by faeces or urine. Semen samples are discarded if contaminated with urine or faeces material (Blanco *et al.*, 2000; Han *et al.*, 2005).

The most widely used test of sperm quality from the initial stages of AI development until the present time has been the assessment of the proportion of normal, progressively moving sperm (Anderson, 1945; Maule, 1962; Salisbury *et al.*, 1978). Ejaculate volume and sperm concentration are the two other critical components of semen evaluation because they determine the number of sperm obtained. Rapid optical density methods for measuring sperm concentration have replaced tedious hemocytometric procedures. Fertility of sperm is the ultimate test of sperm quality.

Semen evaluation includes assessment of volume, colour, pH, concentration, motility and morphology (WHO, 1999). Daniel, (2008) reported the poultry average semen volume of (0.25 ml), pH (7.0), sperm concentration ($118.8 \times 10^9/\text{ml}$). The sperm motility of 81% has also been reported on Philippine native chicken semen (Baguio & Capitan; 2008). In addition, the sperm morphology defects of 7.0 were recorded on indigenous Iran chickens by (Tabatabaei *et al.*, 2009). Sperm survival is defined as the ability to maintain motility rate over time (Coccia *et al.*, 1997). Reproductive performance is critical to able production in poultry and appropriate selection criteria for males based on semen characteristics have been submitted in chickens (McDaniel *et al.*, 1998). To avoid losses in fertility, proper evaluation of semen prior to artificial insemination (AI) or storage is very important (Hammerstedt, 1992). In addition, semen evaluation is extremely important for *in vitro* semen storage and AI. Furthermore, method of semen evaluation which is rapid, economical, objective and strongly predictive of fertility would be beneficial to the poultry industry (Dumpala *et al.*, 2006). Sperm motility rate is an essential factor in the maintenance of fertility (King *et al.*, 2000).

Sperm morphology using nigrosin/eosin staining for live and dead staining of fowl sperm have been reported (Siudzinska, & Lukaszewicz, 2008; Chalah *et al.*, 1999). Sperm morphology has become a basic part of daily semen analysis (Garcia-Herreros *et al.*, 2006). Ejaculated semen with poor sperm morphology is an indicator of decreased fertility in animals and men (Bostofte *et al.*, 1982; Carrel *et al.*, 1994). The few such studies performed to date have not produced consistent relationships between semen analysis results and pregnancy rates, in either sub fertile or general population (Garrett *et al.*, 2003). One of the criteria for a staining method must be

that, the process involved should cause a little change to sperm morphology as possible (Van der Horst & Maree, 2009:iFirst).

2.5 MACROSCOPIC SEMEN EVALUATION

2.5.1 Semen volume: The semen ejaculated was measured by using the graduated tube (falcon[®] 352099, USA).The cockerel produces between 0.1 ml and 1.5 ml per ejaculation, with 0.6 ml being the average ejaculate volume recorded. Different cockerels of the same species often produce different volumes of semen at different times (Anderson, 2001).The average volume ejaculated using the abdominal massage technique is approximately 0.25ml and contains on average 5000×10^6 sperm/ml (Gordon, 2005). It is important to realize that semen volume multiplied by sperm concentration will determine the total number of sperm collected per ejaculation. This could facilitate the determination of the number of insemination doses that can be prepared (Senger, 2003).

2.5.2 Semen pH: Semen pH was measured by inserting the pH meter (Hanna instruments, Portugal) electrode into the semen. The semen pH has been found to vary slightly between different breeds and bird species. Donoghue and Wishart (2000) or Siudzinska and Lukaszewicz (2008), reported that chicken sperm can tolerate a pH ranging from 6.0 to 8.0.

2.6 MICROSCOPIC SPERMEVALUATION

The following techniques were used to microscopically and morphologically evaluate sperms: Swim-up technique and stained and smears sperm technique.

2.6.1 Semen concentration: The sperm concentration is usually defined as the number of sperm per ml of the ejaculated and can normally predict the number of breeding units that can be inseminated (Cole and Cupps, 1977). The colour of semen may depend on the species of bird used, but generally semen should be creamy which indicates a high sperm concentration (Cole & Cupps, 1977). Sperm concentration was measured using the JENWAY 6310 spectrophotometer. The wavelength was set to 650 nm. Add 3 ml of a 2.9% Sodium Citrate solution (pH 7.0) together with 15µl of semen in a cuvette, The absorbance was then converted to sperm concentration, this is the formula used: $(11,170 \times \text{Absorbance}) - 90$, and it was recorded in billion sperm/ml ($\times 10^9/\text{ml}$, Knox *et al.*, 2000). Read the absorbance as soon as the reading stabilizes. This takes about 5 - 10 seconds. Figure 2.5 spectrophotometer.



Figure 2.5: Spectrophotometer

2.6.2 Sperm motility: Visual scoring of sperm motility by microscopy needs only a microscope and microscope glass-slides. However, microscopy provides a very subjective measure of motility that can be greatly influenced by the user. By attaching a video camera to the microscope it is possible to determine the percentage of sperm that are motile in a sample and decrease subjectivity (McDaniel

et al., 1998). The development of the computer assisted sperm analysis[®] (CASA[®]) system used for providing quantitative data on sperm motility, provides more objective sperm motility parameters such as non-progressive motility (NPM), progressive motility (PM), linearity (LIN), velocity on the straight line (VSL), velocity on the average path (VAP), straightness (STR) etc. In addition, it reduced the technical variations (Lavara *et al.*, 2008). Sperm motility analysis on semen quality that is repeatable and is linked to fertility and quality of semen is essential (Bratton *et al.*, 1956). Remarkable advances have been made with the aid of CASA[®] system in the evaluation of various sperm characteristics (Tardif *et al.*, 1997). Sperm motility, velocity and morphometry parameters from different species such as pigeon (Sontakke *et al.*, 2004), rat (Cancel *et al.*, 2000), goat (Sundararaman, & Edwin, 2008), ram (Mortimer, & Maxwell, 2004), turkey (King *et al.*, 2000) and human (Sloter *et al.*, 2006) have been examined by CASA[®] system.

2.6.3 Preparation of sperm swim-up

Collected semen was diluted slowly with BO-wash medium developed by Bracket and Oliphant in 1975 was prepared fresh in Protocol 1 and warmed to 40 °C on a warm plate (Precision Scientific Company, Chicago 47, USA). During semen dilution, 500 µl of BO-wash medium and 10 µl of fresh semen was poured into 15 ml tube (falcon[®] 352099, USA). The tube was then kept for 2-3 minutes at room temperature, and then transferred at the refrigerator (Kelvinator) adjusted at 5°C for 5-6 minutes. Five micro litre of diluted semen were placed on the warm glass slide (~76 x 26 x 1mm, Germany) and placed with a warmed cover slip (22 x 22 mm, Germany) over the microscope-warm plate (Omron) adjusted at 37°C. The sperm motility parameters were evaluated by CASA[®]-Sperm Class Analyser[®] (Microptic, Spain) at

the magnification of 10x (Nikon, China). Before the track sequence was to be analysed or saved, the trajectory of each single sperm identified and captured in the field was then visually assessed to eliminate possible debris and to diminish the risk that unclear tracks were included in the Microsoft-excel sheet of the analysis. The Sperm Class Analyser[®] system which is Computer Assisted Sperm Analysis (CASA) was used to analyse sperm motility parameters. Figure 2.6 Sperm Class Analyser.

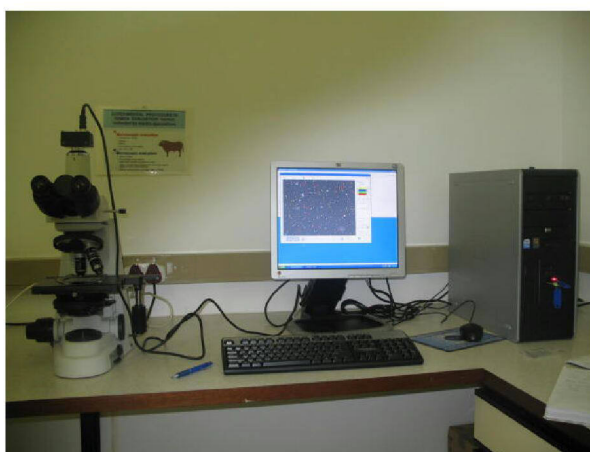


Figure 2.6: Sperm Class Analyser

The Brackett and Oliphant sperm washing solution (BO-WASH) as originally described by Bracket & Oliphant (1975) was prepared in three steps as follows:

Table 2.7: Preparation of Solution A (10X BO stock solution)

Components	Molecular Weight	mM	g/100ml
NaCl	58.44	112.00	6.5453
KCl	74.56	4.02	0.2997
NaH ₂ PO ₄ .H ₂ O	137.99	0.83	0.1145
MgCl ₂ .6H ₂ O	203.30	0.52	0.1057
CaCl ₂ .2 H ₂ O	147.02	2.25	0.3308
Glucose	181.16	13.90	2.5181

Antibiotics

(Penicillin) or

Streptomycin	50mg/ml	5 ml
0.5 % Phenol Red		1.5 ml
Ultra-pure water		Add up to 100 ml
(Sabax)		
Total volume		100 ml

Filter

Store in 4° C

The solution was filtered using craft suction unit (Rocket) and a 250ml filter system.

In the preparation of the BO stock solution A, it is important that the $MgCl_2 \cdot 6H_2O$ and $CaCl_2 \cdot 2H_2O$ be dissolved in water, separately. Water must be added to the tube first, and then the components are added.

Table 2.8: Preparation of solution B (1xBO working solution)

Components	Molecular Weight	mM	g/100ml
NaHCO ₃	84.01	37.0	0.3108
Na-Pyruvate	110.04	1.25	0.0138
BO stock solution A			10 ml

Add water to 100 ml

Store in 4° C

Table 2.9: Preparation of Sperm Washing Solution (BO-WASH with a maximum storage time of 4 weeks)

Components	Molecular Weight	mM	g/100ml
Caffeine	194.20		0.1553
BSA			0.2400

Take out 80 ml of solution B

80 ml

Add water to 100 ml

Store in 4° C

2.6.4 Assessment of kinetics and motility of sperm

Velocity on the curve line (VCL): ($\mu\text{m/s}$), velocity over the actual point to point sperm track, which include all deviations of sperm head movement (King *et al.*, 2000; Holt, & Palomo, 1996; Slotter *et al.*, 2006; Kozdrowski *et al.*, 2007).

Velocity on the average path (VAP): ($\mu\text{m/s}$): velocity over a calculated, smoothed path, measures the sperm head along its spatial average trajectory (King *et al.*, 2000; Holt, & Palomo, 1996; Slotter *et al.*, 2006; Kozdrowski *et al.*, 2007).

Velocity on the straight line (VSL): ($\mu\text{m/s}$): velocity over the straight line distance between the beginning and last detected position of the sperm track (King *et al.*, 2000; Holt, & Palomo, 1996; Slotter *et al.*, 2006; Kozdrowski *et al.*, 2007).

Linearity (LIN): %: the straight line distance divided by the incremental deviations along the actual path, is calculated as $\text{VCL/VAP} \times 100$ (King *et al.*, 2000; Holt, & Palomo, 1996; Slotter *et al.*, 2006; Kozdrowski *et al.*, 2007).

Straightness (STR):%: is the linearity of the sperm average path calculated as $\text{VSL/VAP} \times 100$ (Slotter *et al.*, 2006; Kozdrowski *et al.*, 2007).

Beat Cross Frequency (BCF): (Hz): is the time average rate at which the curvilinear sperm track crosses its average path in either direction (Sloter *et al.*, 2006; Sundararaman & Edwin 2008).

Amplitude of lateral head displacement (ALH): (μm): is the maximum lateral displacement of a sperm head about its spatial average trajectory (Sloter *et al.*, 2006).

Wobble (WOB):% It indicates the wavering of the curvilinear trajectory upon the mean trajectory and it was calculated as $\text{WOB} = \text{VAP} \times 100 / \text{VCL}$ (Schmidt, & Kamp, 2004; Somi *et al.*, 2006).

Progressive motility (PM) %: These are sperm that are moving forward. It is the sperm with progressive movement i.e. with VAP of $75 \mu\text{m}/\text{second}$ and STR of 80% and rapid proportion of rapidly moving sperm (Kozdrowsiki *et al.*, 2007).

Non- Progressive motility (NPM) %: Sperm those are not moving in a straight line.

Total motility (TM) %: is the ratio of motile cells to the total cell concentration expressed as percentage (Kathiravan *et al.*, 2008).

Slow%: These are sperm that are moving at $< 10 \mu\text{m}/\text{second}$.

Medium%: These are sperm that are moving at $< 50 \mu\text{m}/\text{second}$.

Rapid%: These are sperm that are moving at $< 100 \mu\text{m}/\text{second}$.

The kinematic values recorded for each sperm included, in addition to the 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 2.10).

Table 2.10 Sperm Class Analyser[®] (V.5.0) settings used to analyse the sperm motility and velocity characteristics

Parameters	Settings
Contrast	169
Brightness	470
Image/second	50
Optic	pH-
Chamber	Cover slide
Scale	10x
Particle area	5<190 μm^2
Slow	<10 $\mu\text{m/s}$
Medium	<50 $\mu\text{m/s}$
Rapid	<100 $\mu\text{m/s}$
Progressivity	>70% of straightness
Circular	<50% of linearity
Connectivity	18
Velocity on the average path line	7 $\mu\text{m/s}$
Number of images	30

2.7 SPERM MORPHOLOGY

An important part of any breeding soundness exam is an evaluation of sperm morphology. Sperm morphology assessment is one of the most important criteria for determining the quality of a semen sample for humans and animals (WHO1999). In the most fundamental case, the size and shape of the head, midpiece and tail are examined. Moreover many claims have been made about the relation between sperm morphology assessment and fertility (Menkveld *et al.*, 2003). Sperm from different species vary in size and shape and the heads of chicken sperm are spindle-shaped and almost difficult to distinguish from the midpiece. The accuracy of sperm morphology assessment depends on careful preparation, fixation and staining of sperm (Garcia-Herreros *et al.*, 2006), because this procedures can affect sperm dimensions significantly. Sperm morphology seems to be one of the most important qualitative characteristics of semen (Kuster *et al.*, 2004). It may be a basic indicator for predicting the fertilizing ability of spermatozoa, based upon correlation between particular categories of spermatozoa and fertility results (Łukaszewicz, 1988). Success in assessment of sperm morphology depends on stain preparation techniques, stains type and staining methods (Bilgili *et al.*, 1985).

Eosin–nigrosin dye is commonly used in laboratory stain which allows analysis of sperm structure (Bjorndahl *et al.*, 2003). When stained smears are viewed under the oil immersion objective of light microscope, the percentage of viable, live, properly formed spermatozoa, nonviable and also partially-damaged spermatozoa can be determined. Live, normal spermatozoa possess an intact plasma membrane which protects them from penetration of eosin. Under the microscope these spermatozoa appear white, unstained against the purple background of nigrosin. Dead and

damaged spermatozoa have a permeable plasma membrane, which enables eosin penetration of the cell to stain internal organelles pink (Bakst and Cecil, 1997). Live spermatozoa can be categorized into different forms, for example morphologically normal, or with defects in the acrosome (crooked or loose), head (bulb, small, enlarged, looped), Neck (broken at different angle in relation to head), midpiece and tail (swelling, looping, partial or totally lacking) (Blom, 1981 and Edens *et al.*, 1973).

2.7.1 Morphology analysis

Sperm viability is determined by simple microscopic examination combined with nigrosin-eosin stain. Live viable sperm do not take up the pink-coloured eosin stain, remaining white. Dead sperm take up the eosin, and appear pink. When viewed under the microscope at 80-100X magnification, a field of view is measured for live vs. dead sperm (Umesiobi and Iloeje, 1999). At the same time, live abnormal sperm can be counted because these will also probably be incapable of fertilization.

Nigrosin-eosin stain should be prewarmed to body temperature. Pipette a drop of stain onto the end of a slide, and then pipette a small droplet of semen next to the stain. Place the edge of another slide into the drops of stain and semen rock that slide back and forth a few times to mix the sperm and stain, and then smear the second slide across the surface of the first. Dry the slide rapidly by placing in on a warming plate or waving it back and forth in the air. Examine using a bright field microscope (typically using a 40-100X objective lens). The nigrosin-stain stain produces a dark background on which the sperm stand out as lightly colored objects. Normal live sperm exclude the eosin stain and appear white in color, whereas "dead"

sperm (i.e. those with loss of membrane integrity) take up eosin and appear pinkish in colour. The cell morphology was evaluated with a fluorescent microscope (BX51 TF®, Olympus, Japan). Figure 2.7 Fluorescence microscope.



Figure 2.7: Fluorescence microscope

2.7.2 Smears preparation for sperm morphology

At least two slides were prepared per cock for each semen sample. Thereafter, 7 μ l of semen was mixed with 20 μ l of the nigrosin-eosin staining solution into the 0.6 ml micro centrifuge graduated tube (Simport, Canada). A drop of 5 μ l of diluted semen sample was then placed on the clear end of a microscope slide and dragging the drop across the slide (Rubio-Guillen *et al.*, 2007). The resulting smears were allowed to air dry at room temperature before evaluation. The pH of nigrosin-eosin staining solution was adjusted to pH 8.45 and osmolarity to 387. The results of morphological

evaluation will be expressed as the percentage of particular categories of spermatozoa (200 cells = 100%).

2.8 ARTIFICIAL INSEMINATION

Assisted reproduction technologies (ART's), such as artificial insemination (AI) which encompasses the AI deposition of semen in the hen's reproductive tract may contribute to increase poultry production, as it allows a wider use of genetically superior cockerels with a high productive performance. On the other hand, ART's have the potential benefit of allowing the preservation of semen collected from these cockerels for future use and for export if necessary. This is the most commonly used AI procedure and two people are required for this operation. The placement of semen into the hen's reproductive tract is accomplished by inverting the cloaca of the hen to expose the opening of the vagina. The insemination pipette is inserted and sperm delivered to a location near the SST. The cloaca is everted by holding the thighs of the hen between the thumb and forefinger while the body rests in the palm of the left hand (Etches, 1996). Hens have to be inseminated for two consecutive days for the first time, and thereafter once a week, if fertile eggs are required. Cockerel semen has a limited life outside the body, and must be deposited in the hen within 1h of semen collection from the cockerel, in the case of fresh semen. It is best to inseminate hens in the afternoon (14:00 and 16:00) as in the morning hens may have an egg in the oviduct, making it difficult for the sperm to swim up to the ovary. Eggs are normally fertile after the second day of insemination, and can remain fertile for 2 or more weeks (Martin, 2004). It has been shown that hens inseminated with fresh semen produce more fertile eggs than hens inseminated with frozen/thawed sperm (Blanco *et al.*, 2000). The fertility of the eggs can also be

influenced by the transport of sperm. The artificially inseminated pullets will be examined for egg production, fertility (percentage fertilized) and hatchability (percentage hatched) in accordance with the procedures of Molekwa and Umesiobi (2008).

2.8.1 History of Artificial Insemination in chickens

By 1907 Ivanow had studied Artificial Insemination (AI) in domestic farm animals, dogs, foxes, rabbits, and poultry. Artificial insemination has been widely applied to poultry. Semen collection, processing, and AI have been reviewed by Sexton (1979) and Lake (1986) and more recently by (Donoghue & Wishart 2000). Burrows and Quinn (1937) developed the method of abdominal massage and pressure to collect semen. With the ease of collecting poultry semen, and proximity of hens on large breeding farms, AI is used extensively with freshly collected semen. It is used 100% for turkey breeding because mating is difficult. Freshly collected chicken semen was among the first type of semen to be frozen (Shaffner *et al.*, 1941; Polge *et al.*, 1949). However, cryopreserved poultry sperm are less fertile than fresh semen and freezing poultry sperm still is experimental (Gill *et al.*, 1999). Brillard (2003) also recommended that the period of artificial insemination in a given poultry breeder house should not be done around the time of lay for maximum insemination results.

2.8.2 Methods of Artificial Insemination

Artificial insemination technique involves the artificial injection of sperm-containing semen from a male into a female to cause pregnancy. Artificial insemination is often used in animals to multiply the possible offspring of a prized animal and for the breeding of endangered species. Artificial insemination is a vital component of

commercial poultry production. In AI, semen collected from one cock is used to inseminate 20-30 breeder hens, in a ratio of 1 cock to 8-10 hens in natural mating (Bayyari *et al.*, 1990). Intra-uterine insemination produced better fertility than intra-vaginal insemination when breeding quail by artificial insemination (Wentworth & Mellen, 1963; Ogasawara & Huang, 1963). These advantages of artificial insemination include increased number of settable eggs, better overall fertility and hatchability, thus reducing the cost of production per unit of day-old chicks (Brillard, 2003). Several factors must be synchronized for optimum success of artificial insemination and they include breeder stock management, sperm quality and quantity, sperm dosage, depth of insemination, frequency and timing of artificial insemination (Lake, 1978; Van Krey & Siegel, 1980; King *et al.*, 2002). Variations among AI techniques, such as depth of insemination, can greatly influence the rate of fertility. Lorenz *et al.* (1959) recommended deep semen deposition, whereas Rooney *et al.* (1966) found no fertility differences when inseminating tom turkeys and Bronze hens at 1.27 cm or 5 cm.

2.8.3 Fertility rate following Artificial Insemination

Fertility and hatchability are two important factors used to determine the reproductive performance of birds. Orunmuyi *et al.* (2007), Bakst *et al.* (1994) found that, fertility and the number of sperm stored in the hen's oviduct increases with increasing numbers of sperm inseminated. However, the magnitude of this increase is most likely influenced by semen quality. It has been estimated that a weekly artificial insemination dose of 50 to 300 million sperm is needed to maintain fertility in turkeys and chickens (Bakst *et al.*, 1994). To maximize fertility in broiler breeders, Eslick and McDaniel (1992) suggested an insemination dose of 150 million sperm every 5 days.

It is possible that lower insemination doses could be used if semen quality is high. Freshly collected cock semen is normally diluted to obtain the desired spermatozoa concentration for multiple inseminations or for short-term and long-term storage.

2.9 HATCHABILITY

Hatchability refers to the percentage of eggs hatched into viable chicks after 21 days of incubation. Hatchability is either determined on the basis of all eggs set (% hatch) or on the basis of fertile eggs set after candling (% hatchability) (Orunmuyi *et al.*, 2007). To maximize hatch of total eggs set after AI and efficient use of sires, semen of the highest quality is essential (Hammerstedt, 1996).

Hatchability = $100 \text{ [number of chicks hatched]/number of fertile eggs set}$.

2.9.1 Hatchability Traits

Hatching eggs were collected twice a day (8:00-11:00 and 14:00-17:00 h) and marked for individual cock and hen two days after first insemination. Good shaped and sound shell eggs were weighed and dipped in powerful disinfectant solution (Virkon S Disinfectant®, UK). Then eggs were stored over a period of one week in a cool room at 15 to 17 °C and 75 to 80% relative humidity (RH). Proper cleaning, disinfection and fumigation were conducted before setting of eggs. Eggs from a pullet were set adjacent to each other on the same tray. Numbers of eggs set for each individual sire-pullet group of breed were recorded. The eggs were turned by an automatically programmed device at 2 hourly bases. The following temperature and humidity were maintained during incubation period:

- A) Setting temperature 37.7 °C up to 18 days of incubation.
- B) Hatching temperature 0.5 -1°C reduced and 36.5 from 18 to 21 days of

Incubation

- C) Setting relative humidity: 80-85% up to 18 day of incubation.
- D) Hatching relative humidity: 2-5% increased and 87-90% relative humidity from 18 to 21 days of incubation and relative humidity increased to 92% particularly at 21 day.

The eggs were transferred from the setting trays to different pedigree compartment of hatching trays according to the breed and replication in the afternoon of the 18 day of incubation.

2.9.2 Management of day old chickens

On the day 21, the number of hatched chicks including the normal, weak, abnormal chicks and dead chicks after hatch and those the unhatched eggs and pips were counted separately as dead in shell according to breeds and replication were recorded. The chicks, which were undersized, poorly feathered, parrot beaked, micromelia, blind, lameness, open navel etc. was considered as abnormal chicks. After discarding all abnormal chicks the rest of the chicks were considered as normal. The weights of all hatching eggs were taken in gram by using an electronic digital balance (Indiamart® Vernier Caliper, Hong Kong). Then average was calculated. Calculation were made of fertility, hatchability, embryonic mortality, dead in germ, dead in shell, abnormal chicks hatched, normal chicks hatched. The weights of day-old chicks were taken in gram by using an electronic digital balance and then averages were calculated. Hatchery sanitation was strictly maintained during the experimental period.

REFERENCES

- Aganga, A.A., Omphile, U.J., Malope, P., Chabanga, C.H., Motsamai, G.M. & Motsumi, L.G., 2000. Traditional poultry production and commercial broiler alternatives for small-holder farmers in Botswana. *Livestock Research for Rural Development*. (12) 4. <http://www.cipav.org.co/lrrd/lrrd12/4/aga124a.htm>.
- Anderson, J., 1945. The Semen of Animals and its Use for Artificial Insemination. Tech. Comm. Imperial Bureau of Animal Breeding Genetics, Edinburgh.
- Anderson, J., 2001. The semen of animals and its use for artificial insemination. Green world publishers India.
- Baczynski, K., 2008. The effect of sperm mobility phenotype on fertility persistence in layer and broiler hens. College Park (MD): University of Maryland.
- Baguio, S.S. & Capitan, S.S., 2008. Motility, livability and fertility of cock spermatozoa as influenced by day of collection, diluent and cryopreservation. *Journal of Veterinary Medicine*. 45, 109-117.
- Bakst, M.R. & Cecil, H.C., 1997. Techniques for semen evaluation, semen storage, and fertility determination. *Bakst and Cecil editors. Published by the Poultry Science Association, Inc., Savoy, Illinois*.
- Bakst, M.R., Wishart, G.J. & Brillard, J.P., 1994. Oviductal sperm selection, transport and storage in poultry. *Poultry Science Review*. 5, 117-143.
- Bayyari, G.R., Cook, J.R., Harris, G.C., Macy, L.B., Slavik, M.F. & Skeeles, J.K., 1990. Research note: The evaluation of chicken spermatozoa using fluorescent staining in a 96 well format. *Poultry Science*. 69, 1602-1605.

- Bilcik, B. & Estevez, I., 2005. Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. *Applied Animal Behavioural Science*. 92, 307–323.
- Bilgili, S.F., Renden, J.A. & Sexton, K.J., 1985. The influence of staining techniques and examiners on evaluation of the morphology of fowl spermatozoa. *Poultry Science*. 64, 2358–2361.
- Bjorndahl, L., Soderlund, I. & Kvist, U., 2003. Evaluation of the one-step eosin nigrosin staining technique for human sperm vitality assessment. *Human Reproduction*. 18, 813–816.
- Blanco, J.M., Gee, G., Wildt, D.E. & Donoghue, L., 2000. Species Variation in Osmotic Cryoprotectant, and Cooling Rate Tolerance in Poultry, Eagle and Peregrine Falcon Spermatozoa. *Biology of Reproduction*. 63, 1164–1171.
- Blanco, J.M., Wildt, D.E., Hofle, U., Voelker, W. & Donoghue, A.M., 2009. Implementing artificial insemination as an effective tool for ex situ conservation of endangered avian species. *Theriogenology*. 71, 200–213.
- Blesbois, E., Seigneurin, F., Grasseau, I., Limouzin, C., Besnard, J., Gourichon, D., Coquerelle, G., Rault, P. & Tixier-boichard, M., 2007. Semen cryopreservation for ex situ management of genetic diversity in chicken: creation of the French avian cryobank. *Poultry Science*. 86, 555–564.
- Blom, E., 1981. Physiology and pathology of reproduction. *Medycyna Weterynaryjna*. 4, 239–242 (in Polish).
- Bostofte, E., Serup, J. & Rebbe, H., 1982. Relation between morphologically abnormal spermatozoa and pregnancies obtained during a twenty year follow-up period. *International Journal of Andrology*. 5, 379–386.

- Brackett, B.G. & Oliphant, G., 1975. Capacitation of rabbit spermatozoa invitro. *Biology Reproduction*. 12, 260–74.
- Bratton, R.W., Foote, R.H., Henderson, C.R., Musgrave, S.D., Dunbar, R.S., Dunn, H.O. & Beardsley, J.P., 1956. The relative usefulness of combinations of laboratory tests for predicting the fertility of bovine semen. *Journal of Dairy Science*. 39, 1542.
- Brillard, J.P., 2003. Practical aspects of fertility in poultry. *World's Poultry Science Journal*. 59, 441-446.
- Burrows, W.H. & Quinn, J.P., 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*. 16, 185-191.
- Cancel, A.M., Lobdell, D., Mendola, P. & Perreault, S.D., 2000. Objective evaluation of hyperactivated motility in rat spermatozoa using computer-assisted sperm analysis. *Human Reproduction*. 6, 1322-1328.
- Carrel, D.T., Zobell, R.L., Middleton, R.G. & Urry, R.L., 1994. A functional analysis and the potential clinical significance of 7 categories of sperm morphology. *Journal of Urology*. 151, 376-380.
- Chalah, T., Seigneurim, F., Blesbois, E. & Brillard, J.P., 1999. In vitro comparison of fowl sperm viability in ejaculates frozen by three different techniques and relationship with subsequent fertility in vivo. *Cryobiology*. 39, 185-191.
- Coccia, M.E., Becattini, C., Criscuoli, L., Fuzzi, B. & Scarselli, G., 1997. A sperm survival test and in vitro fertilization outcome in the presence of male factor infertility. *Human Reproduction*. 12, 1969-1973.
- Cole, H.H. & Cupps, P.T., 1977. Reproduction in domestic animals. 3rd ed. New York. Academic press.

- Dohner, J.V., 2001. The encyclopedia of historic and endangered livestock and poultry breeds. Yale Univ. Press, New Haven, CT.
- Donoghue, A.M. & Wishart, G.J., 2000. Storage of poultry semen. *Animal Reproduction Science*. 62, 213-232.
- Dumpala, P.R., Parker, H.M. & McDaniel, C.D., 2006. The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen. *International Journal of Poultry Science*. 5, 838-845.
- Edens, F.W., Van Krey, H.P. & Siegel, P.B., 1973. Selection for body weight at eight weeks of age 10. Spermatozoa morphology. *Poultry Science*. 52, 2287–2289.
- El Ghany, F.A.A., El Dein, A., Soliman, M.M., Rezaa, A.M., & El Sodany, S.M., 2011. Relationships between some body measurements and fertility in males of two local strains of chicken. *Egyptian Poultry Science*. 31, 331–349.
- Eslick, M.I. & McDaniel, G.R., 1992. Interrelationships between fertility and hatchability of eggs from broiler breeder hens. *Journal of Applied Poultry Research*. 1, 156–159.
- Etches, R.J., 1996. Reproduction in farm animals. London: Wallingford Publishers.
- Food and Agricultural Organization., 2006. Globalization of livestock markets biggest single factor in erosion of farm animal diversity.
- Fourie, C. & Grobbelaar, J.A.N., 2003. Indigenous poultry breeds. Wing Nut. Krugersdorp. Feb:20-21. Wing Nut Publications.
- Garcia-Herreros, M., Aparicio, I.M., Baron, F.J., Garcia Marin, L.J. & Gil, M., 2006. Standardization of sample preparation, staining and sampling methods for automated sperm head morphometry analysis of boar spermatozoa. *International Journal of Andrology*. 29, 553-563.

- Garrett, C., Liu, D.Y., Clarke, G.N., Rushford, D.D. & Baker, H.W.G., 2003. Automated semen analysis: 'zona pellucidia preferred' sperm morphometry and straight-line velocity are related to pregnancy rate in subfertile couples. *Human Reproduction*. 18, 1643-1649.
- Gebriel, G.M., Kalamah, M.A., El-Fiky, A.A. & Ali, A.F.A., 2009. Some factors affecting semen quality traits in Norfa cocks. *Egyptian Poultry Science*. 29, 677-693.
- Gill, S.P., Hammerstedt, R.H. & Amann, R.P., 1999. Poultry artificial insemination: Procedures, current status and future needs. In: Proc. Annu. Mtg. Soc. Nashville. *Theriogenology*. 353-362.
- Gordon, I., 2005. Reproductive technologies in farm animals. CABI Publishing UK.
- Grobbelaar, J.A.N., 2008. Egg production potentials of four indigenous chicken breeds in South Africa. M-Tech dissertation, Pretoria, Tshwane University of Technology.
- Gueye, E.F., Ndiaye, A. & Branckaert, R.D.S., 1998. Prediction of body weight on the basis of body measurements in mature indigenous chickens in Senegal. LRRD 10: <http://www.cipav.org.co/lrrd10/3/sene103.htm>.
- Hammerstedt, R.H., 1992. Artificial insemination using extended liquid semen: An old technology of great value to modern industry. In: Proceedings of 41st National Breeders Roundtable. Poultry Breeders of America, St. Louis, MO. pp. 163-186.
- Hammerstedt, R.H., 1996. Evaluation of sperm quality: Identification of sub fertile male and courses of action. *Animal Reproduction Science*. 42, 77-87.

- Han, X.F., Liu, F.Z., Nilu, Z.Y. & Yang, C.S., 2005. Effects of diluents, cryoprotectants, equilibration time and thawing temperature on cryopreservation of duck semen. *Poultry Science*. 4, 197-201.
- Holt, W.V. & Palomo, M.J., 1996. Optimization of a continuous real-time computerized semen analysis system for ram sperm motility assessment, and evaluation of four methods of semen preparation. *Reproduction Fertility and Development*. 8(2), 219-30.
- Ivanow, E.I., 1907. De la fe'condation artificielle chez les mammife`res. *Archives Science and Biology*. 12, 377–511.
- Kathiravan, P., Kalatharan, J., Edwin, M.J. & Veerapandian, C., 2008. Computer automated motion analysis of crossbred bull spermatozoa and its relationship with *in vitro* fertility in zona free hamster oocytes. *Animal Reproduction Science*. 104, 9-17.
- King, L.M., Brillard, J.P., Garret, W.M., Bakst, M.R. & Donoghue, A.M., 2002. Segregation of spermatozoa within sperm storage tubules of fowl and turkey hens. *Reproduction*. 123, 79-86.
- King, L.M., Holsberger, D.R. & Donoghue, A.M., 2000. Correlation of CASA velocity and linearity parameters with sperm mobility phenotype in turkeys. *Journal of Andrology*. 21, 65-71.
- Knox, R.V., Tudor, K.W., Rodriguez-Zas, S.L. & Robb, J.A., 2000. Effect of subcutaneous versus intramuscular administration of PG600 on estrual and ovulatory responses of prepubertal gilts. *Journal of Animal Science*. 78, 1732-1737.

- Kozdrowski, R., Dubiel, A., Bielas, W. & Dzięcioł, M., 2007. Two Protocols of Cryopreservation of Goat Semen with the Use of Computer-Assisted Semen Analysis System. *Acta Veterinaria Brno*. 76, 601-604
- Kuster, C.E., Singer, R.S. & Althouse, G.C., 2004. Determining sample size for the morphological assessment of sperm. *Theriogenology*. 61, 691–703.
- Lake, P.E. & Stewart, J.M., 1978. Artificial insemination in poultry. Biochemistry of the domestic fowl. Academic Press London, pp, 1411-1425.
- Lake, P.E., 1986. The history and future of the cryopreservation of avian germplasm. *Poultry Science*. 65, 1–15.
- Lavara, R., Vicente, J.S., Marco-Jimenez, F. & Baselga, M., 2008. Correlation between CASA and ASMA parameters in rabbit semen. *Reproduction 9th World Rabbit Congress*.381-385.
- Lerner, I. M., 1937.Shank length as a criterion of inherent size. *Poultry Science*. 16, 213–215.
- Long, J.A., 2008. Reproductive biotechnology and gene mapping: tools for conserving rare breeds of livestock. *Reproduction in Domestic Animal*. 43, 83-88.
- Lorenz, F.W., Abott, W.K., Asmundson, V.S., Alder, H.A., Kratzer, F.H., Ogasawara, F.X. & Carson, J.D., 1959. Turkey fertility. California, Exptl. Stn. Cir 472.
- Lucas, A.M. & Stettenheim, P.R., 1972. Avian anatomy-Integument.Agriculture Handbook 362 Pts I and II.Washington, D.C., U.S.Government Printing Office.
- Łukaszewicz, E., 1988. Study of diluents for cock's semen storage in the light of laboratory estimation and fertility rates.Zeszyty Naukowe AR Wrocławiu, Zootechnika XXX 168: 43–59 (in Polish).

- Maloney, M.A., Gilbreath, J.C. & Morrison, R.D., 1963. Two-way selection for body weight in chickens. *Oklahoma Agr. Exp. Sta. Tech. Bull.*, T-99.
- Martin, R.D., 2004. Artificial insemination of poultry. *Poultry Essays*, Essay 14, BernalPublishing. <http://www.bernalpublishing.com/poultry/essay14.shtml>.
- Maule, J.P., 1962. *The Semen of Animals and Artificial Insemination*. Commonwealth Agricultural Bureaux, Farnham Royal, U.K.
- Menkveld, R., Huwe, P., Ludwig, M. & Weidner, W., 2003. Morphological sperm alternations in different types of prostatitis. *Journal of Andrology*. 35(5), 288–293.
- McDaniel, C.D., Hannah, J.L., Parker, H.M., Smith, T.W., Schultz, C.D. & Zumwalt, C.D., 1998. Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. *Poultry Science*. 77, 888-893.
- McGary, S., Estevez, I. & Bakst, M.R., 2003. Potential relationships between physical traits and male broiler breeder fertility. *Poultry Science*. 82, 328–337.
- Molekwa, J.T. & Umesiobi, D.O., 2008. Relationships between cock semen viability and the fertility of artificially inseminated South African indigenous chicken breeds. Published Proceedings of the 10th World Conference on Animal Production of the World Association for Animal Production, 23-28 November 2008, International Conference Centre, Cape Town.
- Molekwa, J.T. & Umesiobi, D.O., 2009. Relationships between cock semen viability and the fertility of artificially inseminated South African indigenous chicken breeds. *South African Journal of Animal Science*. 39 (supplement 1), 24-28.
- Mortimer, S.T. & Maxwell, W.M.C., 2004. Effect of medium on the kinematics of frozen-thawed rams spermatozoa. *Reproduction*. 127, 285–91.

- Naidoo, M., 2003. Indigenous poultry production systems in Northern Kwazulu-Natal, South Africa, pp. 66–73. Paper presented at the 1st National Workshop on Indigenous Poultry Development, Nature and Development Group of Africa (NGO Registration No.026-851-NPO).
- Nhleko, M.J., Slippers, S.C. & Nsahlai, I.V., 2003. A preliminary report of on-farm performance of rural chickens kept under farmers' management. Nature and Development group of Africa NGO Registration no. 026-851-NPO. Proceedings of the 1st National Workshop on indigenous poultry development:79-83.
- Njue, S.W., Kasiiti, J.L., Macharia, M.J., Gacheru, S.G., Reddy, P.M., Reddy, V.R., Reddy, C.V., Rap, P.S.P. & Mbugua, H.C.W., 2002. Health management improvement of family poultry production in Africa-Survey results from Kenya. In: Characteristics and parameters of family poultry production in Africa, IAEA Vienna.
- Nwosu, C.C., Obioha, F.C., Omeje, S.S., Gowen, F., Bellonwu, C.T. & Onuora, G.I., 1984. Growth performance of chickens in deep litter systems. *World Review of Animal Production*. 20(2), 17-26.
- Ogasawara, F. & Huang, R., 1963. A modified method of artificial insemination in the production of chicken-quail hybrid. *Poultry Science*.42, 1386.
- Orunmuyi, M., Oni, O.O., Adeyinka, I.A. & Asiribo, O.E., 2007. Genetic parameter estimates for plasma alkaline phosphatase activity and reproductive traits in two strains of Rhode Island chickens. *Asian Journal of Animal Science*. 1, 76-81.

- Parker, T.H., Thompson, D., Ligon, J.D., Schneider, B. & Byrn, F., 2006. Does red jungle fowl comb size predict sperm swimming speed and motility? *Ethology Ecology & Evolution Journal*. 18, 53–60.
- Pizzari, T., Jensen, P. & Cornwallis, C.K., 2004. A novel test of the phenotype linked fertility hypothesis reveals independent components of fertility. *Proceedings of the Royal Society's B, Biological Research Journal*. 271:51–58.
- Polge, C., Smith, A.V. & Parkes, A.S., 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature*. 164, 666-666.
- Ramsy, K., Harris, L. & Kotze, A., 2000. South Africa Landrace Breeds: South Africa's Indigenous and Locally Developed Farm Animals. Pretoria: Farm Animal Conservation Trust.
- Rooney, W.F., Ogasawara, F.X. & Ferebee, D.C., 1996. Insemination technique and timing found important factors in turkey fertility studies on a commercial farm. *California Agric*, 20: 2-4.
- Rubio-Guillén, J., González, D., Garde, J.J., Esteso, M.C., Fernández-Santos, M.R., Rodríguez-Gil, J.E., Madrid-Bury, N. & Quintero-Moreno, A., 2007. Effects of cryopreservation on bull spermatozoa distribution in morphometrically distinct subpopulations. *Reproduction in Domestic Animals*. 42, 354–357.
- Salisbury, G.W., Van Demark, N.L. & Lodge, J.R., 1978. Physiology of Reproduction and Artificial Insemination of Cattle. 2nd ed. W.H. Freeman Co., San Francisco.
- Schmidt, H. & Kamp, G., 2004. Induced hyperactivity in boar spermatozoa and its evaluation by computer-assisted sperm analysis. *Reproduction and Fertility*. 128, 171–179.

- Senger, P.L., 2003. Pathways to pregnancy and Parturition. (99164-6332) 2nd Ed. Pullman, Washington, USA
- Sexton, T.J., 1979. Preservation of poultry semen—a review. In: H. W. Hawk (ed.) Animal Reproduction. Beltsville Symposia in Agricultural Research, No. 3. pp159–170. Allanheld, Osmun & Co., Montclair, NJ.
- Shaffner, C.S., Henderson, E.W. & Card, C.G., 1941. Viability of spermatozoa of the chicken under various environmental conditions. *Poultry Science*. 20, 259–265.
- Siegel, P.B., 1962. Selection for body weight at eight weeks of age. 1. Short term response and heritabilities. *Poultry Science*. 41, 954–962.
- Suidzinska, A. & Lukaszewick, E., 2008. The effect of breed on freezability of semen of fancy fowl. *Animal Science Papers and Reports*. 4, 331-340.
- Sloter, E., Schmid, T.E., Marchetti, F., Eskenazi, B., Nath, J. & Wryobek, A.J., 2006. Quantitative effects of male age on sperm motion. *Human Reproduction*. 21(11), 2868-2875.
- Somi, S.S., Kluger, S., Knapp, E., Klein, D. & Aurich, C., 2006. Effects of semen extender and semen processing on motility and viability of frozen-thawed dog spermatozoa. *Theriogenology*. 66, 173-182.
- Sonaiya, E.B., 2003. Village poultry and its role in rural Africa. Nature and Development group of Africa NGO Registration no. 026-851-NPO. Proceedings of the 1st National Workshop on indigenous poultry development: 16-30.
- Sontakke, S.D., Umaphathy, G., Sivaram, V., Sanjiva, D., Kholkute, & Shivaji, S., 2004. Semen characteristics, cryopreservation, and successful artificial insemination in the Blue rock pigeon (*Columba livia*). *Theriogenology*. 62, 139–153.

- Sundararaman, M.N. & Edwin, M.J., 2008. Changes in motility characteristics of goat spermatozoa during glycerol-equilibration and the relevance to cryopreservation. *Asian Journal of Cell Biology*. 3(1), 22-33.
- Tabatabaei, S., Batavani, R.A. & Talebi, A.R., 2009. Comparison of oviductal sperm age on fertility, hatchability and embryonic death rates in Iranian indigenous and Ross-308 broiler breeder chickens. *Journal of Animal and Veterinary Advances*. 8(1), 85-89.
- Tadelle, D., Alemu, Y. & Peters, K.J., 2000. Indigenous chickens in Ethiopia: genetic potential and attempts at improvement. *World Poultry Science Journal*. 56, 45-54.
- Tardif, R., Charest-Tardif, G., Brodeur, J. & Krishnan, K., 1997. Physiologically based pharmacokinetic modelling of a ternary mixture of alkyl benzenes in rats and humans. *Toxicology and Applied Pharmacology*. 144, 120–134.
- Teguia, A., Ngandjou, H.M., Defang, H. & Tchoumboue, J., 2008. Study of the live body weight and body characteristics of the African Muscovy Duck (*Cairina moschata*). *Tropical Animal Health and Production*. 40, 5-10.
- Udeh, I., Ugwu, S.O.C. & Ogagifo, N.L., 2011. Predicting semen traits of local and exotic cocks using linear body measurements. *Asian Journal of Animal Science*. 5, 268–276.
- Umesiobi, D.O. & Iloeje, M.U., 1999. Effect of sexual teasing and diurnal period of semen collection on reaction time and semen characteristics of Large White boars. *Journal of Sustainable Agriculture & Environment*. 1, 231-235.
- Van der Horst, G. & Maree., 2009. Spermablue[®]: a new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis. *Biotechnical & Histochemistry*. 1-10.

- Van Krey, H.P. & Siegel, P.B., 1980. A revised artificial insemination schedule for broiler breeder hens. *Poultry Science*. 55, 725-728.
- Von Schantz, T., Tufvesson, M., Goöransson, G., Grahn, M., Wilhelmson, M., & Wittzell, H., 1995. Artificial selection for increased comb size and its effects on other sexual characters and viability in *Gallus domesticus* (the domestic chicken). *Heredity*. 75, 518–529.
- Wentworth, B.C. & Mellen, W.J., 1963. Egg production and fertility following various methods of insemination in Japanese quail (*Coturnixcoturnixjapónica*). *Journal of Reproduction and Fertility*. 6, 215.
- Woelders, H., Zuidberg, C.A. & Hiemstra, S.J., 2006. Animal genetic resources conservation in the Netherlands and Europe: poultry perspective. *Poultry Science*. 85, 216-222.
- World Health Organization., 1999. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge, United Kingdom: Cambridge University Press; 4th ed.
- Zuk, M., Johnson, K., Thornhill, R. & Ligon, J.D., 1990. Mechanisms of female choice in red jungle fowl. *Evolution*. 44, 477–485.

RELATIONSHIP BETWEEN LIVE PERFORMANCE AND TESTIS TRAITS OF SOUTH AFRICAN INDIGENOUS COCKERELS

M.B Makhafola^{1, 2, 4}, D.O Umesiobi² and T.L Nedambale^{1, 3, 5*}

¹Agricultural Research Council, Animal Production Institute, Germplasm Conservation and Reproductive Biotechnologies, Private Bag X 2, Irene, 0062

²Central University of Technology, Free State Faculty of Health and Environmental Science School of Agriculture and Environmental Science, Private Bag X 20539 Bloemfontein 9300

³Tshwane University of Technology, Department of Animal Sciences, Private Bag X 680, Pretoria 0001, South Africa

⁴University of South Africa, College of Agriculture & Environmental Science; Department of Agriculture., Animal Health & Human Ecology; Florida Campus Private Bag X 6, Florida 1710

⁵University of the Free State, Department of Animal, Wildlife and Grassland Sciences, P.O. Box 339, Bloemfontein, South Africa

3.1 Abstract

The aim of the study was to find the relationship between live performance and testis traits of South African indigenous cockerels on Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK). A total of 33 cockerels were used in this study; Ovambo (n=11), Potchefstroom Koekoek (n=11) and Naked Neck (n=11). The body weights of Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) cockerels were recorded individual at weekly intervals. The cockerels shank, comb and wattle lengths were recorded with a vernier calliper. Breed significantly ($P < 0.05$) affected the body weight of Ovambo (2.5 ± 0.4 kg), Naked Neck (2.0 ± 0.3 kg) and Potchefstroom Koekoek (2.3 ± 0.3 kg) cockerels. There were no significant differences on the shank length (SL), comb length (CL) and wattle length in all the breeds. Table 3.2, Ovambo (11.45 ± 0.9^a g) had the highest live weight of testis, it was significantly different from Naked Neck (9.2 ± 0.8^b g) and Potchefstroom Koekoek (8.9 ± 0.8^b g). For

average length of the testis there was a significant difference amongst all breeds, Ovambo (4.0 ± 0.5^b cm), Naked Neck (3.4 ± 0.3^c cm) and Potchefstroom Koekoek (3.7 ± 0.4^b cm). In conclusion, there is no correlation between live performance and testis traits. Though this study suggests that body weight and testicular length are good indicators of sperm production. Simple and indirect method of estimation is still needed to predict the fertility of the breeding cockerels.

Keywords: Ovambo, Naked Neck, Potchefstroom Koekoek, live performance, testis

*Corresponding Author: Email: lucky@arc.agric.za. Tel: +27 (0)12 672 9210. Fax: +27(0)12 665 1604.

3.2 INTRODUCTION

Poultry plays an important role in producing animal protein most effectively with the shortest possible time (Hosseinzadeh, 2010). Indigenous chicken have several valuable characteristics that are not found in “exotic” chicken, and are appropriate traditional low input/low output farming system. These indigenous chickens have a good adaptability for climate and tolerance against diseases. Therefore conservation of these genetic resources is essential and other relevant breeding strategies.

Accurate and reliable prediction equations for estimating semen characters from *in vivo* measurements are required by poultry breeders. The most accurate method for the evaluation of semen measurements are collection of semen followed by microscopical examination. However, this method is stressful for the bird, time consuming and costly. Thus, simple, reliable and indirect methods for *in vivo* estimation of semen attributes based upon the correlation between particular traits

and parameters were needed. Behavioural studies in sexual selection of red jungle fowl show female preference for males with larger combs (Zuk *et al.*, 1995; Ligon and Zwartjes, 1995). Additionally, male comb area may indicate health status. Positive correlation between wattle length, comb length and fertility indicates that the development of secondary sexual characters may convey male reproductive quality in some genetic strains (McGary *et al.*, 2003).

The reproductive organs of male chicken are intra abdominal and located on either side of the mid-line attached to the roof of the coelom. The organs consist of a pair of testis, epididymis and vas deferens which terminates in the local copulatory apparatus (Lake, 1957). The main function of the testis is sperm production via spermatogenesis, the epididymis transport and that of the vas deferens, storage of sperm for maturation. Gray (1937) reported that the right testis was 10% larger than the left. Meanwhile Latimer (1924) had reported that the left testis of leghorn cockerels below six months of age was 17% larger than the right. Heavier breeds of cocks have larger testis than lighter breeds (Parker *et al.*, 1942; Kumaran & Turner, 1949). Therefore the aim of the study was to find the relationship between live performance and testis traits of South African indigenous cockerels on Naked Neck, Ovambo and Potchefstroom Koekoek.

3.3 MATERIALS & METHODS

Experimental site

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

Production Institute campus is located at 25° 55' South; 28° 12' East and is located in the Highveld region of RSA and situated at an altitude of 1525m above sea level.

Experimental chickens

Indigenous Ovambo (n=11), Naked Neck (n=11) and Potchefstroom Koekoek cockerels (n=11) were used in this study. At the onset of the trial the males were 32 weeks of age. The cockerels had been vaccinated at hatching against Marek's disease, infectious bronchitis and Newcastle disease using live vaccines. At day 14 all cockerels were vaccinated against infectious bursal disease and the Newcastle disease virus, with live vaccines (Immuno-Vet Services CC, Johannesburg, RSA). At 32 weeks of age, all cockerels were kept in individual battery cages and fed layer commercial diet and water *ad libitum*. The cockerels were weighed once a week for 6 weeks. The experimental diet met the National Research Council (1994) requirements. The cockerels were exposed to photoperiod of 16 h followed by 8 h of darkness (16 Light: 8 Darkness). The experimental birds were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (APIEC10/04).

Measurements and observation: Body weights were recorded at 40 weeks of age. Also, shank length was determined on live birds by measuring the length of the tibiotarsus (from the top of hock joint to the foot pad) with a digital calliper. Head appendages (comb and wattle) were measured using a measuring tape as a distance between the upper and the lower point of the organ. The length, width and thickness of the testis were measured with a vernier calliper.

TABLE 3.1: The nutrient composition diet from Epol® for the chickens

Item	Diet		
	Maxi-chicks starter g/kg	Maxi-chicks grower g/kg	Maxi-chicks layers g/kg
Protein	200	150	150
Moisture	120	120	120
Fat	25	25	25
Fibre	50	70	70
Calcium	10-12	10-12	28-30
Phosphorus	5	5	5
Total lysine	10.5	5.7	7

3.4 STATISTICAL ANALYSIS

Data was analysed using the General Linear Models (GLM) procedure of SAS User's Guide, 2001.

3.5 RESULTS

The body weight of Ovambo (2.54 ± 0.41 kg) was significantly ($P < 0.05$) higher compared to the Naked Neck (2.02 ± 0.34 kg) and Potchefstroom Koekoek (2.27 ± 0.33 kg) cockerels. Data presented in Table 3.2 showed that the live performance parameters as affected by breed. There was no significant differences on the shank length (SL), comb length (CL) and wattle length (WL) of Naked Neck (8.4, 5.4 and 5.6 cm), Ovambo (8.3, 5.8 and 6.0 cm) and Potchefstroom Koekoek (7.7, 5.9 and 5.6 cm). In Table 3.3, Ovambo (11.45 ± 0.9^a g) had the highest live weight of testis, it was significantly different from Naked Neck (9.2 ± 0.8^b g) and Potchefstroom Koekoek

(8.9 ± 0.8^b g). For average length of the testis there was a significant difference amongst all breeds; Ovambo (4.0 ± 0.5^b cm) Naked Neck (3.4 ± 0.3^c cm) and Potchefstroom Koekoek (3.7 ± 0.4^b cm). For average breadth of the testis there was no significant difference amongst the breeds, Ovambo (1.9 ± 0.4^b cm), Naked Neck (1.8 ± 0.3^b cm) and Potchefstroom Koekoek (1.8 ± 0.3^b cm).

Table 3.2 Live performance traits (Mean±SD) of cockerels of NN, OV and PK

Cockerels	Live performance Traits			
(n)	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-c}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 and PK=Potchefstroom Koekoek.

Table 3.3 Testis traits (Mean±SD) of cockerels of NN, OV and PK

Testis	Weight (g)	Length (cm)	Breath (cm)
NN (left)	9.8±0.8 ^b	3.4±0.3 ^c	1.8±0.3 ^b
NN (right)	8.6±0.8 ^b	3.4±0.3 ^c	1.7±0.3 ^b
OV (left)	12.3±0.9 ^a	4.1±0.5 ^a	2.0±0.4 ^b
OV (right)	10.6±0.9 ^a	3.9±0.5 ^a	1.8±0.3 ^b
PK (left)	9.6±0.8 ^b	3.7±0.4 ^b	1.8±0.3 ^b
PK (right)	8.2±0.8 ^b	3.7±0.4 ^b	1.7±0.3 ^b

^{a-c}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 and PK= Potchefstroom Koekoek

3.6 DISCUSSION

Ovambo had the highest body weight compared to Potchefstroom Koekoek and Naked Neck cockerels. Number of spermatozoa per ejaculate and body weight were positively correlated (Wilson *et al.*, 1979). This means that when the body weight is high the sperm concentration is also high which will lead to more number of semen doses for artificial insemination programs. However, the study also demonstrated that there was no significant difference on the live performance traits among cockerel's breeds Naked Neck, Ovambo and Potchefstroom Koekoek. For Yoruba and Fulani ecotype chickens (Olawunmi *et al.*, 2008) comb length was also not significantly ($p>0.05$) different being 6.53 and 7.09 cm, respectively. Numerous studies (El- Sayiad *et al.*, 1994 & Abdellatif, 1999) have recommended the use of comb and wattle length as good indicators for selection of quantitative traits in chickens. Larger combs may reliably indicate roosters with greater semen production (Burrows and Titus, 1939). The mean testicular weight for the three indigenous cockerels was (9.2 ± 0.8^b g), (11.45 ± 0.9^a g) and (8.9 ± 0.8^b g) for Naked Neck, Ovambo and Potchefstroom Koekoek, respectively. It was comparable to the values (9-13 g) for White Plymouth (Kumaran & Turner, 1949) but lower than the mean values for the light and heavy breeds (16.5-31 g) of white leghorn (Parker *et al.*, 1942). The weights and dimensions of the left testis were larger than those of the right testis. In the sexually active cocks, the length of the bean shaped testis was 2.66 – 4.55 cm long, which is comparable to the findings in this study, Naked Neck (3.4 ± 0.3^c cm), Ovambo (4.0 ± 0.5^b cm) and Potchefstroom Koekoek (3.7 ± 0.4^b cm) and also compared favourably to the 3.25 – 5.60 cm reported by King (1975).

3.7 CONCLUSION

Body weight and length of testis were positive indicators of good sperm production. Comb and wattle length has been recommended to be good indicators for selection of quantitative traits in chickens. Larger combs may indicate greater semen production. Based on these results, it was suggested that in village communities of South Africa where most farmers live and facility for microscope evaluation of semen are not available, simple and indirect method of estimation is still needed to predict the fertility of the breeding cockerels. Further studies are needed to increase more knowledge on the reproductive traits of these Indigenous cockerels.

3.8 ACKNOWLEDGEMENTS

This study was supported by grants from Department of Agriculture Forestry and Fisheries, National Research Foundation and Agricultural Research Council. The author would like to express thanks to Agricultural Research Council, Germplasm Conservation & Reproductive Biotechnologies staff for their assistance during the experiment.

3.9 REFERENCES

- Abdellatif, M.A., 1999. Inheritance of comb and wattle lengths and it's as indicators to select cockerels in a population of Dandarawi chicken. *Assiut Journal of Agricultural Science*. 30, 123-131.
- Burrows, W.H. & Titus, H.W., 1939. Some observation on the semen production of the male fowl. *Poultry Science*. 64 (suppl. 1):73 (Abstract).
- EL-Sayiad, G.H., Hossari, M.A., Yamani, K.A., Sabri, H.M. & Abdel-Warith, A., 1994. A genetical study on Egyptian Fayoumi fowl. *Egyptian Poultry Science*. 14, 285-315.

- Gray, J.C., 1937. The anatomy of the male genital ducts in fowl. *Journal of Morphology*. 60, 393–405.
- Hosseinzadeh, M.H., Ebrahimmnezhad, Y., Janmohammadi, H., Ahmadzadeh, A.R. & Sarikhan, M., 2010. Poultry by product meal: Influence on performance and egg quality traits of layers. *International Journal of Agricultural and Biology*. 12, 547-550
- King, A.S., 1975. The Aves Urogenital system. In: Robert Getty (ed), *Sisson and Grossman's Anatomy of the Domestic Animals*, 5th ed. Vol. II., pp: 1919–27. Saunder Company
- Kumaran, I.D.S. & Turner, C.W., 1949. The normal development of testis in the White Plymouth Rock. *Poultry Science*. 28, 511–20.
- Lake, P.E., 1957. The male reproductive tract of fowl. *Journal of Anatomy*. 91, 116–1299.
- Latimer, H.B., 1924. Postnatal growth of the body, systems and organs of the single-comb white leghorn chicken. *Journal of Agricultural Research*. 29, 363–97.
- Ligon, J.D. & Zwartjes, P.W., 1995. Ornate plumage of male red jungle fowl does not influence mate choice by females. *Animal Behaviour*. 49, 117-125.
- McGary, S., Estevez, I., Bakst, M.R. & Pollock, D.L., 2003. Phenotypic traits as reliable indicators of fertility in male broiler breeders. *Poultry Science*. 81, 102-111.
- NRC. (1994). Nutrient Requirements of Poultry. 9th rev. National Research Council, National Academy Press, Washington, D.C. USA.

- Olawunmi, O.O., Salako, A.E. & Afuwape, A.A., 2008. Morphometric differentiation and assessment of function of the Fulani and Yoruba ecotype indigenous chickens of Nigeria. *International Journal of Morphology*. 26(4), 975-980.
- Parker, J.E., Mchenzie, F.F. & Kempster, H.I., 1942. Fertility in the male domestic fowl. Missouri Agr. Expt. Sta. Res. Bull. No 347.
- SAS Institute, 2001. SAS/STAT User's Guide Version 8.2ed: Statistics. SAS Institute Inc., Cary, NC.
- Wilson, H.R., Piesco, N.P., Miller E.R. & Nesbeth, W.G., 1979. Prediction of the fertility potential of broiler breeder males. *World's Poultry Science Journal*. 35, 95-118.
- Zuk, M., Pompa, S.L. & Johsen, T.S., 1995. Courtship displays, ornaments, and female mate choice in captive red jungle fowl. *Behaviour*. 132, 821-836.

CHARACTERIZATION OF SPERM MOTILITY RATE OF SOUTH AFRICAN INDIGENOUS COCKEREL SEMEN FOLLOWING ANALYSIS BY SPERM CLASS ANALYSER

M.B Makhafola^{1,2*}, D.O Umesiobi² M.L Mphaphathi^{1,3}, M.B Masenya^{1,2} and T.L Nedambale^{1,3}

¹Agricultural Research Council, Animal Production Institute, Germplasm Conservation and Reproductive Biotechnologies, Private Bag X 2, Irene, 0062

²Central University of Technology, Free State School of Agriculture and Environmental Science, Private Bag X 20539 Bloemfontein 9300

³Tshwane University of Technology, Department of Animal Sciences, Private Bag X680, Pretoria 0001, South Africa

4.1 Abstract

The Sperm Class Analyser (SCA) system (CASA) is advantageous and provides precise additional information that cannot be attained through the subjective manual sperm assessment methods. The aim of the study was to characterise Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) cockerels semen macroscopically and microscopically. A total of 198 ejaculates were collected from 33 cockerels; Ovambo (n=11), Potchefstroom Koekoek (n=11) and Naked Neck (n=11) by means of the abdominal massage technique. Semen was analysed macroscopically (colour, semen volume & semen pH) and microscopically (sperm concentration, morphology, sperm motility and velocity rate). The sperm motility rate was evaluated with the aid of the Sperm Class Analyser (SCA) system. The sperm morphology was performed by staining sperm with nigrosin-eosin. Breed significantly ($P < 0.05$) affected the body weight of Ovambo ($2.5 \pm 0.4\text{kg}$), Naked Neck ($2.0 \pm 0.3\text{kg}$) and

Potchefstroom Koekoek (2.3 ± 0.3 kg) cockerels. The semen volume of Naked Neck (0.5 ± 0.2 ml) breed was significantly higher, when compared to Ovambo (0.3 ± 0.2 ml), but similar to Potchefstroom Koekoek (0.4 ± 0.2 ml) breed. The overall sperm motility rate of Ovambo ($95.0 \pm 7.2\%$), Potchefstroom Koekoek ($86.0 \pm 13.7\%$) and Naked Neck ($76.4 \pm 22.2\%$) group was significantly ($P < 0.05$) different. The normal sperm morphology in the Naked Neck (69.9 ± 20.2) was lower when compared to Potchefstroom Koekoek (79.9 ± 13.7) and Ovambo (75.5 ± 14.02) group of cockerel. A positive correlation existed between body weight and semen volume for Potchefstroom Koekoek ($r=0.1477$) cockerels only. Moreover, there was a positive correlation between the body weight and total sperm motility for Naked Neck ($r=0.3848$), Ovambo ($r=0.4871$) and Potchefstroom Koekoek ($r=0.2230$) cockerels. However, a negative correlation existed between body weight and semen volume for Naked Neck ($r=-0.4502$) and Ovambo ($r=-0.1244$). In conclusion, body weight positively affected semen volume and sperm motility rate but this was breed dependent. The Sperm of Potchefstroom Koekoek and Ovambo resulted in a better motility and morphology rate. The Sperm Class Analyser or CASA provided more precise, repeatable and objective information. Evaluation of the sperm motility rate of South African indigenous cockerels holds potential for future use in semen evaluation.

Keywords: Ovambo, Naked Neck; Potchefstroom Koekoek; sperm; indigenous

*Corresponding Author: Email: lucky@arc.agric.za. Tel: +27 (0)12 672 9210. Fax: +27(0)12 665 1604.

Published: Journal of Animal Science Advances., 2012, 2(4):416-424.

4.2 INTRODUCTION

To date, no research work has been done on the characterization of South African indigenous cockerel semen such as the Ovambo (Figure 4.1a), Naked Neck (Figure 4.1b) and Potchefstroom Koekoek (Figure 4.1 c) using newly improved Computer Assisted Sperm Analyser (CASA) also known as Sperm Class Analyser (SCA). The Ovambo, Naked Neck and Potchefstroom Koekoek cockerels are native to South Africa. The Ovambo is a dark coloured breed and smaller in size, which helps to camouflage and protect it from predators. The Ovambo is generally very aggressive and agile with an average weight at 20 weeks of 2.16 kg for males and 1.54 kg for females. The hens reach sexual maturity at 143 days of age and the average egg weight is 52.5g (Joubert, 1996). The Dutch East Indian company introduced the Naked Neck chickens in the 17th century to the Cape of Good Hope in South Africa (Fourie & Grobbelaar, 2003). Currently, Naked Neck is recognized as an indigenous breed to South Africa. The Naked Neck is an adaptable breed and can be found all South African regions even under diverse environmental conditions (Grobbelaar, 2008). The chickens have very colourful white, red and black feather combinations (Joubert, 1996). The Naked Neck chickens have 30% less feathers than the fully feathered birds and can therefore produce the same body weight with less feed. The Naked Neck reaches sexual maturity at 155 days of age with an average weight of 1.95 kg for the males and 1.4 kg for the females at 20 weeks of age. Finally The Potchefstroom Koekoek was bred from crosses between the Black Australorp and the White Leghorn, and is recognised as a developed breed in South Africa. It is characterised by a relatively high egg production, and adaptability

for household production. The average egg weight is 55.7g and the colour of the eggs is brown (Ramsey *et al.*, 2000). It reaches sexual maturity at 130 days, much earlier than the Ovambo and Naked Neck cockerels.

There is a need to preserve the local chicken breeds in South Africa both in in-situ and ex-situ. Consequently, the Agricultural Research Council (ARC) established the Farm Animal Conservation Trust (FACT) in 1994, to facilitate and promote in-situ conservation of live native animal genetic resources known as Fowls for Africa®. Three of the South African native chicken lines (Naked Neck, Ovambo and Venda chicken) and one locally developed breed (Potchefstroom Koekoek) have then been listed by the FACT (Ramsey *et al.*, 2000). However, there has been no ex-situ (sperm, ovum and embryo) conservation strategy initiated with male reproductive fertility defined as the ability to produce an ejaculate with sperm that are viable and able to fertilize eggs (Umesiobi, 2008a).

There are many methods of assessing sperm quality and estimating their fertilizing ability. Some of these are regarded as highly subjective microscope evaluation which is bias, while others require more sophisticated special laboratory facilities (Hazary & Wishart, 2001). Emphasis has been placed on the use of objective methods such as Computer Aided Sperm Analysis® (CASA) system (Ferrara *et al.*, 1997). In order to standardize the manual analysis of sperm, the Sperm Class Analyser system was developed to provide a more precise, accurate and objective information on the kinetics of

sperm. Additionally, semen analyses include the evaluation of sperm concentration, motility rate, morphology and semen volume. The advantage of this recently developed SCA system is that a series of videotaped microscope images can be digitalised and computer programmes used to evaluate the rate of sperm motility, velocity and morphological characteristics (Ferrara *et al.*, 1997). Accurate semen analysis can then also assist in the conservation of viable male gametes of endangered indigenous poultry breeds. Moreover, sperm morphology using nigrosin/eosin staining for estimating the live/dead ratio was previously reported (Chalah *et al.*, 1998; Suidzinska and Lukaszewicz, 2008), and it became a basic part of the sperm analyses. The aim of the study was to characterize Naked Neck, Ovambo and Potchefstroom Koekoek cockerels semen macroscopically and microscopically for later preservation studies.

4.3 MATERIALS AND METHODS

Experimental site and Management of chickens

The study was conducted in the Republic of South Africa (RSA) at the Agricultural Research Council (Germplasm Conservation & Reproductive Biotechnologies Unit). The Agricultural Research Council, Animal Production Institute campus is located at 25° 55' South; 28° 12' East and is located in the Highveld region of RSA and situated at an altitude of 1525m above sea level. Indigenous Ovambo cockerels (n=11), Naked Neck cockerels (n=11) and Potchefstroom Koekoek cockerels (n=11) were used in this study. At the onset of the trial the males were 32 weeks of age. The chickens had been vaccinated at hatching against Marek's disease, infectious bronchitis and

Newcastle disease using live vaccines. At day 14 all cockerels were vaccinated against infectious bursal disease and the Newcastle disease virus, with live vaccines (Immuno-Vet Services CC, Johannesburg RSA). At 32 weeks of age, all chickens were kept in individual battery cages and fed layer commercial diet (Table 4.1) and water *ad libitum*. The cockerels were weighed once a week for 6 weeks. The experimental diet fed met the National Research Council requirements (1994). The chickens were exposed to photoperiod of 16 h followed by 8 h of darkness (16 Light: 8 Darkness). The experimental birds were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (APIEC10/04).

TABLE 4.1: The nutrient composition diet from Epol® for the chickens

Item	Diet		
	Maxi-chicks starter g/kg	Maxi-chicks grower g/kg	Maxi-chicks layers g/kg
Protein	200	150	150
Moisture	120	120	120
Fat	25	25	25
Fibre	50	70	70
Calcium	10-12	10-12	28-30
Phosphorus	5	5	5
Total lysine	10.5	5.7	7

Semen collection and analysis

The cockerels were trained for semen collection following 2 weeks of adaptation. The abdominal massage technique as described by Burrows & Quinn (1937), was used for semen collection. Semen was collected 2 times per week (Tuesday & Thursday). Individual ejaculates were collected 6 times from each individual cockerel into a 15 ml tube (falcon® 352099, USA). During collection, the semen tube was maintained at 40°C in a thermo flask. After semen collection, the semen was transported to the laboratory within 15 min of collection, care being taken during semen collection to minimize contamination by faeces or urine.

Semen volume was measured by using the graduated falcon tube, while semen pH using the litmus paper. Sperm concentration was determined using the grey Beckman spectrophotometer (Jenway 6310 spectrophotometer, Bibby Scientific, England) the wavelength being set to 650nm. Thereafter 3 ml of 2.9% Sodium Citrate solution (pH 7.0) added to a square cuvette with 15 µl of cockerel semen. The cuvette was placed back in the spectrophotometer and the absorbance read as soon as the reading stabilized. The absorbance was then converted to sperm concentration, using the formula used: $(11,170 \times \text{Absorbance}) - 90$, and it was recorded in billion sperm/ml ($\times 10^9/\text{ml}$, Knox *et al.*, 2000). Collected semen was evaluated using the swim-up (10 µl of semen added to 500 µl of swim-up medium) technique, for evaluation of sperm motility rate by a Sperm Class Analyser® (Microptic, Barcelona, Spain) at a magnification of X 10 (Nikon, Japan). Following sperm swim-up preparation, 5 µl of diluted semen sample were placed on a warm glass slide (~76 X 26 X 1mm, Germany) and covered with warmed cover slip (22 X 22 mm, Germany)

on the microscope-warm stage (Omron) adjusted to 37° C. The kinematic values recorded for each sperm included, in addition to the 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 4.2).

Sperm morphology

The eosin/nigrosin (eosin-Y 11.1 mg plus nigrosin 66.7 mg/ml distilled water; Sigma, St. Louis, MO, USA) stain was used to prepare slide smears for percentage live/dead sperm. At least 100 sperm per slide in each individual cockerel ejaculate were counted, using a fluorescence microscope (BX51 Olympus) at X 100 magnification, with oil immersion. The underlying principle on which these techniques are based is that spermatozoa with structurally intact cell membranes—supposedly live spermatozoa—are not stained, while dead spermatozoa with disintegrating cell membranes take up the stain (Burke, 1984). Figure 2: Stained cockerel spermatozoa (a, d and f) live normal sperm (b & c) live abnormal sperm and (e) dead sperm. The experiment was replicated 6 times for each treatment group.

Table 4.2 Sperm Class Analyser[®] (V.5.0) settings used to analyse the sperm motility and velocity characteristics

Parameters	Settings
Contrast	169
Brightness	470
Image/second	50
Optic	pH-
Chamber	Cover slide
Scale	10x
Particle area	5<190 μm^2
Slow	<10 $\mu\text{m/s}$
Medium	<50 $\mu\text{m/s}$
Rapid	<100 $\mu\text{m/s}$
Progressivity	>70% of straightness
Circular	<50% of linearity
Connectivity	18
Velocity on the average path line	7 $\mu\text{m/s}$
Number of images	30

4.4 STATISTICAL ANALYSIS

Data were analysed using the General Linear Model. The significance of differences between means was compared by Student's t-LSD (Least Significant Difference). Differences with a probability of values $P < 0.05$ were considered significant. The correlation of body weight with semen volume,

concentration, pH, sperm motility and total percentage of live cell was performed using the SAS statistical software. The Pearson two sided analysis was used to determine the correlation between body weight and the variables (Snedecor & Cochran, 1980).

4.5 RESULTS

The mean body weight of Ovambo (2.5 ± 0.4 kg) was significantly ($P < 0.05$) higher compared to Naked Neck (2.0 ± 0.3 kg) and Potchefstroom Koekoek (2.3 ± 0.3 kg) cockerels (Table 4.3). The values of the macroscopic semen evaluations are set out in Table 4.3. Semen volume of the Naked Neck (0.5 ± 0.2 ml) breed was significantly higher than Ovambo (0.3 ± 0.2 ml) but similar ($P < 0.05$) to Potchefstroom Koekoek (0.4 ± 0.2 ml) cockerels while the semen pH of Ovambo (7.7 ± 0.9) cockerels was significantly higher compared to Naked Neck (6.8 ± 1.3) and Potchefstroom Koekoek (6.6 ± 2.2) groups. The semen concentration of the Potchefstroom Koekoek ($8.0 \pm 4.0 \times 10^9$ /ml) was significantly higher when compared to Ovambo ($3.8 \pm 6.2 \times 10^9$ /ml) and Naked Neck ($6.3 \pm 3.2 \times 10^9$ /ml) cockerels. The differences in the ejaculate volume, pH & sperm concentration were considered significant ($P < 0.05$) in less than 5% of the cases. The percentages of live, dead sperm with morphological defects are set out in Table 4.4. No significant differences were recorded in the percentage of dead sperm, and the abnormalities on the head and tail of the sperm, for all the breeds. Motility rate characteristics of three Indigenous cockerel breeds are presented in Table 4.5. There was a significant difference on total sperm motility, progressive, rapid, medium, slow & static (immotile) sperm were recorded in all the breeds. The Pearson correlation coefficient between body weight and the semen characteristics of Indigenous cockerel

breeds are set out in Table 4.6. A positive correlation was recorded between body weight and semen volume of the Potchefstroom Koekoek ($r=0.1477$). A positive correlation was recorded between body weight and total sperm motility in the Naked Neck ($r=0.3848$), Ovambo ($r=0.4871$) and Potchefstroom Koekoek ($r=0.2230$), as well as for total live normal sperm in the Naked Neck ($r=0.2086$), Ovambo ($r=0.1711$) and Potchefstroom Koekoek ($r=0.1865$). However, a negative correlation existed between body weight and semen volume of the Naked Neck ($r=-0.4502$) and Ovambo ($r=-0.1244$). In addition, a non significant negative correlation existed between body weight and sperm concentration of Naked Neck ($r=-0.2854$), Ovambo ($r=-0.5010$) and Potchefstroom Koekoek ($r=-0.1726$) cockerels as well as semen pH of Naked Neck ($r=-0.1782$), Ovambo ($r=-0.0919$) and Potchefstroom Koekoek ($r=-0.3105$).

FIGURE LEGENDS

Figure 4.1(A) Indigenous chickens used in this study, a) Ovambo, b) Naked Neck and c) Potchefstroom Koekoek.

(A)



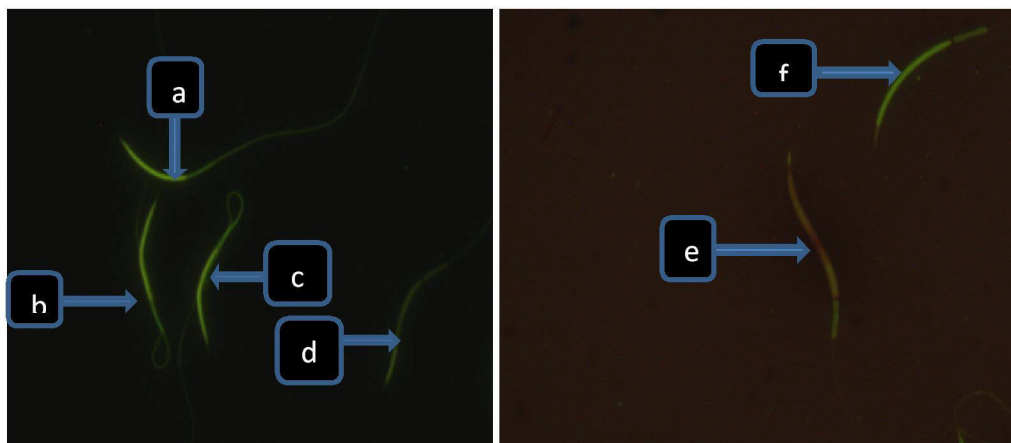
a) Ovambo

b) Naked Neck

c) Potchefstroom Koekoek

Figure 4.2 (B) Stained cockerel spermatozoa (a, d and f) live normal sperm (b & c) live abnormal sperm and (e) dead sperm.

(B)



Stained cockerel spermatozoa (a, d and f) live normal sperm, (b & c) live abnormal sperm and (e) dead sperm

Table 4.3 The means ($\pm$ SD) of macroscopic evaluation of cockerel semen characteristics

Cockerel breeds (n)	Number of ejaculates	Body weight (kg)	Ejaculate volume (ml)	Semen pH	Sperm Concentration ($\times 10^9$ /ml)
NN (11)	66	2.0 ± 0.3^c	0.5 ± 0.2^a	6.8 ± 1.3^b	6.3 ± 3.2^a
OV (11)	66	2.5 ± 0.4^a	0.3 ± 0.2^b	7.7 ± 0.9^a	3.8 ± 6.2^b
PK (11)	66	2.3 ± 0.3^b	0.4 ± 0.2^{ab}	6.6 ± 2.2^b	8.0 ± 4.0^a

^{a-c} Values within each column with different superscript differ significantly * $P < 0.05$

NN = Naked Neck, OV= Ovambo and PK=Potchefstroom Koekoek

Table 4.4 Comparison of sperm morphological defects (mean±SD) of different cockerel breeds

Cockerel breeds (n)	Number of spermcounted	Live Normal sperm (%)	Dead sperm (%)	Live sperm abnormalities (%)		
				Head	Midpiece	Tail
NN (11)	600	69.9±20.0 ^b	19.8±16.9 ^a	3.2±3.1 ^a	6.5±7.5 ^a	0.6±1.3 ^a
OV (11)	600	75.5±14.0 ^{ab}	17.4±16.2 ^a	2.5±3.0 ^a	3.8±4.6 ^b	0.8±1.0 ^a
PK (11)	600	79.9±13.7 ^a	14.5±9.2 ^a	1.9±1.5 ^a	2.9±5.6 ^b	0.8±0.8 ^a

^{a-c} Values within each column with different superscripts differ significantly * P < 0.05

NN = Naked Neck, OV= Ovambo and PK=Potchefstroom Koekoek

Table 4.5 Characterization (mean±SD) of sperm motility rate of cockerels of different breeds

Cockerel breeds (n)	TM (%)	PM (%)	NPM (%)	RAP (%)	MED (%)	SLW (%)	STATIC
NN (11)	76.4±22.2 ^c	32.1±20.8 ^b	42.6±17.0 ^a	28.3±24.0 ^b	17.7±11.4 ^b	30.9±16.4 ^b	23.4±22.3 ^b
OV (11)	95.0±7.2 ^a	49.7±11.0 ^a	45.4±10.3 ^a	53.3±18.7 ^a	24.3±9.3 ^b	18.8±9.6 ^b	4.6±7.3 ^c
PK (11)	86.0±13.7 ^b	42.2±18.5 ^a	43.8±16.6 ^a	34.2±21.4 ^b	33.7±20.7 ^a	34.2±21.4 ^a	33.7±20.7 ^a

^{a-c}Values within each column with different superscripts differ significantly *(P < 0.05)

NN = Naked Neck, OV= Ovambo and PK=Potchefstroom Koekoek

TM= total motility, PM= progressive motility, NPM= non progressive motility, RAP= rapid, MED= medium and SLW= slow

Table 4.6 Pearson Correlation Coefficients between body weight and semen characteristics of Indigenous cockerel breed

Breed	Characteristics	Body weight	Ejaculate volume (ml)	Semen Concentration ($\times 10^9/\text{ml}$)	Semen pH	Total Motility (%)	Live Normal (%)
Naked Neck	Body weight	1.00					
	Ejaculate volume (ml)	-0.45025	1.00				
	Semen Concentration ($\times 10^9/\text{ml}$)	-0.28542	0.54265	1.00			
	Semen pH	-0.17822	0.32381	0.04071	1.00		
	Total Motility (%)	0.38486	-0.78841	-0.08566	0.8004	1.00	
	Live Normal (%)	0.20867	-0.26444	-0.04459	-0.42604	-0.00077	1.00
Ovambo	Body weight	1.00					
	Ejaculate volume (ml)	-0.12443	1.00				
	Semen Concentration ($\times 10^9/\text{ml}$)	-0.50104	0.60230	1.00			
	Semen pH	-0.09192	0.67745	0.41720	1.00		
	Total Motility (%)	0.48716	-0.08106	-0.52064	0.06745	1.00	
	Live Normal (%)	0.17114	-0.21209	0.15713	-0.03899	0.25765	1.00
Potchefstroom Koekoek	Body weight	1.00					
	Ejaculate volume (ml)	0.14775	1.00				
	Semen Concentration ($\times 10^9/\text{ml}$)	-0.17261	0.25396	1.00			
	Semen pH	-0.31054	0.67231	0.47640	1.00		
	Total Motility (%)	0.22305	0.23041	-0.08515	0.22656	1.00	
	Live Normal (%)	0.18653	-0.21490	0.24336	-0.35119	-0.28264	1.00

NN = Naked Neck, OV= Ovambo and PK=Potchefstroom Koekoek

4.6 DISCUSSION

This study revealed the first time basic sperm characteristics of South African indigenous cockerels and demonstrated that a positive correlation existed between body weight and semen volume of the Potchefstroom Koekoek ($r=0.1477$). Furthermore, there was also a positive correlation (not significant) between body weight and total sperm motility of the Naked Neck ($r=0.3848$), Ovambo ($r=0.4871$) and Potchefstroom Koekoek ($r=0.2230$). Interestingly, body weight was also positively correlated with the total normal live sperm of Naked Neck ($r=0.2086$), Ovambo ($r=0.1711$) and Potchefstroom Koekoek ($r=0.1865$) cockerels. The ejaculated volume of Ovambo (0.3 ml), Naked Neck (0.5 ml) and Potchefstroom Koekoek (0.4 ml) was found to be acceptable as it was higher than the acceptable standard limit which ranges from 0.25-0.35 ml (Burke, 1984) and 0.26-0.35 ml (Bakst, 1990). Further an average semen volume of 0.3 ml was reported in the indigenous Shikabrown breed by Gabriel *et al.* (2009), which is similar to the Ovambo breed in this study. Podgórný *et al.* (1976) Found that White Rock roosters produce an average of 0.42 ml semen which is similar to Potchefstroom Koekoek breed. The semen pH has been found to vary slightly between different breeds and bird species. Donoghue & Wishart (2000) and Suidzinska & Lukaszewicz (2008), reported that chicken sperm can tolerate a pH ranging from 6.0 to 8.0-which is in agreement with this study where pH of Naked Neck was 6.8, Ovambo 7.7 and Potchefstroom Koekoek 6.6. The sperm concentration is usually defined as the number of sperm per ml of the ejaculated and can normally predict the number of breeding units that can be inseminated (Cole & Cupps, 1977). The sperm concentration of Naked Neck ($6.3 \times 10^9/\text{ml}$) and Potchefstroom Koekoek ($8.0 \times 10^9/\text{ml}$)

was higher than ($3.8 \times 10^9/\text{ml}$) reported Cecil and Bakst (1988). This may have been due to breed differences.

It has been reported that the high percentage of dead and abnormal cells reduces the total progressive motility of the sperm (Omeje & Marire, 1990) which is in agreement with the current study where the Naked Neck breed had the lowest total sperm motility rate - as a result of the higher number of dead and abnormal cells recorded. It is predicted that a high fertility rate could therefore be achieved with the South African Indigenous chicken breeds, used in this study. Similarly in the study by (Donoghue & Walker-Simmons 1999), reported a high positive correlation to exist between progressive sperm motility and fertility in chicken. Comparable observations were reported by Tabatabaei *et al.* (2009), who found that sperm motility and viability rate to be significantly higher in sperm from indigenous roosters than from the Ross breed of males. It was also reported that that cockerels with a heavier body weight had significantly higher sperm motility (Holcman *et al.*, 1993; Soliman, 1996), which is in agreement with the current study where the heavier Ovambo cockerels had higher ($P < 0.05$) total sperm motility rate. Interestingly, the lighter body weighing cockerels have been reported to have higher ejaculate volumes (Holcman *et al.*, 1993 and Soliman, 1996) and a lower live sperm count (Holcman *et al.*, 1993) similarly to the observed results of Naked Neck cockerels in this study.

4.7 CONCLUSION

This is the first study that provided baseline information on indigenous Naked Neck, Ovambo and Potchefstroom Koekoek cockerel sperm motility characteristics, using the Sperm Class Analyser. The body weight positively affected the semen volume

and sperm motility rate, but it was breed dependent. The Sperm of Potchefstroom Koekoek and Ovambo resulted in a better motility and morphology rate. The percentage of live normal sperm rate was higher in Potchefstroom Koekoek compared to other groups. However the Ovambo group recorded higher motility rates. The Sperm Class Analyser provided precise, accurate and objective sperm motility characteristics and it is suggested that it may be a very reliable technique for evaluating the fertility of cockerel sperm.

4.8 ACKNOWLEDGEMENTS

The author would like to express thanks to Agricultural Research Council (ARC), Germplasm Conservation & Reproductive Biotechnologies (GCRB) staff for assistance during the experiment. This study was supported by grants from (ARC), National Research Foundation (NRF), Department of Agriculture Forestry & Fisheries (DAFF) and Central University of Technology (CUT).

4.9 REFERENCES

- Bakst, M.R., 1990. Preservation of avian cells. In Crowford, R.D. (Ed.), Poultry breeding and genetics, Elsevier Science Publishing Co. Inc., New York, 91-98.
- Burke, W.H., 1984. Avian Reproduction In: Duke's Physiology of Domestic Animals. 10th edition, M.J. Swenson (ed) Cornell University Press, New York, 847-862.
- Burrows, W.H. & Quinn, J.P., 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*. 16, 185-191.

- Cecil, H.C. & Bakst, M.R., 1988. Daily output of spermatozoa and extra gonadal spermatozoa reserves in turkeys. *Poultry Science*. 67, 327-332.
- Cole, H.H. & Cupps, P.T., 1977. Reproduction in domestic animals. 3rded. New York Academic press.
- Chalah, T. & Brillard, J.P., 1998. Comparison of assessment of fowl sperm viability by eosin-nigrosin and dual fluoresce (SYBR-14/PI). *Theriogenology*. 50, 487-493.
- Donoghue, A.M. & Walker-Simmons, M.K., 1999. The influence of wheat dehydration-induced proteins on the function of turkey spermatozoa after twenty-four hours in vitro storage. *Poultry Science*. 78, 235-241.
- Donoghue, A.M. & Wishart, G.J., 2000. Storage of poultry semen. *Animal Reproduction Science*. 62, 213-232.
- Ferrara, F.R., Daverio, G., Mazzini, P., Bonini & Banfin, G., 1997. Automation of human sperm analysis by flow Cytometry. *Clinical Chemistry*. 43, 801-807.
- Fourie, C. & Grobbelaar, J.A.N., 2003. Indigenous poultry breeds. Wing Nut. Feb:20-21. Publications. Krugersdorp.
- Gabriel, G.M., Kalamah, M.A., El-fiky, A. & Ali, F.A., 2009. Some factors affecting semen quality traits in Norfa cocks. *Egyptian Poultry Science*. 29, 677-693.
- Grobbelaar, J.A.N., 2008. Egg production potentials of four indigenous chicken breeds in South Africa. M Tech dissertation, Pretoria, Tshwane University of Technology.

- Hafez, E.S.E., 1995. Artificial Insemination. In: E.S.E. Hafez (ed) Reproduction in Farm Animals. 6th edition Lea and Febiger, Philadelphia., U.S.A. PP: 424.
- Hazary, R.C. & Wishart, G.J., 2001. Assay of sperm quality in the domestic fowl by MTT reduction. *British Poultry Science*. 42, 111–114.
- Holcman, A.R., Fabijan, & Kovac, M., 1993. The effect of 2-Way selection for 56 day body weight in fowls on some reproductive traits of cocks. Zbornik Biotehniske. Fakultete.Univerze. Ed Varda. Kardelja. V. Ljubljani, Kmetijstvo. No. 62, 143–151:6.
- Joubert, J.J., 1996. The story of the indigenous domestic animals in South Africa Agricultural Research Centre, Private Bag X2, Irene, 0062. South Africa. (unpublished)
- Knox, R.V., Tudor, K.W., Rodriguez-Zas, S.L. & Robb, J.A., 2000. Effect of subcutaneous versus intramuscular administration of PG600 on estrual and ovulatory responses of prepubertal gilts. *Journal of Animal Science*. 78, 1732-1737.
- National Research Council., 1994. Nutrient requirements of poultry - ninth revised edition. National Academy Press, Washington, USA.
- Omeje, S.S.I. & Marire, B.N., 1990. Evaluation of the semen characteristics of adult cocks of different genetic backgrounds. *Theriogenology*. 34, 1111-1118.
- Podgórný, E., Wöyk, S. & Ada-Gorzowska, A., 1976. Variations in cocks sperm quality throughout the year. VIII Int. Congr. Animal Reproduction and Artificial Insemination, 1057–1060 in Proc. Cracow, Poland. M. Tischner and J. Pilch, ed. Wroclawska Drukarnia Naukowa, Wroclaw, Poland.

- Ramsey, K., Harris, L. & Kotze, A., 2000. Landrace breeds: South Africa's indigenous and locally adapted developed farm animals. Ramsey, Harris & Kotzé (eds). Farm Animal Conservation Trust, Pretoria.
- Suidzinska, A. & Lukaszewicz, E., 2008. The effect of breed on freezability of semen of fancy fowl. *Animal Science Papers and Reports*. 26, 331-340.
- Tabatabaei, S., Batavani, R.A. & Talebi, A.R., 2009. Comparison of semen quality in indigenous and Ross broiler breeder roosters. *Journal of Animal and Veterinary Advances*. 8, 90-93.
- Soliman, M.M., 1996. Some factor affecting fertility and hatchability using artificial insemination in chickens. M.Sc. Thesis, Fac. Agric. Minofiya Univ., Egypt.
- Snedecor, G.W. & Cochran, W.G., 1980. Statistical methods. 7th edition, Iowa State University Press, Ames, Iowa.
- Umesiobi, D.O., 2008a. Measures of servicing capacity of boars and their effects on subsequent fertility in artificially inseminated gilts. *Journal of Applied Animal Research*. 34, 9-12.

RELATIONSHIP BETWEEN PHENOTYPIC AND SPERM TRAITS OF SOUTH AFRICAN INDIGENOUS COCKERELS

M.B Makhafola^{1,2} D.O Umesiobi² and T.L Nedambale^{1,3,4}

¹Agricultural Research Council, Animal Production Institute, Germplasm Conservation & Reproductive Biotechnologies, Irene, RSA

²Central University of Technology, Free State Faculty of Health and Environmental Science School of Agriculture and Environmental Science, Private Bag X 20539 Bloemfontein, 9300, South Africa

³Tshwane University of Technology, Faculty of Science, Department of Animal Sciences, Pretoria, RSA

⁴University of the Free State, Department of Animal, Wildlife and Grassland Sciences, Bloemfontein, RSA

5.1 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, & 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.

Keywords: Ovambo, Naked Neck, Potchefstroom Koekoek, phenotypic traits, sperm quality

*Corresponding Author: Email: lucky@arc.agric.za. Tel: +27 (0)12 672 9210. Fax: +27(0)12 665 1604.

Published: International Journal of Livestock Production, 2012. 3(6):61-65.

5.2 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 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 kg 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 e.g. 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 predicts 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, i.e. 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.

5.3 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 & Reproductive Biotechnologies Unit.

Experimental chickens

Thirty three 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 hours daylight and 8 hours darkness, the cockerels were fed layer commercial diet (Table 5.1) 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).

TABLE 5.1: The nutrient composition diet from Epol[®] for the chickens

Item	Diet		
	Maxi-chicks starter g/kg	Maxi-chicks grower g/kg	Maxi-chicks layers g/kg
Protein	200	150	150
Moisture	120	120	120
Fat	25	25	25
Fibre	50	70	70
Calcium	10-12	10-12	28-30
Phosphorus	5	5	5
Total lysine	10.5	5.7	7

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 is between 40.6° and 41.7°C (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 (X 10⁹/ml), 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 X 26 X 1mm, Germany) and covered with warmed cover slip (22 X 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 X (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 5.2).

Live performance traits

Measurements and observation: The cockerels were weighed weekly for 6 weeks. Shank, wattle & comb length were measured once a week for 6 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 as a distance between the upper and the lower point of the organ.

Table 5.2 Sperm Class Analyser[®] (V.5.0) settings used to analyse the sperm motility and velocity characteristics

Parameters	Settings
Contrast	169
Brightness	470
Image/second	50
Optic	pH-
Chamber	Cover slide
Scale	10x
Particle area	5<190 μm^2
Slow	<10 $\mu\text{m/s}$
Medium	<50 $\mu\text{m/s}$
Rapid	<100 $\mu\text{m/s}$
Progressivity	>70% of straightness
Circular	<50% of linearity
Connectivity	18
Velocity on the average path line	7 $\mu\text{m/s}$
Number of images	30

5.4 Statistical analysis

Data were analysed using the SAS Institute, Inc. (1999), SAS/STAT 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 Breed with 3 levels (NN, OV and PK) with 11 Cocks randomly selected from each breed. Shapiro, S.S. and Wilk, M.B. (1965); An Analysis of Variance Test for Normality (complete samples). *Biometrika*, 52, 591-611. A significance level of 0.05% was used ($P < 0.05$).

5.5 Results

There were no significant differences on the shank, comb and wattle length of all three breeds as indicated in Table 5.3. 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 5.4. 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 ($8.0 \pm 5.0 \times 10^9/\text{ml}$) 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 5.5 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.

Table 5.3 Live performance traits (Mean±SD) of cockerels of NN, OV and PK

Cockerels (n)	Live performance Traits			
	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-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 and PK=Potchefstroom Koekoek.

Table 5.4 Comparison of Indigenous cockerels breed on macroscopic evaluation of cock semen characteristics (Mean±SD)

Cockerels (n)	Ejaculate volume (ml)	Semen pH	Sperm Concentration (x 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-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 and PK=Potchefstroom Koekoek.

Table 5.5: Pearson Correlation Coefficients of semen characteristics and live performance traits for Indigenous cock breeds

Variables	Weight	Shank Length	Wattle Length	Comb Length	Vol(ml)	Concentration X10 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 X10 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
TotalMotility%	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
LiveNormal%	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 $\alpha=0.05$

5.6 DISCUSSION

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 MM. Soliman, Minufiya University, Egypt, M.Sc Thesis) 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 correlation between body weight and semen volume.

5.7 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.

5.8 ACKNOWLEDGEMENTS

This study was supported by grants from Department of Agriculture Forestry and Fisheries, National Research Foundation and Agricultural Research Council. The author would like to express thanks to Agricultural Research Council, Germplasm Conservation & Reproductive Biotechnologies staff for their assistance during the experiment.

5.9 REFERENCES

- Adenokun, S.D. & Sonaiya, E.B., 2001. Comparison of the performance of Nigerian chickens from three agro-ecological zones. *Livestock Research for Rural Development*. 13: 2.
- Bakst, M.R. & Cecil, H.C., 1997. Techniques for semen evaluation, semen storage, and fertility determination. Bakst and Cecil editors. Published by the Poultry Science Association, Inc., Savoy, Illinois.
- Bakst, M.R., Wishart, G.J. & Brillard, J.P., 1994. Oviductal sperm selection, transport and storage in poultry. *Poultry Science Review*. 5, 117-143.
- Burrows, W.H. & Quinn, J.P., 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*. 16, 185-191.

- Debby, I., Maya, F. & Haim, B., 2012. Mechanism of sperm capacitation and the acrosome reaction: role of protein kinases. *Asian Journal of Andrology Advance*.10, 1038.
- Donoghue, A.M. & Wishart, G.J., 2000. Storage of poultry semen. *Animal Reproduction Science*. 62, 213-232.
- Fan, Y.K., Ju, J.C., Lee, S.L., Chen, C.F., Peh, H.C., Hsu, J.C. & Lee, Y.P., 2004. High Ejaculation Frequency Enhances Semen Production in Taiwan Country Chickens. *Asian-Australian Journal of Animal Science*. (17), 7: 924-929.
- Galal, A., 2007. Predicting semen attributes of Naked Neck and normally feathered male chickens from live performance traits. *International Journal of Poultry Science*. 6(1): 36-42.
- Holcman, A., Fabijan, R., & Kovac, M., 1993. The effect of 2-Way selection for 56 day body weight in fowls on some reproductive traits of cocks.ZbornikBiotehniske.Fakultete.Univerze. Ed Varda. Kardelja. V. Ljubljani, Kmetijstvo: No. 62: 143–151; 6 ref.
- Ifeanyichukwu, U., 2012. Use of factor scores for determining the relationship between body measurements and semen traits of cocks. *Open Journal of Animal Science*. 2, 41-44.
- Kelso, K.A., Cerolini, S., Noble, R.C., Sparks, N.H.C. & Speake, B.K., 1996. Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. *Journal of Reproduction Fertility*. 106, 201-206.
- Makhafola, M.B., Umesiobi, D.O., Mphaphathi, M.L., Masenya, M.B. & Nedambale, T.L., 2012. Characterization of Sperm Motility Rate of Southern African Indigenous Cockerel Semen following Analysis by Sperm Class Analyser. *Journal of Animal Science Advances*. 4, 416-424.

- McGary, S., Estevez, I., Bakst, M.R. & Pollock, D.L., 2003. Phenotypic traits as reliable indicators of fertility in male broiler breeders. *Poultry Science*. 81(1), 102-111.
- Peters, S.O., Omidji, E.A., Ikeobi, C.O.N., Ozoje, M.O. & Adebambo, O.A., 2004. Effect of Naked Neck and Frizzled Genes on Egg Traits, Fertility and Hatchability in Local Chicken. In: Self Sufficiency of Animal Protein in Nigeria. Proceedings of the 9th Annual Conference of Animal Science Association. Nigeria, Ebonyi State University, Abakaliki, Nig. September, 13-16th:262-264.
- Scogin, V., Harris, G.C., Sellers, Jr R., Parker, J. & Goto. K., 1982. Effects of environmental temperature, feeding program and body weight on semen production and certain blood parameters of broiler breeders. *Poultry Science*. 61, 1395 (Abstract).
- Shapiro, S.S. & Wilk, M.B., 1965a. Testing the normality of several samples. *Biometrika*. 52, 3-4
- Siudzinska, A. & Lukaszewicz, E., 2008b. Effect of semen extenders and storage time on sperm morphology of four chicken breeds. *Applied Poultry Research*. 17, 101-108.
- Weaver, Jr W.D., 2002. Poultry housing. In: Commercial chicken meat and egg production, 5th edition, (Ed. Donald D. Bell and William D. Weaver Jr.). pp. 102:103.

QUANTIFICATION OF INDIGENOUS COCKERELS SPERM TRAITS MEASURED BY COMPUTERASSISTED SPERM ANALYSIS (CASA) TECHNOLOGY AND THE RELATIONSHIP TO FERTILITY

M.B Makhafola^{1,2} D.O Umesiobi² and T.L Nedambale^{1,3,4}

¹Agricultural Research Council, Animal Production Institute, Germplasm Conservation and Reproductive Biotechnologies, Private Bag X 2, Irene, 0062

²Central University of Technology, Free State Faculty of Health and Environmental Science School of Agriculture and Environmental Science, Private Bag X 20539 Bloemfontein 9300

³Tshwane University of Technology, Department of Animal Sciences, Private Bag X 680, Pretoria 0001, South Africa

⁴University of the Free State, Department of Animal, Wildlife and Grassland Sciences, P.O. Box 339, Bloemfontein, South Africa

6.1 Abstract

Increase use of artificial insemination (AI) in poultry industry emphasizes the need of good quality sperm distribution. The objective of the study was to investigate the relationship between South African indigenous cockerel sperm traits measured by CASA and fertility rate (hatchability, fertility, chick hatched weight, and survivability) following artificial insemination. A total of 90 hens and 33 indigenous cockerels were used in this study. The abdominal massage technique was used to collect semen. Sperm characteristics was analysed by CASA before artificial insemination to correlate the CASA results and that of the insemination. Following analysis, 0.25 ml undiluted semen dose from Naked Neck (NN, 11), Ovambo (OV, 11) and Potchefstroom Koekoek (PK, 11) cockerels was introduced into the oviduct of each NN (30), OV (30) and PK (30) hens once per week. Data was analysed using the general linear model. Semen volume of NN (0.5 ± 0.2 ml) was significantly higher when compared to OV (0.3 ± 0.2 ml), but similar to PK (0.4 ± 0.2 ml) cockerels. Total

sperm motility rate of OV ($95.0 \pm 7.2\%$), PK ($86.0 \pm 13.7\%$) NN ($76.4 \pm 22.2\%$) cockerels were significantly different. Following AI, PK significantly produced eggs that had highest average egg weight (55.5 g), fertility (68.2 %), hatchability (78.6 %), normal chicks (80.1%) and chick weight (37.9 g) compared to OV(40.4 g, 52.1 % , 70.2 %, 71.5 %, 32.8 g) and NN (37.1 g, 50.6 %, 71.1 %, 75.8 % and 23.5 g). Chicks hatched from larger eggs had higher body weight. It is therefore suggested that breeders should select bigger egg size of 50-55g for breeding purposes.

Key word: Cocks, semen viability, fertility, hatchability, indigenous chicken breeds

*Corresponding Author: Email: lucky@arc.agric.za. Tel: +27(0)12 672 9210. Fax: +27(0)12 665 1604.

6.2 INTRODUCTION

Poultry plays an important role in producing animal protein most effectively within the shortest possible time (Hosseinzadeh *et al.*, 2010). This is because of their prolificacy and fast growth rate. There is overwhelming evidence that indigenous chicken production plays a significant role in the socio-cultural, economic and nutritional aspects of the lives of rural households in Africa. Indigenous chicken breeds have been a product of their environment and survived under the harsh conditions for many generations (Umesiobi, 2000 & Fourie *et al.*, 2004).

The reproductive potential of the indigenous chicken has not been fully exploited in South Africa compared to other exotic chicken breeds. A proper semen analysis is empirical for selection and preserving their genetic materials through *ex-situ* and *in-situ*. The assessment of semen quality characteristics of poultry gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability rate of eggs (Peters *et al.*, 2004).

Assessment of the proportion of normal and progressively moving sperm is the most widely used test of sperm quality from the initial stages of artificial insemination (AI) development (Maule, 1962 and Salisbury *et al.*, 1978). To maximize hatchability of total eggs after AI, the efficient use of cock semen of the highest quality is essential (Hammerstedt, 1996).

Recent studies found positive correlations between sperm motility and fertility on hatched eggs (Froman and McLean, 1996 and McDaniel *et al.*, 1998). However, many seminal attributes such as sperm concentration, live morphological normal sperm and motility are essential to achieve full fertilizing potential. Semen containing a high percentage of viable sperm with all the essential functional attributes was noted to be of high fertility (Amann and Hammerstedt, 1993 and Hammerstedt, 1996).

Artificial insemination is a vital component of commercial poultry production. During AI, semen is collected from one cock then 20-30 breeder hens are inseminated in a ratio of 1 cock to 8-10 hens in natural mating (Bayyari *et al.*, 1990). A number of other factors including egg age (Tarongoy *et al.*, 1990), storage condition, age of flock (Buhr, 1995), system of husbandry and rearing technology (Weis, 1991), mating system (Gebhardt-Henrich and Mark, 1991), incubation relative humidity and eggs turning angle (Permsak, 1996) have been shown to influence the hatchability of poultry eggs. Fertility and hatchability are major parameters of reproductive performance which are most sensitive to environmental and genetic influences (Stromberg, 1975). Fertility refers to the percentage of incubated eggs that are fertile while hatchability is the percentage of fertile eggs that hatch. According to

Tabatabaei *et al.* (2009), fertility and hatchability depends on genetic, physiological, social and environmental factors. These are interrelated heritable traits which may vary between chicken breeds. The supply of day-old chicks is very important for the success of the poultry production chain. The objective of the study was to check the relation between cock sperm traits, egg traits, hatchability, fertility, chick hatch weight, and chick survivability of artificial inseminated South African indigenous chicken breeds (Naked Neck, Ovambo and Potchefstroom Koekoek).

6.3 MATERIALS AND METHODS

Experimental site

The study was conducted in the Republic of South Africa (RSA) at the Agricultural Research Council (ARC), Animal Production Institute API), Germplasm Conservation & Reproductive Biotechnologies (GCRB) Unit. The ARC, Animal Production Institute campus is located at 25° 55' South; 28° 12' East and is located in the Highveld region of RSA and situated at an altitude of 1525m above sea level.

Experimental chickens

Indigenous Ovambo (n=11), Naked Neck (n=11) and Potchefstroom Koekoek cockerels (n=11) were used in this study. At the onset of the trial the males were 32 weeks of age. The cockerels had been vaccinated at hatching against Marek's disease, infectious bronchitis and Newcastle disease using live vaccines. At day 14 all cockerels were vaccinated against infectious bursal disease and the Newcastle disease virus, with live vaccines (Immuno-Vet Services CC, Johannesburg, RSA). At 32 weeks of age, all cockerels were kept in individual battery cages and fed layer commercial diet (Table 6.1) and water *ad libitum*. The cockerels were weighed once

a week for 6 weeks. The experimental diet fed met the National Research Council (1994) requirements. The cockerels were exposed to photoperiod of 16 h followed by 8 h of darkness. The experimental chickens were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (APIEC10/04).

TABLE 6.1: The nutrient composition diet from Epol[®] for the chickens

Item	Diet		
	Maxi-chicks starter g/kg	Maxi-chicks grower g/kg	Maxi-chicks layers g/kg
Protein	200	150	150
Moisture	120	120	120
Fat	25	25	25
Fibre	50	70	70
Calcium	10-12	10-12	28-30
Phosphorus	5	5	5
Total lysine	10.5	5.7	7

Semen collection and analysis

Cockerels were trained for semen collection at 28 weeks. After adaptation period of 2 weeks, semen was collected by abdominal massage technique as described by Burrows and Quinn (1937). Semen was collected 2 times per week (Tuesday and Thursday). Individual ejaculates were collected 6 times from each individual cockerel into a 15 ml tube (falcon[®] 352099, USA). During collection the semen tube was maintained at 40°C in a thermo flask. After semen collection, the semen was transported to the laboratory within 15 min of collection. Care was taken during semen collection to minimize contamination by faeces or urine.

Semen volume and pH was measured by using the graduated falcon tube and litmus paper respectively. Sperm concentration was determined using the grey Beckman spectrophotometer (Jenway 6310 spectrophotometer, Bibby Scientific, England) the wavelength being set to 650nm, Knox *et al.*, (2002). Thereafter 3 ml of 2.9% Sodium Citrate solution (pH 7.0) was added to a square cuvette with 15 µl of cockerel semen. The cuvette was placed back in the spectrophotometer and the absorbance read as soon as the reading stabilized. The absorbance was then converted to sperm concentration, using the formula: $(11,170 \times \text{Absorbance}) - 90$, and it was recorded in billion sperm/ml ($\times 10^9/\text{ml}$), (Knox *et al.*, 2002).

Collected semen was also evaluated using the swim-up (10 µl of semen added to 500 µl of swim-up medium) technique for analysis of motility and velocity rate by a Computer Aided Sperm Analysis called 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 X 26 X 1mm, Germany) and covered with warmed cover slip (22 X 22 mm, Germany) on the microscope-warm stage adjusted to 37° C. The sperm motility characteristics were analysed by the Sperm Class Analyzer, Makhafola *et al.* (2012)[®] (Microptic, Spain), at a magnification of X 10 (Nikon, Japan). The kinematic values recorded for each sperm included, in addition to the 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 6.2).

Artificial insemination

The most reliable and successful routine of insemination in poultry is by depositing semen directly in the mid-vaginal area. To achieve this, the vaginal orifice must be

everted, by using gentle abdominal pressure (Cole & Cupps, 1977). The females were inseminated intra-vaginally with the syringe to a depth of 8 cm. Each hen was inseminated twice a week with 0.25ml semen doses with approximately 6.0×10^9 /ml sperm.

Vaginal insemination

The placement of semen into the hen's reproductive tract is accomplished by inverting the cloaca of the hen to expose the opening of the vagina. The insemination pipette is inserted and sperm delivered to a location near the storage sperm tubule (SST). The cloaca is everted by holding the thighs of the hen between the thumb and forefinger while the body rests in the palm of the left hand (Etches, 1996). Hens have to be inseminated for two consecutive days for the first time, and thereafter once a week, if fertile eggs are required. Cockerel semen has a limited life outside the body, and must be deposited in the hen within 1 hour of semen collection. It is recommended to inseminate hens in the afternoon (14:00 and 16:00) as in the morning hens may have an egg in the oviduct, making it difficult for the sperm to swim up to the ovary. Eggs are normally fertile after the second day of insemination, and can remain fertile for 2 or more weeks (Martin, 2004). It has been shown that hens inseminated with freshly collected semen produce more fertile eggs than hens inseminated with frozen/thawed semen (Blanco *et al.*, 2000).

Hatchability

All eggs for incubation were sorted against cracks, morphological deformities, and dirty (soiled). A total of 163 NN, 170 OV and 169 PK chicken eggs were assessed respectively. It is important to note that fertilised eggs and successful hatching

depends on how they are taken care of from laying till setting. The broad end of an egg has an air sac through which the egg breathes. Eggs should be stored with the broad end facing upwards. The egg shell is porous and has little holes which if blocked may suffocate the embryo (baby chick). To prevent rotting, eggs must be stored in a clean and dry place.

Management of day old chicks

The weight of all hatching eggs was measured in grams by using an electronic digital balance, Teraoka, South Africa (Pty) Ltd. Hatchery sanitation was strictly maintained during the experimental period. To prevent high mortality, chicks were kept in a safe, warm and clean environment. On day 21, the number of hatched chicks including the normal, weak, abnormal and dead unhatched chicks eggs and pips were counted and recorded separately per breed. The chicks that were underdeveloped, poorly feathered, parrot-beaked, blind, lame, open-naveled etc. were considered abnormal chicks. After discarding all abnormal chicks the rest of the chicks were considered normal.

6.4 STATISTICAL ANALYSIS

Statistical analyses were performed using the general linear models procedure of the Statistical Analysis System (SAS, 1982). Percentage data were subjected to arcsine or square root transformation for analyses. Duncan's multiple range test was used to indicate differences ($P < 0.05$) between means.

Table 6.2 Sperm Class Analyser[®] (V.5.0) settings used to analyse the sperm motility and velocity characteristics

Parameters	Settings
Contrast	169
Brightness	470
Image/second	50
Optic	pH-
Chamber	Cover slide
Scale	10x
Particle area	5<190 μm^2
Slow	<10 $\mu\text{m/s}$
Medium	<50 $\mu\text{m/s}$
Rapid	<100 $\mu\text{m/s}$
Progressivity	>70% of straightness
Circular	<50% of linearity
Connectivity	18
Velocity on the average path line	7 $\mu\text{m/s}$
Number of images	30

6.5 RESULTS

There was a significant ($P>0.05$) effect of body weight on all three breeds. Statistically from Table 6.3, semen volume of NN and PK were similar ($P>0.05$), while that of NN and OV were entirely different ($P<0.05$). Semen pH of OV was higher differed significantly ($P<0.05$) from those NN and PK. PK had the highest sperm concentration which compared ($P<0.05$) with that of NN but differed ($P<0.05$) from sperm concentration of OV (Table 6.3). From Table 6.3, Egg hatchability of PK, OV and NN were statistically similar ($P<0.05$).

Table 6.3 Means of macroscopic evaluation of Indigenous cockerels semen characteristics (Mean±SD)

Cockerel breeds (n)	Number of ejaculates	Body weight (kg)	Ejaculate volume (ml)	Semen pH	Sperm Concentration (x 10 ⁹ /ml)	Total Motility (%)
NN (11)	66	2.0±0.3 ^c	0.5±0.2 ^a	6.8±1.3 ^b	6.3±3.2 ^a	76.4±22.2 ^c
OV (11)	66	2.5±0.4 ^a	0.3±0.2 ^b	7.7±0.9 ^a	3.8±6.2 ^b	95.0±7.2 ^a
PK (11)	66	2.3±0.3 ^b	0.4±0.2 ^{ab}	6.6±2.2 ^b	8.0±4.0 ^a	86.0±13.7 ^b

^{a-b}Means in the same column with different letters are significantly different at a 5% level of significance.

NN= Naked Neck, OV= Ovambo and PK=Potchefstroom Koekoek.

Table 6.4 Least square means ($\pm$ SE) of fertility traits of South African Indigenous hens following artificial insemination

Breeds	Average egg weight (g)	Fertility	Hatchability on set eggs (%)	Live chicks (%)	Normal chicks (%)	Chick weight (g)
Naked Neck	37.1 $\pm$ 0.5 ^c	50.6 $\pm$ 0.3 ^b	71.1 $\pm$ 0.6	80.2 $\pm$ 0.5 ^a	75.8 $\pm$ 0.9 ^b	23.5 $\pm$ 0.1 ^c
Ovambo	40.4 $\pm$ 1.0 ^b	52.1 $\pm$ 0.3 ^b	70.2 $\pm$ 0.7	75.4 $\pm$ 0.4 ^a	71.5 $\pm$ 0.3 ^c	32.8 $\pm$ 0.5 ^b
Potchefstroom Koekoek	55.5 $\pm$ 0.6 ^a	68.2 $\pm$ 0.5 ^a	78.6 $\pm$ 0.5	66.0 $\pm$ 0.7 ^c	80.1 $\pm$ 0.3 ^a	37.9 $\pm$ 0.3 ^a

^{a-b}Means in the same column with different letters are significantly different at a 5% level of significance.

NN= Naked Neck, OV= Ovambo and PK=Potchefstroom Koekoek.

6.6 DISCUSSION

Potchefstroom Koekoek (PK) had highest average egg weight, fertility, highest but not significant egg hatchability, normal chicks and chick weight compared to OV and NN. Live chicks of NN and OV were similar but different for PK. However; Naked Neck (NN) had highest semen volume compared to PK and OV. Moreover, PK had the highest sperm concentration while OV had the highest motile sperm. Semen volume obtained in this study (NN = 0.5 ml, OV = 0.3 ml and PK = 0.4 ml) was lower than 0.7 ml reported by Bajpai (1963), and 0.58-0.80 ml reported by Nwaguet *et al.* (1996). Differences in the volume of semen observed can be attributed to breed, age and environmental factors (Ezekwe and Machebe, 2004).

Sperm concentration of Ovambo ($3.8 \pm 6.2 \times 10^9/\text{ml}$) and Naked Neck ($6.3 \pm 3.2 \times 10^9/\text{ml}$) was comparable to the results reported by ($7.90 \pm 1.19 \times 10^9/\text{ml}$) by Cecil and Bakst (1988). However, PK ($8.0 \pm 4.0 \times 10^9/\text{ml}$) had higher sperm concentration compared to their reported results. The difference in sperm concentration might also have caused by different breed used. The semen pH results obtained on this study is in agreement with the previous reported results (Etches, 1998). Moreover, the performance of cockerels used in this study with respect to semen characteristics was within the reported range in literature. A proper evaluation of semen prior to Artificial Insemination is vital to avoid losses in fertility (Peters, 2008).

The mean egg weights of the three indigenous chickens used in the present study were comparably to the results obtained by Senapati *et al.* (1996). According to Msoffe *et al.* (2001) egg weights of Tanzanian local chicken ecotypes range from 37.65 to 45.61 g for medium and heavy breeds. Odula *et al.* (2009) reported egg weight of 45 and 48 g in local chickens in Kenya. The higher egg weight in PK than NN and OV could be attributed to establishment of the breed which was locally

developed, listed by FACT (Ramsey *et al.*, 2002). The curvilinear relationship between egg weight and hatchability was reported previously (Shatokhina, 1975). Moreover, Shatokhina, (1975) recorded hatchability of eggs weighing between 46 and 50g to be 10.5%. It was comparably with Potchefstroom Koekoek with an average egg weight of 55.5 g and had the highest hatchability. Previously, it was indicated that there is a positive correlation between egg weight and hatchability in chicken (Senapati *et al.*, 1996).

6.7 CONCLUSION

Naked Neck (NN) had highest semen volume compared to PK and OV cockerels. However, PK had the highest sperm concentration. In contrast, PK had highest average egg weight, fertility, egg hatchability, normal chicks and chick weight compared to OV and NN. Live chicks of NN and OV were similar but different for PK. Chicken hatched from larger eggs had higher body weight, hatchability and fertility; therefore, breeders or poultry farmers should select bigger egg sizes such as 50-55g for breeding programmes.

6.8 ACKNOWLEDGEMENTS

This study was supported by grants from Department of Agriculture Forestry and Fisheries, National Research Foundation and Agricultural Research Council. The author would like to express thanks to Agricultural Research Council, Germplasm Conservation & Reproductive Biotechnologies staff for their assistance during the experiment.

6.9 REFERENCES

- Amann, R.P. & Hammerstedt, R.H., 1993. In vitro evaluation of sperm quality. An opinion. *Journal of Andrology*. 14, 397-406.
- Bajpai, P.K., 1963. The effect of photoperiodicity on semen characteristics of poultry. *Poultry Science*. 42, 462-465.
- Blanco, M.J., Gee, G., Wildt, D.E. & Donoghue, A.M., 2000. Species variation in osmotic, Cryoprotectant, and cooling rate tolerance in poultry, eagle, and peregrine spermatozoa. *Biology Reproduction*. 63, 1164-1171.
- Bayyari, G.R., Cook, J.R., Harris, G.C., Macy, L.B., Slavik, M.F. & Skeeles, J.K., 1990. Research note: The evaluation of chicken spermatozoa using fluorescent staining in a 96 well format. *Poultry Science*. 69, 1602-1605.
- Buhr, R.J., 1995. Incubation relative humidity effects on allantoic fluid volume and hatchability. *Poultry Science*. 74, 874-884.
- Burrows, W.H. & Quinn, J.P., 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*. 16, 185-191.
- Cecil, H.C. & Bakst, M.R., 1988. Daily output of spermatozoa and extragonadal spermatozoa reserves in turkeys. *Poultry Science*. 67, 327-332.
- Cole, H.H. & Cupps, P.T., 1977. Reproduction in domestic animals. 3rd ed. New York. Academic press.
- Etches, R.J., 1996. Reproduction in poultry. 1st Ed. CAB International, Cambridge, UK.
- Etches, R.T., 1998. Reproduction in poultry. CAB Int., Wallingford, pp: 318.

- Ezekwe, A.G. & Machebe, N.S., 2004. Ejaculate characteristics of two strains of local cocks (Naked-Neck and Frizzle) in Nigeria. In: Proc. 29th Annual Conference. *Nigerian Journal of Animal Production*. 29, 92-95.
- Fourie, H.J., Swatson, H.K., Grobbelaar, J.A.N., Molalagotla, M.N. & Joosten, F.A., 2004. Fowls for Africa. In: Proc. XXII World Poultry Congress, Istanbul, June 8–13.
- Froman, D.P. & McLean, D.J., 1996. Objective measurement of sperm motility based upon sperm penetration of Accudenz[®]. *Poultry Science*. 75, 776-784.
- Gebhardt-Henrich, S.G. & Mark, H.L., 1991. The effect of switching males among caged females on egg production and hatchability in Japanese quail. *Poultry Science*. 70, 1845-1847.
- Hammerstedt, R.H., 1996. Evaluation of sperm quality: Identification of subfertile male and courses of action. *Animal Reproduction Science*. 42, 77-87.
- Hosseinzadeh, M.H., Ebrahimnezhad, Y., Janmohammadi, H., Ahmadzadeh, A.R. & Sarikhan, M., 2010. Poultry by product meal: Influence on performance and egg quality traits of layers. *International Journal of Agriculture and Biology*. 12, 547-550.
- Knox, R.V., Tudor, K.W., Rodriguez-Zas, S.L. & Robb, J.A., (2002). Effect of subcutaneous versus intramuscular administration of PG600 on estrual and ovulatory responses of prepubertal gilts. *Journal of Animal Science*. 78, 1732-1737.
- Makhafola, M.B., Umesiobi, D.O., Mphaphathi, M.L., Masenya, M.B. & Nedambale T.L., 2012. Characterization of Sperm Motility Rate of Southern African

- Indigenous Cockerel Semen following Analysis by Sperm Class Analyser. *Journal of Animal Advances*. 2(4), 416-424.
- Martin, R.D., 2004. Artificial insemination of poultry. Poultry Essays. Essay 14.
- Maule, J.P., 1962. The semen of animals and artificial insemination. Commonwealth Agricultural Bureaux England.
- McDaniel, C.D., Hannah, J.L., Parker, H.M., Smith, T.W., Schultz, C.D. & Zumwalt, C.D., 1998. Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. *Poultry Science*. 77, 888-893.
- Msoffe, P.L., Minga, U.M., Olsen, J.E., Yongolo, M.G., Juul-Madsen, H.R., Gwakisa, P.S., & Mtambo, M.M., 2001. Phenotypes including immune competence in scavenging local chicken ecotypes in Tanzania. *Tropical Animal Health and Production*. 33, 341-354.
- Nwagu, B.J., Rekwot, P.I., Abubakar, B.Y., Oni, O.O., Adeyinka I.A. & Egbuedu, U.C., 1996. Semen characteristics of Rhode Island Red and White breeder cocks. *Nigerian Journal of Animal Production*. 23, 1-4.
- OdulaOlwande, P., Ogara, W.O., Okuthe S.O., Muchemi, G., Okoth, E., Odindo, M.O., & Adhiambo, R.F., 2009. Assessing the productivity of indigenous chickens in an extensive management system in Southern Nyanza, Kenya. *Tropical Animal Health and Production*. 41.pg
- Permsak, S., 1996. Effect of water spraying and eggs turning angle to efficiency of duck hatchability. Proceedings of the 34th Kasetsart university annual conference, Bangkok (Thailand), pp: 517, 22- 26.

- Peters, S.O., Shoyebo, O.D., Ilori, B.M., Ozoje, M.O., Ikeobi, C.O.N. & Adebambo, O.A., 2008. Semen quality traits of seven strain of chickens raised in humid tropics. *International Journal Poultry Science*. 7, 949-953.
- Ramsey, K., Harris, L., & Kotze, A., 2000. Landrace breeds: South Africa's indigenous and locally adapted developed farm animals. Ramsey, Harris and Kotzé (eds). Farm Animal Conservation Trust, Pretoria.
- Salisbury, G.W., Van Demark, N.L. & Lodge, J.R., 1978. Physiology of Reproduction and Artificial Insemination of Cattle. 2nd ed.W.H Freeman and Company.
- SAS Institute, Inc. (1982). *SAS user's guide: Statistics, 1982 Edition*. Cary, NC: SAS Institute, Inc.
- Senapati, P.K., DaskMadal, K.G. & Chatterjee, A.K., 1996.Relationship between egg weight, shape index, fertility and hatchability of Japanese quail eggs. *Environmental and Ecological Statistics*. 14, 574-577.
- Shatokhina, S.T., 1975. Relationship of morphological traits of eggs with embryonic and post-embryonic development of different lines of laying hens.Thesis of Candidate of Agricultural Sciences, Kuban Agricultural University, Krasnodar, Russia.
- Snedecor, G.W. & Cochran, W.G., 1980.Statistical methods.7th edition, Iowa State University Press, Ames, Iowa.
- Stromberg, J., 1975. A Guide to Better Hatching.Stromberg Publ. Co. Iowa, USA, pp: 8-25.

- Tabatabaei, S., Batavani, R.A. & Talebi, A.R., 2009. Comparison of oviductal sperm age on fertility, hatchability and embryonic death rates in Iranian indigenous and ross-308 broiler breeder chickens. *Journal of Animal Veterinary Advances*. 8, 85-89.
- Tarongoy, Jr., Eduave, F. & Gemota, E.K., 1990. Age as a factor of hatchability. *SWUCA-J. Agric. Res. (Philippines)*. 5, 22-26.
- Umesiobi, D.O., 2000. Animal Production. I. Basic Principles and Practices. Bean Blaise Publication, Owerri, Nigeria.
- Weis, J., 1991. Analysis of fertility, hatchability and egg quality indices in reproduction breeding of guinea fowls .*Acta Zootechnical Universitatis Agriculturae (CSFR)*. Number 47: 5-15.