ASSESSMENT OF SEMEN QUALITY PARAMETERS OF THREE TANZANIAN NATIVE CHICKENS

A dissertation submitted to Sokoine University of Agriculture in Fulfillment of the Requirements for the Degree of Master of Science in Animal Reproduction and Biotechnology

By

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EXTENDED ABSTRACT

Tanzania has a total chicken population of approximately 92.8 million, of which about 42.7 million are native breeds (*Gallus gallus domesticus*) and 50.1 million are exotic breeds kept primarily for commercial purposes. The poultry industry plays an important role in terms of food security, source of income, and meeting economic and social obligations for the household, especially for poor families. Despite their importance, research on improving productivity of the native chicken's strains is lacking. Therefore, the present study assessed semen quality parameters of freshly collected semen of three different native chicken ecotypes; Ching'wekwe, Kuchi and Morogoro-medium and also investigated the effect of synthetic Gonadotropin releasing hormone (GnRH) on semen quality parameters of Tanzanian native chickens. For assessment of semen quality, twelve roosters (four from each ecotype) with two age groups (11-15 and 24-28 months) were used as semen donors and a total of 192 semen samples were collected from 12 roosters (four from each ecotype) using the abdominal massage technique at weekly interval for four consecutive months. Evaluation of the effect of GnRH treatment on semen quality parameters in three ecotypes of Tanzanian native chickens was performed; where a total of thirty-six mature cockerels from Tanzanian native chicken ecotypes were used. Thirty cockerels (ten from each ecotype) were intramuscularly injected with 0.2 ml of GnRH (Factrel®) once in a week for five consecutive weeks while six (two from each ecotype) were used as a control group only receiving normal saline solution. Semen was collected at weekly interval by abdominal massage technique starting immediately after last GnRH injection for five consecutive weeks. Semen characteristics of individual samples were evaluated. For assessment of semen parameters, volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the ecotypes varied from 0.42±0.04 to 0.52±0.03mL, 7.01±0.00 to 7.02±0.00, 72.81±1.27 to 76.63±1.35%, 3.90±0.98 to 4.12±1.96 x 10^9/mL, 86.16±0.55 to 89.38±0.80% and 88.06±1.13 to 90.97±0.81% respectively. However, only the variations in proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the ecotypes were significant ($P<0.05$). The semen volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the two age groups varied from 0.44±0.03 to 0.52±0.03mL, 7.01±0.00 to 7.02±0.00, 73.88±1.13 to75.92±0.99%, 3.80±0.45 to 4.28±0.32 x 10^9/mL, 87.02±0.58 to 88.15±0.64%, 88.27±0.77 to 89.83±0.77% respectively. Nevertheless, only the variations in semen volume among the two age groups were significant ($P<0.05$). The Pearson correlation coefficients between semen volume and other semen quality characteristics were mostly low to medium with positive values ranging from 0.01-0.51 between semen volume and sperm motility and between morphological normal spermatozoa and proportion of live spermatozoa, respectively. Regarding the effect of GnRH on semen quality, semen parameters increased significantly ($p<0.05$) between control and treatment groups; including semen volume (0.48±0.02 mL versus 0.55±0.02 mL), sperm motility (74.90±0.76% against 80.02±0.30%), concentration (4.04±0.18 × 10^9/mL versus 4.80±0.14 × 10^9/mL), proportion of morphological normal spermatozoa (87.58±0.43% versus 91.25±0.3%) and proportion of live spermatozoa (89.05±0.55% against 91.65±0.31%) but semen pH did not change between control and treatment groups. It can be concluded that although there is minimal variation in semen quality among ecotypes and age groups, all the ecotypes might still be used in breeding purposes to maintain native chickens and semen quality parameters can be improved by injecting GnRH to cockerels and therefore increasing productivity in the poultry industry.

DECLARATION

I, **JULIUS DONATUS LUVANGA,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This study is dedicated to my father the late Donatus Julius Luvanga and my lovely mother Rose Paul Lyimo for their profound love, care and encouragements throughout my studies.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BFAP	Bureau of Food and Agricultural Policy
DPRTC	Directorate of Postgraduate studies, Research, Technology transfer and Consultancy
FSH	Follicle Stimulating Hormone
GIP	Genomics to Improve Poultry
GnRH	Gonadotropin Releasing Hormone
KCAL	Kilo Calories
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
MLDF	Ministry of Livestock Development and Fisheries
SEM	Standard Error of Mean
SPSS	Statistical Product and Service Solutions
SUA	Sokoine University of Agriculture
TAJAS	Tanzania Journal of Agricultural Sciences
URT	United Republic of Tanzania
USA	United States of America

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Tanzania has a total chicken population of approximately 92.8 million, of which about 42.7 million are native breeds (*Gallus gallus domesticus*) and 50.1 million are exotic breeds kept primarily for commercial purposes (URT, 2022). The poultry industry plays an important part in food availability, national income and meeting the needs for poor people (Swai *et al.*, 2007). Native chickens accounts for about 94% of poultry kept by farmers in rural areas contributing to nearly 100% of the poultry meat consumed in the rural areas and 20% of eggs consumed in urban areas (Mushi *et al*., 2020). Native chicken breeds are comparatively adapted to and robust to stressful tropical circumstances of harsh climate and diseases (Msoffe *et al*., 2002) and can be produced with marginal resources of housing, food and veterinary services (Mkpughe and Bratte, 2015).

Therefore, selection of male birds for reproduction is of great importance for poultry industry. It is thus mandatory to monitor semen quality traits routinely to evaluate their reproductive capacity (Banaszewska *et al.*, 2015). The importance of fresh semen evaluation to identify males of different fertilizing abilities is regularly accomplished (Wishart, 2009). Poultry semen quality significantly affects fertility which is in turn affected by genetic composition (Tabatabaei *et al.,* 2009). Semen quality evaluation involves quantitative (macroscopic) and qualitative (microscopic) measures of semen quality parameters. These parameters include the appearance and volume of the semen, concentration, motility, viability and morphology of the sperm (Galal, 2007). Several studies have reported that the semen quality in roosters is influenced by various factors which include nutrition (Tadondjou *et al*., 2013), season (Elagib *et al.,* 2012), frequency of ejaculation, hormones (Fathi, 2000), duration of photoperiods (Almahdi *et al.,* 2014), breed or strain (Oke and Ihemeson, 2010; Tarif, 2013) and age (Shanmugam *et al.,* 2012).

1.2 Reproductive Physiology of Roosters

Spermatogenesis is initiated by sufficient secretion of Gonadotropin Releasing Hormone (GnRH) from the hypothalamus, the secretion of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) by the anterior lobe of the pituitary gland and the secretion of the gonadal steroids (testosterone and estrogen). LH acts on the Leydig cells within the testes to excite the production of progesterone, which is converted to the male sex hormone testosterone (Etches, 1996). Testosterone within the seminiferous tubules is vital for spermatogenesis, while the Leydig cells become insensitive sustaining high levels of LH (Etches, 1996).

Development of the secondary sex characteristics and normal mating behaviour in the males requires testosterone. This hormone also helps in spermatocytogenesis, transport of sperm and deposition of sperm in the female reproductive tract (Hafez and Hafez, 2000). Once cockerel reaches maturity, the secretion of testosterone is prompted by the increasing concentration of circulating gonadotropins (Etches, 1996). LH is important in stimulating the Leydig cells to secrete testosterone and other androgens (Hafez and Hafez, 2000). FSH supports spermatogenesis to the secondary spermatocytes phase by acting on the germinal cells in the seminiferous tubules of the testis.

1.3 Spermatogenesis

Spermatogenesis is the process of both cell division and differentiation by which spermatozoa are produced in the seminiferous tubules of the testes and it comprises of two stages, namely spermatocytogenesis and spermiogenesis (Hafez and Hafez, 2000). The number of spermatozoa produced is reliant on the number of nurse cells and interstitial cells present. The Golgi apparatus is among of the cell organelles, situated near the sperm nucleus and which give rise to the subcellular organelle known as the acrosome. The acrosome progresses and forms a cap over the anterior portion of the nucleus and spreads until it covers two-thirds of the anterior nucleus (Etches, 1996). Maturation phase is characterized by the spermatids being completely differentiated with the final formation of the flagella, assembly of mitochondria in the midpiece and the neck piece and complete condensation and shaping of the nucleus (Hafez and Hafez, 2000).

1.4 Role of GnRH

Gonadotropin Releasing Hormone (GnRH) is the key controller of the reproductive hormonal cascade and was initially isolated from mammalian hypothalamus (Sharp and Gow, 1983). The other name of GnRH is Luteinizing Hormone Releasing Hormone (LHRH). GnRH controls the reproductive system by stimulating the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Sharp and Gow, 1983). GnRH after been produced in the hypothalamus it is carried in a pulsatile fashion to the anterior pituitary gland through the hypothalamo-hypophyseal portal axis to bring about the release of the gonadotropins, LH and FSH, which, in turn, affect gonodal function (Clayton, 1989). The action of GnRH is accomplished by binding to and activation of its high affinity receptors on the pituitary (Clayton, 1989). The pulsatile programed and concentration levels of GnRH are vital for the maintenance of steroids hormone synthesis and for normal reproductive function (Conn *et al*., 1985). Testicular function in avian species and human is controlled by gonadotropin releasing hormones (GnRH) secretion which is responsible for stimulating the cells of the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Sharp and Gow, 1983). Therefore, the administration of a synthetic GnRH may result in the continued release of LH from the anterior pituitary gland and the production Leydig cell enzymes capable of converting cholesterol into testosterone, hence affecting semen quality parameters in roosters.

1.5 Factors Affecting Semen Quality Parameters

Semen quality parameters in chicken are affected by different factors such as breed, age, feed and environmental stressors like temperature and humidity (Karaca *et al*., 2002; Shanmugam *et al*., 2012). There are other several issues that may influence the production of semen and therefore thorough knowledge of the physiology of rooster reproduction is essential to enable an understanding of male fertility (Anderson, 2018). There are also numerous external and internal influences that may affect the production of semen in roosters. Some of the external factors affecting reproductive efficiency in the cocks includes; feed, management, the normal physiological processes that regulate the activities of spermatogenesis and factor that influence the degree to which the birds will respond to the abdominal massage technique during semen collection (Maule, 1962). Others are breed and seasonal differences (Saeid and Al-Soudi, 1975). According to Wishart, (2009) it was found that semen of older birds had significantly lower motility, viability and mass movement than younger birds. Also according to (Long *et al.*, 2010) stated that poultry semen quality decreased with age. Reports on Iranian indigenous broiler (Tabatabaei *et al*., 2009) and 8 pedigreed lines (Long *et al.,* 2010)

indicated that younger chickens produce semen of greater motility than older chickens. Other researchers investigated the effect of nutrition on semen quality and they found that dietary manipulation especially mineral supplementation influences semen output and functions in domestic chickens (Okoro *et al*., 2016).

1.6 Semen Volume

Chicken semen volume compared to other livestock is relatively low because avian species lack sex accessory glands which are well established in mammals (Almahdi *et al.*, 2014). Although semen volume does not necessarily relate to fertilizing ability or viability of the spermatozoa, the volume cannot be abandoned in semen evaluation for the semen serves as the transportation medium for the spermatozoa. According to (Tamanini, 2002) roosters produces between 0.1 and 1.5 mL per ejaculation, with 0.6 mL being the average ejaculate volume recorded. Sometimes different roosters of the same species often produce different volumes of semen at different times (Anderson, 2018). The mean volume ejaculated using the abdominal massage method is around 0.25mL (Hafez and Hafez, 2000). According to (Bah *et al.,* 2001) they found the average semen volume ejaculated by abdominal massage technique to be 0.28 ± 0.14mL. Though, the documented semen volume was found to range between 0.37 ± 0.02 and 0.73 ± 0.01 mL (Peters *et al.,* 2008). Low semen volume (hypospermia) is regularly linked with several influences like blockage of vas deferens or seminal vesicle and hormonal imbalance. Conversely, high semen volume (hyperspermia) is a signal of hormonal disparity due to the presence of certain steroids (Rengaraj *et al*., 2015). According to Okoro *et al.* (2016) dietary manipulation especially mineral and vitamin supplementation influences semen output and functions in domestic chicken. Furthermore, semen quality and volume are influenced by breed and strain of chicken (Haunshi *et al*., 2011;Shanmugam *et al*., 2012). Age is another factor which affects the semen volume. Researchers reported an increase in semen volume in broiler roosters starting from from 24 to 48 weeks of age (Shanmugam *et al.,* 2012) and a decline in semen volume in White Leghorn cocks with advancing age (46 weeks and above) (Clark and Sarakoon, 1967).

1.7 Semen pH

The semen pH varies to a smaller extent between different bird strains and species. The optimum semen pH ranges between 7.0 and 7.4. Variations in semen pH may be caused by many factors. The pH, especially that of ejaculated semen is dependent on several secretions involved. Poor quality semen generally contains large amounts of fluid from the accessory glands, which increases the semen pH (Salisbury *et al*., 1978). Semen samples that contain many dead spermatozoa may evolve to ammonia, which will also increase the pH (Salisbury *et al*., 1978). According to Haunshi *et al.* (2010) they found that there were no significant differences between different genetic groups and chicken strain on semen pH.

1.8 Sperm Concentration

The number of spermatozoa found per unit volume (mL) of semen defines the sperm concentration (Malejane *et al.,* 2014). Conferring to Tamanini (2002), concentration can be used when scheduling artificial insemination in a flock to forecast the number of breeding hens to be inseminated. Semen collected from domestic cockerel contains an average sperm concentration of 3000 - 7000 $\times 10^6$ sperm/mL (Hafez and Hafez, 2000). Sperm concentration tends to decrease with age (Cerolini *et al.,* 1997) although a study done by Long *et al*. (2010) showed that sperm count per ejaculate increased with age. Studies on the injection of three doses of GnRH analogue (gonadorelin diacetate

tetrahydrate) every 2 days have been found to increase testosterone concentration and have reduced the semen collection period and increased the sperm concentration in the ejaculate of camels (Monaco *et al.,* 2015).

1.9 Sperm Morphology

Evaluation of sperm morphology is of great value in poultry breeding since it is regularly used in estimating the fertilizing capability of the spermatozoa (Lukaszewicz, 1988). Generally the spermatozoon comprises of an acrosome, head, midpiece and tail. The sperm head contains the nucleus which is responsible for carrying the genetic information to the next generation. According to Bakst and Brillard (1994) only sperms with normal morphology can go up through the vagina of the hen to the sperm storage tubules. The sperm morphology of poultry semen differs from that of mammals. However a difference also exists between birds, even though the shape and size of the spermatozoa are similar(Hafez and Hafez, 2000). In poultry the spermatozoon is surrounded by the cytoplasmic membrane and the acrosome has an inner spine surrounded by a conical shaped cap. The head of the sperm contains the genetic material of the gamete, while the midpiece consists of mitochondria. The midpiece of cockerel sperm is considerably longer, compared to other species, approximately one quarter longer and this property makes poultry sperm to have more midpiece bending than other species. According to Alkan *et al.* (2002) the in vitro assessment of morphological sperm defects include; neck bending (mid piece bending), mid piece damage, acrosome damage (bending, swelling, knotting or rounding), total head swelling and tail defects. A study performed by Feyisa *et al.* (2018) reported that breed differences can also influence the morphology of chicken spermatozoa. Moreover, the highest percentages of the observed morphological defects are at the head and tail segments of the spermatozoa in all breeds studied (Feyisa *et al.,* 2018). (Feyisa *et al.,* 2018) also reported that bent, coiled, detached, broken and knotted were common specific morphological defects in all breeds studied.

1.10 Sperm Motility

Sperm motility can be classified as progressive (forward direction) or non-progressive (random movement) movement. Generally, progressive motility is determined subjectively at room temperature using a microscope at low magnification or objectively using a computer-assisted semen analysis system (Long *et al.,* 2010). Evaluation of sperm motility is an indicator of sperm viability and quality of semen sample collected. Evaluation of sperm motility can be performed with fresh and diluted semen, and then observed under the light microscope (Hafez and Hafez, 2000). With fresh semen it is difficult to observe individual sperm motility patterns. Studies on the administration of two daily doses of GnRH for 7 weeks in rams have been found to increase testosterone concentration, scrotal circumference and the percentage of sperm with progressive motility in the ejaculate (Schanbacher and Lunstra, 1977). Moreover, it was also shown that exogenous GnRH was shown to improve sexual behavior and increase the quality of frozen/thawed spermatozoa in fertile stallions during the non-breeding season.

1.11 Sperm Viability

When a sperm is viable it means it possess an intact cell membrane and is regarded to be functional. In short, sperm viability is the proportion of live sperm in the semen sample. Cell membrane integrity is often determined by using either a dead cell or a live cell stain alone or simultaneously. The dead cell stains are excluded by sperm with an intact cell membrane but stain dead sperm possessing a disrupted cell membrane. Live cell stains permeate the intact sperm membrane and become visible only after reacting

with cytosolic enzymes or interacting with sperm nuclear proteins. Eosin-Nigrosin stain is a commonly used method to determine sperm viability (Long *et al.,* 2010). In a nutshell, sperms are stained with Nigrosin/Eosin and a smear of the stained sperm is made on a slide. When observed under a bright field microscope the viable sperm remain colourless, while eosin will stain dead sperm a pink to magenta colour. The Nigrosin works as a background to enhance differentiation between the non-viable and viable sperm. According to Wishart (2009) it was found that semen of older birds had significantly lower viability than younger birds. The number of live sperms was found to increase from early age to mid age in broiler breeder roosters (Shanmugam *et al.,* 2012).

1.12 Problem Statement and Justification

Semen assessment/evaluation is considered as the most important clinical test for identifying and predicting distinct cases of fertility, infertility, or potential subfertility. Several studies on semen quality evaluation in domestic chicken have reported that, genetic composition, age and hormonal treatment can significantly affect semen quality parameters. There is, however, lack of data on effects of genetic composition, age and hormonal treatment on semen quality parameters of Tanzanian native chickens. Therefore, the present study addressed the effect of ecotype and age on semen quality parameters of three native chicken ecotypes commonly kept in Tanzania and evaluated the effects of hormone treatment on semen quality parameters.

1.13 Objectives

1.13.1 Main objective

The main objective of this study was to assess and compare the semen quality parameters of three different native chicken ecotypes namely Kuchi, Ching'wekwe and Morogoro-medium.

1.13.2 Specific objectives

i. Assessing the effect of ecotype and age on semen quality parameters of three local chicken ecotypes
ii. Assessing the effect of Gonadotropin releasing hormone (GnRH) analogue on semen quality parameters of three local chicken ecotypes

1.14 Organization of the Dissertation

This dissertation was prepared based on "publishable manuscripts" format of the Sokoine University of Agriculture. Chapter one contains the introduction, literature review, problem statement and justification, and objectives of the study. Chapter two contains a paper published in peer-reviewed scientific journal; the paper addressed the first specific objective. Chapter three contains a paper submitted in peer-reviewed scientific journal and this paper addressed the second specific objective. The final chapter of the dissertation draws overall discussions, conclusions and recommendations.

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CHAPTER TWO

PAPER I

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Effect of ecotype and age on semen characteristics of three Tanzanian native chickens

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Abstract

Several findings on the semen characteristics of domestic chickens have revealed that ecotype and age significantly affect semen quality. There is, however, lack of data on effects of ecotype and age on semen characteristics of Tanzanian native roosters. This study evaluated the effect of ecotypes (Ching'wekwe, Morogoro-medium and Kuchi) and ages (11-15 and 24-28 months) on semen quality. A total of 192 semen samples were collected from 12 roosters (four from each ecotype) using the abdominal massage technique at weekly interval for four consecutive months. Semen characteristics of individual samples were evaluated. The semen volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the ecotypes varied from 0.42±0.04 to 0.52±0.03mL, 7.01±0.00 to 7.02±0.00, 72.81±1.27 to 76.63±1.35%, 3.90±0.98 to 4.12±1.96 x 10^9/mL, 86.16±0.55 to 89.38±0.80% and 88.06±1.13 to 90.97±0.81% respectively. However, only the variations in proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the ecotypes were significant ($P<0.05$). The semen volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the two age groups varied from 0.44±0.03 to 0.52±0.03mL, 7.01±0.00 to 7.02±0.00, 73.88±1.13 to75.92±0.99%, 3.80±0.45 to 4.28±0.32 x 10^9/mL, 87.02±0.58 to 88.15±0.64%, 88.27±0.77 to 89.83±0.77% respectively. However, only the variations in semen volume among the two age groups were significant ($P<0.05$). The Pearson correlation coefficients between semen volume and other semen quality characteristics were mostly low to medium with positive values ranging from 0.01-0.51 between semen volume and sperm motility and between morphological normal spermatozoa and proportion of live spermatozoa, respectively. Although there is minimal variation in semen quality among ecotypes and age groups, all the ecotypes might still be used in breeding purposes to maintain native chickens, because the results found were within the reference range for chickens.

Keywords: *Ecotype; Eosin-nigrosin; Gallus gallus domesticus; local chickens, semen quality*

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Introduction

Tanzania has a total chicken population of approximately 92.8 million, of which about 42.7 million are native breeds (*Gallus gallus domesticus*) and 50.1 million are exotic breeds kept primarily for commercial purposes (URT, 2022). Native chickens accounts for about 94% of poultry kept

by farmers in rural areas contributing to nearly 100% of the poultry meat consumed in the rural areas and 20% of eggs consumed in urban areas (Mushi *et al.*, 2020). Native chicken breeds are comparatively adapted to and robust to stressful tropical circumstances of harsh climate and diseases (Msoffe *et al.*, 2002) and can be produced with marginal resources, such as housing, food and veterinary services (Mkpughe & Bratte, 2015). In developing countries like Tanzania, poultry industry plays an important part in food availability, national income and meeting the needs for poor people (Swai *et al.*, 2007). Despite their significance, study on improving production of the native chicken's strains is lacking (Kondombo *et al.*, 2003). Tanzania has more than 17 ecotypes of native chickens (Msoffe *et al.*, 2004; Guni *et al.*, 2013) and majority of these ecotypes have not been well studied and most importantly their semen output potential is poorly known.

Selection of cocks/roosters for breeding is of importance for poultry business and semen quality evaluation is one of the components that should not be overlooked. Hence it is important to evaluate semen quality routinely to assess the reproductive ability of males that will be used for breeding purposes (Banaszewska *et al.*, 2015). Semen evaluation involves measures of semen quality parameters such as semen colour, volume, sperm motility, concentration, viability and morphology of spermatozoa (Galal, 2007).

Studies have reported that semen quality in chicken is affected by various aspects such as breed or strain (Oke and Ihemeson, 2010; Tarif *et al.*, 2013), age (Shanmugam *et al.*, 2012), type of feed (Tadondjou *et al.*, 2013), season (Elagib *et al.*, 2012), endocrine disrupting chemicals (Rengaraj *et al.*, 2015) and duration of photoperiod (Almahdi *et al.*, 2014). Therefore, the purpose of the current study was to evaluate the effect of ecotype and age on semen quality parameters of freshly collected semen from three native chicken ecotypes namely; Kuchi, Ching'wekwe and Morogoro-medium kept in Tanzania.

Materials and methods

Study area

The current study was conducted at the experimental poultry farm of the Sokoine University of Agriculture (SUA), Morogoro, Tanzania. SUA is located 3 km south from the centre of Morogoro town. Morogoro town is in the eastern part of Tanzania with Latitude of 6°49'15" S and Longitude of 37°39'40" E, elevation above sea level is 504m, and with mean annual temperature and rainfall of 24.3 °C (16.6-32.7°C) and 935 mm respectively. The mean annual relative humidity is 68% (62.62-84.87%).

Experimental birds

Three ecotypes of native chicken namely; Ching'wekwe, Morogoro-medium and Kuchi were used in this study. A total of 12 roosters (four from each chicken ecotype) of two different age groups (11-15 and 24-28 months) (6 roosters from each age group) were randomly selected from a heterogeneous native chicken population of 50 birds maintained at the experimental poultry farm. This population originated from the lake (Kuchi), eastern (Ching'wekwe and Morogoro-medium), central (Kuchi) and northern (Ching'wekwe and Morogoro-medium) zones of Tanzania. The body weight of Ching'wekwe, Kuchi and Morogoro-medium roosters at the beginning of the experiment ranged between 1.7-2.5, 1.8-3.5 and 2.0-3.1kg, respectively. The chosen roosters were matured enough (11 to 28 months old), apparently healthy and without any physical faults.

Ethical clearance

Ethical clearance on the use of birds was provided by the College of Veterinary Medicine, Sokoine University of Agriculture Ethical Committee Approval reference number DPRTC/R/186/F26.

Management of experimental birds

Experimental roosters used in this study were kept in separate breeder cages (40 × 40 × 60 cm) in an open-sided house with natural light hours (12 hours). The roosters were offered home-made feed (18% crude protein and 2800 Kcal Kg^{-1} metabolizable energy) and fresh water *ad libitum* throughout the experimental duration. All birds were routinely vaccinated against Newcastle

Disease, Fowl pox and Infectious bursal disease and were dewormed after every three months.

Figure 1. Photographs of A -Ching'wekwe, B- Morogoro-medium and C-Kuchi cock ecotypes, Morogoro, Tanzania

Semen collection

Semen was collected at weekly interval from each rooster for four consecutive months starting from November 2021 to February 2022. Semen was collected in a graduated plastic tube using a non-invasive method of massaging the abdomen as previously explained by Burrows and Quinn, (1937). Semen was collected at around 08:00 to 09:00 hours on each day of semen collection and immediately after collection, tubes with semen were kept in a water bath maintained at 37°C and the analysis started just after two to three minutes. To avoid investigator bias, a single researcher was used to collect and examine semen during the whole study period. Semen sample collection and assessment was done at room temperature.

Semen evaluation

Semen volume was evaluated using graduated (millilitre) plastic tubes. The pH of semen was assessed using a calibrated pH meter (Ultra Basic-5, Denver Instrument) immediately after semen collection.

Sperm motility

Motility was evaluated on the principle of percentage of sperm showing frontward motion as previously described by Tadondjou *et al.*, (2013). In summary, 2 µL of neat semen was mixed with 100 µL of phosphate-buffered saline on a clean; grease free, warmed glass slide (37^{O}C) and a cover slip was put on top before examination under light microscope at 400x magnification. The proportion of motile spermatozoa was individually assessed to the nearest 1% on a scale of 0 to 100% and at least 3 microscopic fields were observed. For each sample, motility was expressed as the percentage of motile spermatozoa with moderate to rapid progressive forward movement.

Sperm concentration

Sperm concentration (billions per millilitre) in the semen was assessed by the direct cell count technique using Neubauer counting chamber (Haemocytometer). Before assessment, semen sample was diluted with phosphate-buffered saline at a ratio of 1:100. The haemocytometer was then loaded with diluted semen through the capillary action of the pipette and loaded haemocytometer was finally observed under microscope at 400x magnification. The head of the sperm that fell within the smaller squares at the four edges and centre of the haemocytometer were counted. The concentration of spermatozoa per millilitre was calculated using the formula; Concentration of spermatozoa per millilitre = 50, 000 x Number of spermatozoa counted x Dilution factor, as formerly explained by Ax *et al.*, (2000).

Viability and abnormal sperm

The proportion of live and dead spermatozoa was assessed by differential staining method using Eosin–Nigrosin stain (5% eosin, 10% nigrosin) as formerly explained by Campbell *et al.*, (1953). In brief, 5 µL of semen sample was mixed with 100 µL of Eosin-Nigrosin stain then thin smears were prepared from this mixture and fixed by air-drying the slide at room temperature. For each particular slide, about 200 spermatozoa were observed at 1000x magnification using oil immersion. The spermatozoa which appeared pink in colour (stained with eosin) were regarded as dead while spermatozoa which appeared colourless (no penetration of eosin stain) were regarded as live. Furthermore, the thin Eosin-Nigrosin stained smears were also used to assess spermatozoa morphological defects. The abnormalities of the head, mid-piece and tail of the spermatozoa were examined and at least 200 spermatozoa were observed from each sample. A morphologically normal spermatozoon was considered to be free from any acrosome, head, mid-piece and tail defects.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version: 20.0.0 software was used to analyse the data. Analysis of variance (ANOVA) was used to look for an overall variation in rooster semen quality parameters across ecotypes and age groups. Thereafter, statistically important main effects (ecotype and age) were matched with Tukey's post hoc multiple comparisons. The data were portrayed as Mean± SEM and the differences in parameters were regarded as significant when the $P<0.05$. Estimates of correlation coefficients were used to establish relationships between roosters' body weight and semen parameters, as well as between parameters themselves.

Results

Semen quality parameters among roosters of the three ecotypes

Comparative effect of ecotypes (*i.e.*, Ching'wekwe, Kuchi and Morogoro-medium) on the semen quality is presented in Figure 2 and 3. The mean of semen volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa of Ching'wekwe, Kuchi and Morogoro-medium were 0.42±0.04mL, 7.01±0.00, 72.81±1.27%, 4.11±1.96 x 10^9/mL, 86.16±0.55% and 88.13±0.79%; 0.51±0.03mL, 7.02±0.00, 76.63±1.35%, 3.90±0.98 x 10^9/mL, 89.38±0.80% and 90.97±0.81%; and 0.52±0.03mL, 7.02±0.00, 75.25±1.26%, 4.12±0.87 x 10^9/mL, 87.22±0.79% and 88.06±1.13%, respectively. However, only the variations in proportion of spermatozoa with normal morphology and proportion of live spermatozoa among ecotypes of roosters were significant ($P<0.05$). Kuchi ecotype had the highest proportion of spermatozoa with normal morphology (89.38±0.80%) followed by Ching'wekwe (87.22±0.79%) and Morogoro-medium (86.16±0.55%). Similarly, Kuchi ecotype again had the highest proportion of live spermatozoa followed by Ching'wekwe and then Morogoro-medium with corresponding mean values of 90.97±0.81, 88.13±0.79 and 88.06±1.13% respectively. Representative image of live/dead spermatozoa of three Tanzanian native roosters is shown in Figure 4. Regarding semen volume, although the variation was not statistically significant ($P > 0.05$), Morogoro-medium ecotype had the highest semen volume (0.52±0.03mL) followed by Kuchi (0.51±0.03mL) and then Ching'wekwe (0.42±0.04mL). Furthermore, Morogoro-medium ecotype again had the highest sperm concentration followed by Ching'wekwe and Kuchi with corresponding mean value of 4.12±0.87, 4.11±1.96 and 3.90±0.98 x 10^9/mL, respectively. In addition, Kuchi ecotype had the highest sperm motility followed by Morogoro-medium and then Ching'wekwe with corresponding mean values of 76.63±1.35, 75.25±1.26 and 72.81±1.27% respectively. The means of semen pH showed insignificant variation ($P>0.05$) between ecotypes. All ecotypes' semen pH was slightly alkaline, ranging from 7.01±0.00 for Ching'wekwe to 7.02±0.00 for Kuchi and Morogoro-medium ecotypes.

Semen quality parameters among roosters of two age groups

The mean of semen volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa in 11-months age group were 0.52±0.03mL, 7.01±0.00, 75.92±0.99%, 4.28±0.32 x 10^9/mL, 88.15±0.64% and 89.83±0.77%

respectively. The mean of semen volume, pH, sperm motility, sperm concentration, proportion of spermatozoa with normal morphology and proportion of live spermatozoa in 24-months age group were 0.44±0.03mL, 7.02±0.00, 73.88±1.13%, 3.80±0.45 x 10^9/mL, 87.02±0.58% and 88.27±0.77% respectively.

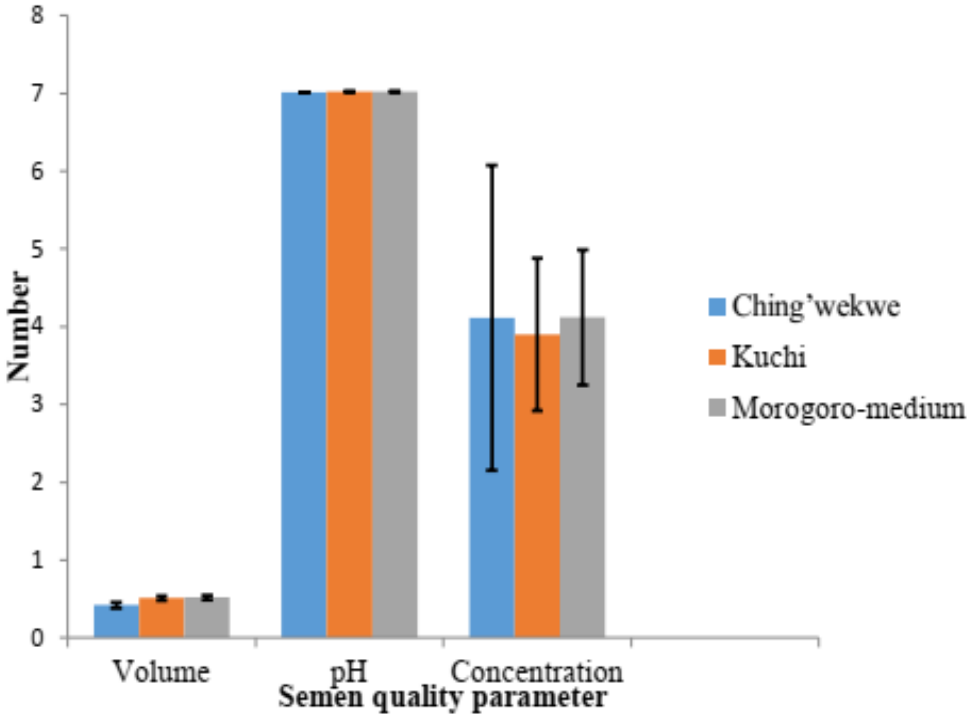

Figure 2. Comparison of ejaculate volume, semen pH and sperm concentration (nx10⁹/mL) among the three rooster ecotypes, Morogoro, Tanzania

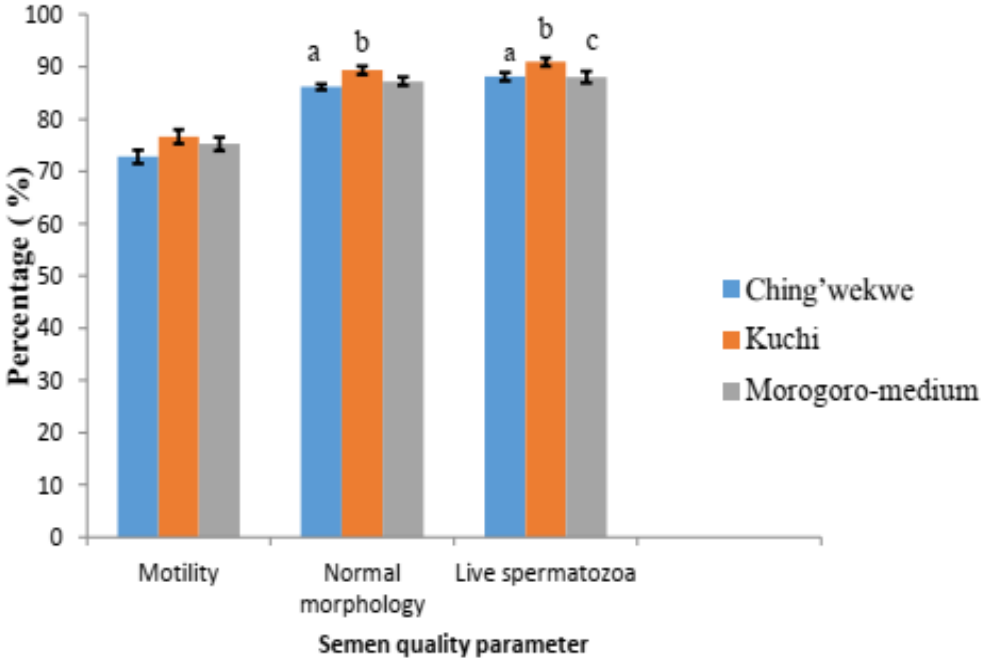

Figure 3. Comparison of sperm cell motility, proportion of morphological normal and of live spermatozoa in fresh semen among three Tanzanian native rooster ecotypes.
^{a,b,c} Mean values with dissimilar letters differ significantly ($p<0.05$), and error bars denote SEM

For the influence of age on semen quality, the results showed that the age of native roosters, either between 11 and 15 months or between 24 and 28 months had significant influence on semen volume ($P < 0.05$) and no other semen quality parameters (Figures 5 and 6). Roosters of studied ecotypes of between 11 to 15 months had the highest semen volume than those of between 24 to 28 months with corresponding mean values of 0.52±0.03 and 0.44±0.03mL respectively. All other parameters decreased with the age of the roosters although the dissimilarity was not statistically significant ($P > 0.05$).

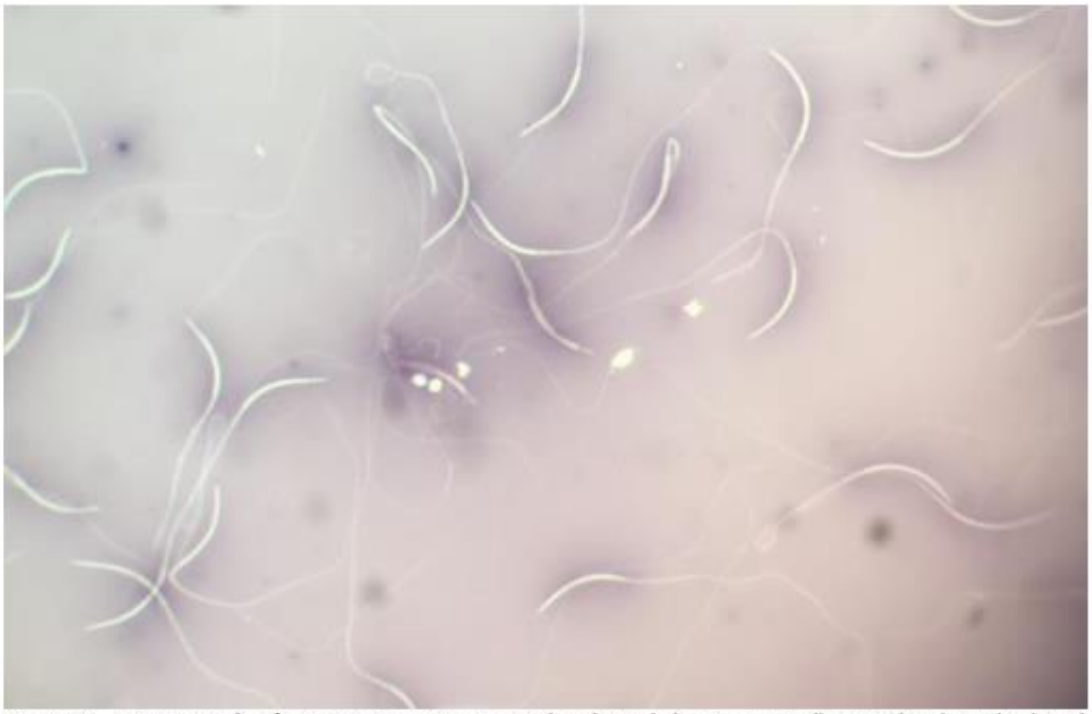

Figure 4. Micrograph of rooster spermatozoa, pink coloured (Eosin stained) considered as dead and colourless (without eosin penetration) considered as live (Eosin Nigrosin Stain, 1000X)

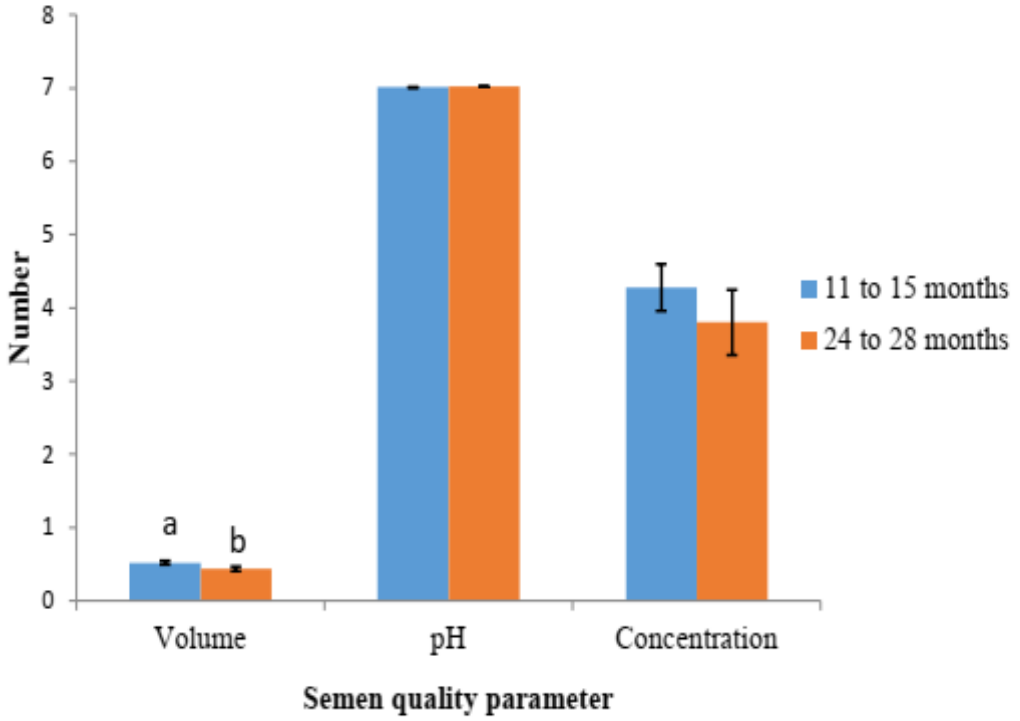

Figure 5. Comparison of ejaculate volume (mL), semen pH and sperm concentration (nx10⁹/mL) among the two age groups of Tanzanian native rooster ecotypes
[a,b,c] Mean values with dissimilar letters differ significantly ($p<0.05$), and error bars denote SEM

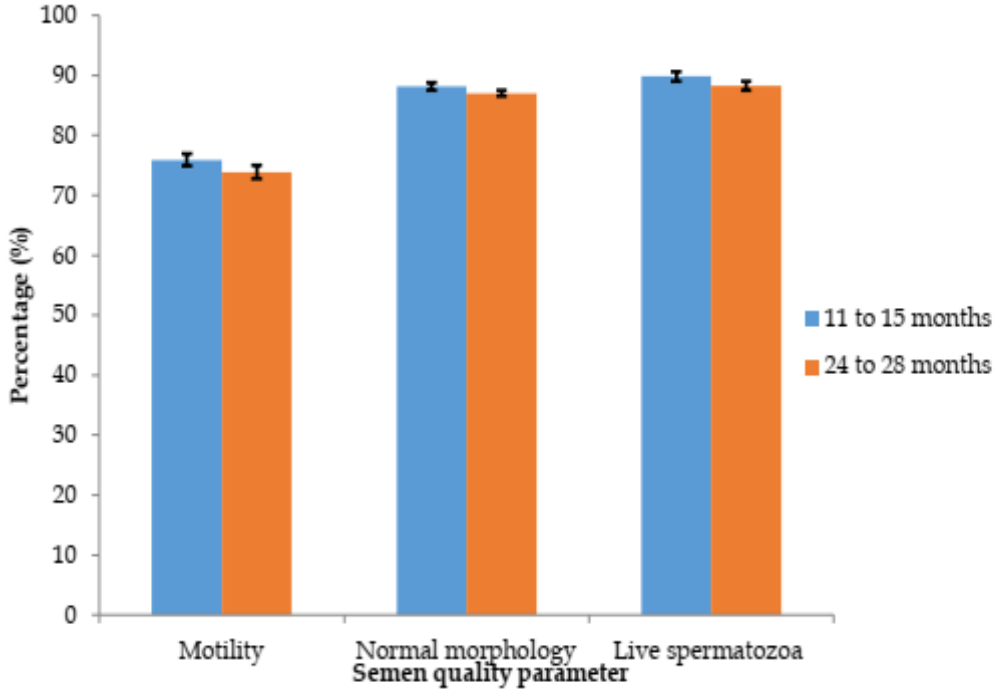

Figure 6. Comparison of sperm motility, proportion of morphological normal and proportion of live spermatozoa among the two age groups of Tanzanian native rooster ecotypes

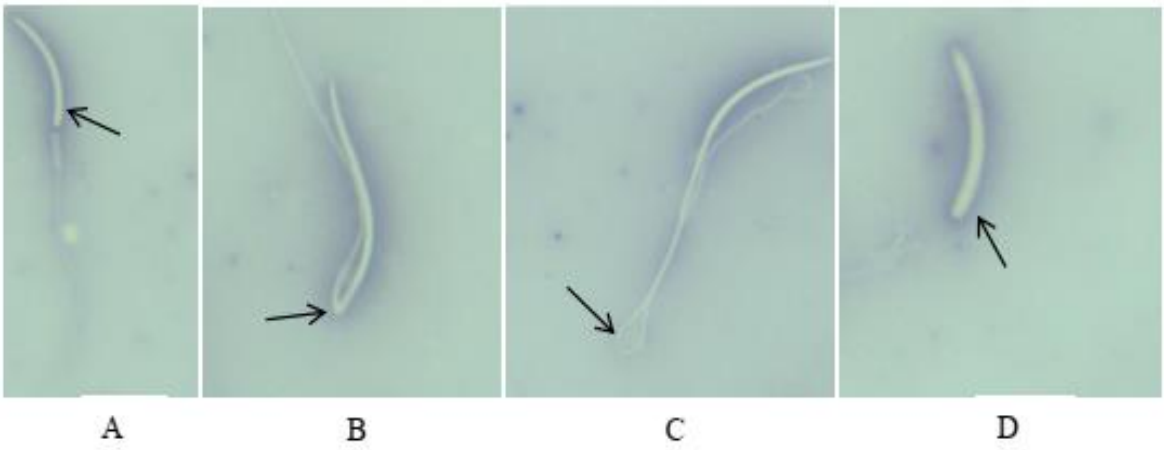

Figure 7. Micrograph of normal and abnormal morphology of Tanzanian native rooster spermatozoa (Eosin Nigrosin stain, 1000X immersion oil) (A) Normal morphology, (B) Bent midpiece, (C) Coiled tail, (D) Loose head/Detached head

Percentage of spermatozoa morphological abnormalities recorded in three Tanzanian native chicken ecotypes

The proportions of total spermatozoa morphological defects recorded in this study in all three ecotypes are summarized in Table 1. The normal and some common abnormalities of Tanzanian native rooster spermatozoa are shown in Figure 7.

Table 1. Percentage of total sperm cell abnormalities recorded in three Tanzanian native chicken ecotypes

Sperm cell abnormality	Percentage abnormality (%)
Bent midpieces	50
Coiled tails	20
Bent tails	15
Detached heads	6
Swollen heads	3
Knotted head	3
Detached tails	3

Ecotype and age interaction effect on semen quality parameters

For semen volume and sperm motility, a substantial interaction ($p< 0.05$) between ecotype and age existed (Table 2). Morogoro-medium had highest semen volume (0.59±0.04mL) at 24-28 months of age than Ching'wekwe and Kuchi ecotypes of the same age. Highest sperm motility of 78.25±1.78% was recorded at 11-15 months of age for the Morogoro-medium whereas the Kuchi and Ching'wekwe roosters had highest sperm motility at 11-15 and 24-28 months of age respectively ($p < 0.05$). A significant interaction ($p< 0.05$) between ecotype and age for the

proportion of live spermatozoa existed (Table 3). Kuchi had highest proportion of live spermatozoa (91.75±1.26%) at 11-15 months of age than Ching'wekwe and Morogoro-medium ecotypes. ***Correlation between semen quality traits and roosters' body weight***

The volume of collected semen increased in proportion to the roosters' body weight. (Pearson correlation coefficient (r) =0.17) and the correlation was statistically significant ($p < 0.05$), while other parameters like sperm concentration and morphological normal spermatozoa had no correlation with body weight (r=0.00). Sperm motility and a proportion of live spermatozoa had very low correlation with body weight of chicken (r=0.01).

Correlation coefficients of semen quality parameters of the three chicken ecotypes

Pearson correlation coefficients of semen quality parameters of the three chicken ecotypes are displayed in (Table 4). The coefficients between semen volume and motility and morphological normal spermatozoa and proportion of live spermatozoa were very low to medium, with positive values ranging from 0.01-0.51. Positive and significant correlations were found between sperm concentration and motility (r = 0.25), morphological normal spermatozoa and motility (r = 0.3), proportion of live spermatozoa and motility (r = 0.31), proportion of live spermatozoa and concentration (r = 0.11), and proportion of live spermatozoa and morphological normal spermatozoa (r = 0.51). The association between sperm concentration and morphological normal spermatozoa was low but significant (r = 0.08).

Discussion

For the effect of ecotype on semen characteristics, the findings from the current study indicate that there was no statistically significant effect of ecotype on semen quality among ecotypes of roosters except for proportion of spermatozoa with normal morphology and proportion of live spermatozoa. In the current study, proportion of live spermatozoa significantly differed among ecotypes of roosters, this finding was similarly reported by (Tarif *et al.*, 2013). The proportion of live spermatozoa in semen sample varied from 88.06 to 90.97% in this study. However, lower proportion of live spermatozoa (72 to 82%) in rooster semen has been stated by Siudzińska and Łukaszewicz, (2008). The difference in proportion of live spermatozoa among ecotype in the current study may be due to genetic disparities in tolerance to stains used for processing. However, the proportion of live spermatozoa in our research was good for breeding purposes in poultry.

The proportion of morphologically normal spermatozoa in rooster semen under this study ranged from 86.16 to 89.38% which is similar to the observation made by Tarif *et al.*, (2013) who reported 87.2 to 90.1% morphologically normal spermatozoa in rooster semen. Nevertheless, the proportion of spermatozoa with normal morphology reported in this study significantly varied among ecotypes of roosters, this finding agrees with that reported elsewhere (Feyisa *et al.*, 2018; Łukaszewicz *et al.*, 2008). However, our findings on sperm morphology mismatch with others (Shanmugam *et al.*, 2012; Tarif *et al.*, 2013; Almahdi *et al.*, 2014; Ameen *et al.*, 2014) who reported insignificant variation in the sperm morphology in different breeds/strains of cockerels. Furthermore, Siudzińska and Łukaszewicz, (2008) reported higher (91 to 94%) morphologically normal spermatozoa obtained from 4 breeds of domestic fowl.

Bird's semen volume is comparatively low than mammals because birds lack sex accessory glands which are well developed in mammals (Almahdi *et al.*, 2014). The semen volume reported in this study ranged from 0.42 - 0.52 mL and the volume did not significantly differ between ecotypes. The semen volume collected is in agreement with the finding of 0.2 to 0.5 mL reported elsewhere (Getachew, 2016). Morogoro-medium and Kuchi ecotypes recorded semen volume of 0.52 and 0.51mL respectively; this can be attributed by their body size because there is a positive association between the body weight and semen volume (Adeyamo *et al.*, 2007). Overall; the strains of roosters with heavier body weights and larger testes produce more spermatozoa and thus may lead to larger semen volume (Adeyamo *et al.*, 2007).

Table 2. Ecotype and age interaction effect on semen volume, semen pH and sperm motility among three Tanzanian native rooster ecotypes

	Semen volume (mL)			pH			Sperm motility (%)		
Ecotype	C	K	M	C	K	M	C	K	M
Age (Months)									
11 to 15	0.56±0.04[a]	0.57±0.04[b]	0.45±0.04[b]	7.01±0.00	7.02±0.00	7.02±0.00	71.38±1.78[a]	78.13±1.78[b]	78.25±1.78[c]
24 to 28	0.28±0.04[a]	0.46±0.04[b]	0.59±0.04[b]	7.01±0.00	7.02±0.00	7.03±0.00	74.25±1.78[a]	75.13±1.78[b]	72.25±1.78[c]
Ecotype x Age (p values)		0.000			0.853			0.044	

[ab]Means on the same row not sharing a common superscript, for each quality trait, differ significantly ($p < 0.05$). Values in the table are mean ± SEM. C- Ching'wekwe, K- Kuchi and M- Morogoro-medium

Table 3. Ecotype and age interaction effect on sperm concentration, proportion of spermatozoa with normal morphology and of live spermatozoa among three Tanzanian native rooster ecotypes.

	Sperm concentration (n × 10^9)/mL			Morphological normal spermatozoa (%)			Live spermatozoa (%)		
Ecotype	C	K	M	C	K	M	C	K	M
Age (Months)									
11 to 15	4.59±0.00	4.15±0.00	4.11±0.00	85.94±1.01	89.75±1.01	88.75±1.01	87.13±1.26[a]	91.75±1.26[b]	90.63±1.26[c]
24 to 28	3.62±0.00	3.66±0.00	4.14±0.00	86.38±1.01	89.00±1.01	85.69±1.01	89.13±1.26[a]	90.19±1.26[b]	85.50±1.26[c]
Ecotype x Age (p values)		0.532			0.216			0.022	

[ab]Means on the same row not sharing a common superscript, for each quality trait, differ significantly ($p < 0.05$). Values in the table are mean ± SEM. C- Ching'wekwe, K- Kuchi and M- Morogoro-medium.

Table 4. Correlation coefficient matrix of semen quality parameters of three Tanzanian native chicken ecotypes

Item	Semen volume	pH	Sperm motility	Sperm concentration	Morphological normal spermatozoa	Live sperm
Semen volume	1.00	0.01	0.01	0.01	0.00	0.03
pH	0.01	1.00	0.00	0.00	0.01	0.00
Sperm motility	0.01	0.00	1.00	0.25*	0.3*	0.31*
Sperm concentration	0.01	0.00	0.25*	1.00	0.08*	0.11*
Morphological normal spermatozoa	0.00	0.01	0.3*	0.08*	1.00	0.51*
Live sperm	0.03	0.00	0.31*	0.11*	0.51*	1.00

*P < 0.05

The variations in semen volume which were observed by different researchers could be attributed by the age of the cocks and breed differences (Peters *et al.*, 2008; Elagib *et al.*, 2012; Tarif *et al.*, 2013; Ajayi *et al.*, 2014), chicken line (Tarif *et al.*, 2013), environmental factors (Saeid and Al-Soudi, 1975) and nutrition (Tadondjou *et al.*, 2013). The insignificant difference between ecotype reported in our study is in line with Sonseeda *et al.*, (2013) who reported that breed had no effect on semen volume in Thai indigenous chickens. However, Ameen *et al.*, (2014) reported a significant variation in semen volume collected from five different Nigerian cockerel ecotypes. The insignificant effect of ecotype on semen volume might be caused by a close genetic make-up of the ecotypes and the same level of management provided to the roosters. Sperm motility and other sperm motion traits are considered to be vital in fertilization capacity of male animals (Verstegen *et al.*, 2002) as they suggest for the ability of sperms to swim from the site where semen is deposited to the storage tubules of the hen. In this study, sperm motility of the three ecotypes ranged from 72% to 76% which is within reference range of 60-80% reported for cockerels (Getachew, 2016). There was no significant difference on sperm motility between ecotypes, the observation which concurs with the report of Sonseeda *et al.*, (2013) who stated that breeds had no effect on sperm motility among the Thai native cocks. However, our findings are contrary to the results reported elsewhere (Tarif *et al.*, 2013; Ajayi *et al.*, 2014) revealing a significant difference in the sperm motility among the chicken lines. Several factors can affect sperm motility subsequent to semen dilution. Bird sperm motility can be afflicted by the quantity of oxygen and Calcium cations in semen (Parker and McDaniel, 2007). Moreover, decreased sperm motility has been associated with abnormal spermatogenesis and epididymal sperm maturation problems (Rengaraj *et al.*, 2015).

The semen pH recorded was slightly alkaline (7.01 – 7.02) in all ecotypes. The semen pH in the present study was within the range stated for chicken semen (Etches, 1996) and was not influenced by the ecotype of the rooster which agrees with other studies (Peters *et al.*, 2008; Haunshi *et al.*, 2010) which stated that there was insignificant dissimilarities in pH between genetic groups. Slight variations in pH (7.01 and 7.02) recorded in our study could be caused by genetic and environmental factors.

In terms of the effect of age on sperm quality parameters, the current study found that the age of native roosters, either between 11 and 15 months or between 24 and 28 months, had no significant influence on semen quality except for

semen volume where roosters of 11to 15 months' age group recorded a significant higher volume than roosters of 24 to 28 months' age group. This finding agrees with Long *et al.*, (2010) who reported that semen volume decreased with the age of the roosters. This finding in our study was due to the fact that normal physiological processes regulating spermatogenesis tend to decrease with age. All other semen quality traits decreased by the age of the roosters although the differences were not statistically significant, this also agrees with Long *et al.*, (2010) who stated that poultry semen characteristics decreased with age, but our findings is contrary to that reported with Sonseeda *et al.*, (2013) who revealed that age had no impact on semen quality among the Thai native cocks. Cerolini *et al.*, (1997) considered the impact of age on semen concentration and reported that concentration keep on increasing significantly from 6th month to 10th month of age; but did not differ significantly between the 10th and 13th month; and was at the lowest concentration in the 18th month. Again, according to Shanmugam *et al.*, (2012) the proportion of live spermatozoa was found to increase from younger age to middle age in broiler roosters and decreased afterwards. Also (Wishart, 2009) reported that semen of older birds had significantly lower motility, viability and mass movement than younger birds. Tabatabaei *et al.*, (2009) observed an increase in rate of sperm morphological defect in Iranian indigenous broiler breeder chickens with aging of roosters; this finding agrees with our findings on sperm morphological defects.

The link between semen volume, pH, sperm motility, sperm concentration, proportion of morphological normal spermatozoa and proportion of live spermatozoa is very important because, to a large degree it define the potential fertility of the ejaculate. The positive and significant correlation (r=0.51) between the proportion of morphological normal spermatozoa and proportion of live spermatozoa was due to the fact that live, normal spermatozoa possess an intact plasma membrane which protects them from penetration of eosin while 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). A positive and significant correlation between the proportion of live spermatozoa and sperm motility existed because spermatozoa will only be able to move when they are alive and sperm motility is an indicator of sperm viability. The positive and significant correlation existed between morphological normal spermatozoa and motility, this finding was similar with the report of Feyisa *et al.*, (2018) who reported that viable and morphological normal spermatozoa in four Korean native chickens breeds were associated with good motility and this scenario existed because according to Bakst, (2009) only sperms with normal morphology can swim properly from the vagina of the chicken to the semen storage tubules. The positive and significant correlation existed between sperm concentration and sperm motility in this study and our finding is similar with the study of Peters *et al.*, (2008) who also reported a correlation coefficient of 0.25 between sperm concentration and sperm motility.

Conclusion

It can be concluded that only the variations in proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the three ecotypes of roosters were significant. Age of indigenous roosters had no significant influence on semen quality except for semen volume and only semen volume showed a positive and a significant correlation with increasing body weight of the roosters. The Pearson correlation coefficients between semen volume and other quality characteristics were mostly low to medium with positive values between semen volume and sperm motility and morphological normal spermatozoa and proportion of live spermatozoa respectively.

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CHAPTER THREE

MANUSCRIPT II

Effect of Gonadotropin-Releasing Hormone (GnRH) analogue on Semen Characteristics of Three Ecotypes of Tanzanian Native Chickens

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Abstract

Several studies have reported the effect of hormone treatment on semen quality and reproductive performance in male animals. However, limited information is available on effect of Gonadotropin releasing hormone (GnRH) treatment on semen quality in galliform species. For that reason, this study examined the effect of synthetic GnRH on semen quality parameters in three ecotypes of Tanzanian native chickens. A total of thirty-six mature cockerels from Tanzanian native chicken ecotypes namely; Ching'wekwe, Morogoro-medium and Kuchi were used in this study. Thirty cockerels (10 from each ecotype) were intramuscularly injected with 0.2 ml of GnRH (Factrel®) once in a week for five consecutive weeks while six (two from each ecotype) were used as a control group only receiving normal saline solution. Semen was artificially collected at weekly interval by abdominal massage technique starting immediately after last GnRH injection for five consecutive weeks. Semen parameters increased significantly ($p<0.05$) between control and treatment group including semen volume (0.48±0.02 mL versus 0.55±0.02 mL), sperm motility (74.90±0.76% against 80.02±0.30%), concentration (4.04±0.18 × 10^9/mL versus 4.80±0.14 × 10^9/mL), proportion of morphological normal spermatozoa (87.58±0.43% versus 91.25±0.3%) and proportion of live spermatozoa (89.05±0.55% against 91.65±0.31%). Semen pH did not change in all samples between control and treatment groups. It can be concluded that semen quality parameters can be improved by injecting GnRH to cockerels and therefore increasing productivity in the poultry industry.

Keywords: Cockerels, Eosin Nigrosin, *Gallus gallus domesticus*, Gonadorelin hydrochloride, Local chickens, Semen quality.

Introduction

Tanzania has an estimated 92.8 million chicken population, of which 75.1 million are on small scale farms and 17.7 million on large scale farms (URT, 2022), with a constant yearly growth rate of 3.9% (BFAP and SUA, 2018). However, poultry industry outputs in terms of meat and egg production is still below potential due to various elements such as the low genetic potential of native chickens, disease and diet (MLDF, 2015). Chickens native to Tanzania show a broad range of genetic and phenotypic diversity, including plumage colour and type, body shape and size, and productivity (Msoffe *et al*., 2001; Minga *et al*., 2004; Msoffe *et al*., 2004). Among the approximately 200 native chicken ecotypes found in Tanzania, Ching`wekwe, Kuchi, and Morogoro Medium have potential in terms of both productivity and disease resistance (Msoffe *et al*., 2002).

Spermatogenesis is a complex reproductive process involving the split of spermatogonial stem cells and the eventual production of spermatozoa while keeping the stem cell population (Thurston and Korn, 2000). This complex event occurs within the seminiferous epithelium and relies on the neurosecretory action of the hypothalamic nucleus with the secretion of gonadotropin-releasing hormone (GnRH) (Hezarjaribi *et al*., 2016). GnRH is primarily involved in the development and function of the reproductive axis in mammals, including birds. Gonadotropin-releasing hormone regulates gonadotropin secretion in mammals by stimulating gonadotropin cells in the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Pawson and McNeilly, 2005). Both FSH and LH regulate germ cell development, release androgens by Leydig cells required for the production of mature spermatozoa, and stimulate interstitial cells to produce testosterone to support spermatogenesis. Thus, administration of synthetic GnRH may result in continuous release of LH from the anterior pituitary gland and production of Leydig cell enzymes that can convert cholesterol to testosterone, thereby affecting semen quality traits.

Semen evaluation is considered the most important clinical test for identifying and predicting distinct cases of fertility, infertility, or potential subfertility. Several studies have demonstrated the effects of GnRH treatment on semen quality in male animals, including cattle (Malak and Thibier, 1985; Gabor *et al*., 1998), buffalo bulls (Sajjad *et al*., 2007), rabbits (Ukar *et al*., 2021) and goats (Giriboni *et al*., 2018). However, there is limited information on the effect of GnRH treatment on semen quality in galliform species although Fathi *et al*. (2000) reported semen quality improvement in naked neck roosters after long-term treatments with GnRH. Therefore, the present study, investigated the effect of synthetic GnRH on semen quality characteristics of three ecotypes of Tanzanian native chickens.

Materials and methods

Study area

The current study was conducted at the experimental poultry farm of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. SUA is located on the slope of the Uluguru Mountains, 3 kilometres from the centre of Morogoro. Morogoro town is located in eastern Tanzania, with a latitude of 6°49′15″ S and a longitude of 37°39′40″ E, an elevation of 504m above sea level, and mean annual temperatures and rainfall of 24.3 °C and 935 mm, respectively.

Experimental birds

A total of thirty-six Tanzania native chicken ecotypes namely; Ching'wekwe, Morogoro-medium and Kuchi were used in this study. Twelve cockerels of 11 months of age and of nearly the same body weight from each ecotype were randomly selected from a heterogeneous native chicken population maintained separately at the experimental poultry farm. The selected cockerels were matured (11 months old), apparently healthy and without any physical abnormalities. Ethical clearance on the use of birds was provided by the University ethics committee.

Management of experimental birds

The experimental birds used in this study were kept in individual breeder cages (40 × 40 × 60 cm) in an open-sided house with natural light hours. The birds were given home-made feed (18% crude protein and 2800 Kcal Kg^{-1} metabolizable energy) and fresh water *ad libitum* throughout the experimental duration. All cockerels were routinely vaccinated against Newcastle, Fowl pox and Infectious bursal disease and were

regularly dewormed.

Treatments

Six cockerels (two from each ecotype) were used as a control group while thirty cockerels (ten from each ecotype) were used as treatment group. The treatment group received 0.2 ml per Kg body weight of GnRH (Factrel® - 50 mg gonadorelin hydrochloride per ml Zoetis Animal Health, New York, USA) intramuscularly as previously described by (Hezarjaribi *et al*., 2016) while the control group was injected with normal saline solution. The treatment group was injected GnRH once in a week for five consecutive weeks and semen collection started immediately after the last injection of hormone (Week 0).

Semen collection

Semen was artificially collected at weekly interval by abdominal massage technique (Burrows and Quinn 1937) starting immediately after last GnRH injection for five consecutive weeks (Week 0, 1, 2, 3 and 4). Semen was collected at around 08:00 to 10:00 hours on each day of collection and just after collection it was stored in a graduated plastic tube, measured to the nearest 0.01 ml and instantly kept in a water bath maintained at 37°C for further analysis.

Semen evaluation

Semen volume was evaluated using graduated (millilitre) plastic tubes. The pH of semen was assessed with a calibrated pH meter (Ultra Basic-5, Denver Instrument) immediately after semen collection.

Sperm motility

Motility was evaluated on the principle of percentage of sperm showing frontward motion as previously described by Tadondjou *et al.* (2013). Briefly, 2 µL of semen was mixed with 100 µL of phosphate-buffered saline on a clean; grease free and warmed glass slide (37^{O}C), and a cover slip was put on top before examination under light microscope at 400x magnification. The proportion of motile sperm was individually assessed on a scale of 0 to 100% and at least 3 microscopic fields were observed, for each sample motility was stated as the percentage of spermatozoa with moderate to rapid progressive movement.

Sperm concentration

Sperm concentration (billions per millilitre) in the semen was assessed by the direct cell count technique using Neubauer counting chamber (Haemocytometer). Before assessment, semen sample was diluted with phosphate-buffered saline at a ratio of 1:100. The haemocytometer was then loaded with diluted semen through the capillary action of the pipette and loaded haemocytometer was finally observed under microscope at 400x magnification. The head of the sperm that fell within the smaller squares at the four edges and centre of the haemocytometer were counted. The concentration of spermatozoa per millilitre was calculated using the formula; Concentration of spermatozoa per millilitre = 50, 000 x Number of spermatozoa counted x Dilution factor, as formerly explained by Ax *et al*., (2000).

Sperm viability and morphology

The proportion of live and dead sperm was assessed by differential staining method using Eosin–Nigrosin stain (5% eosin, 10% nigrosin) as formerly explained by Campbell *et al*. (1953). In brief, 5 µL of semen sample was mixed with 100 µL of Eosin-nigrosin

stain then thin smears were prepared from this mixture and fixed by air-drying the slide at room temperature. For each particular slide, about 200 spermatozoa were examined by using oil immersion at a total magnification of 1000. To determine the percentage of live spermatozoa, 10 fields per slide (at least 100 sperms per field) were directly counted using light microscope (1000x magnification). The spermatozoa which looked pink in colour (stained with eosin) were regarded as dead while spermatozoa which were colourless (no penetration of eosin stain) were regarded as live. Furthermore, the thin Eosin-Nigrosin stained smears were also used to assess spermatozoa morphological defects. The defects on the acrosome, head, mid-piece and tail of the spermatozoa were examined and at least 200 spermatozoa were observed from each sample.

Statistical Analysis

Statistical Product and Service Solutions (SPSS version: 20.0.0) software was used to analyse the data. Analysis of variance (ANOVA) was used to look for an overall variation in rooster semen quality parameters between the control and treatment groups. The data were portrayed as Mean± SEM and the differences in parameters were regarded as significant when the $P< 0.05$.

Results

Throughout the study duration, a total of 180 semen samples (150 from treated group and 30 from control group) were analysed. Semen volume and sperm concentration increased significantly ($p<0.05$) in a treated group except for semen pH (Figure 3.1). Specifically semen volume, and sperm concentration between the control and treated groups varied from 0.48±0.02 to 0.55±0.02 mL, and 4.04±0.18 to 4.80±0.14 × 10^9/mL respectively. Semen pH remained the same between the control and the treated group (7.02±0.00).

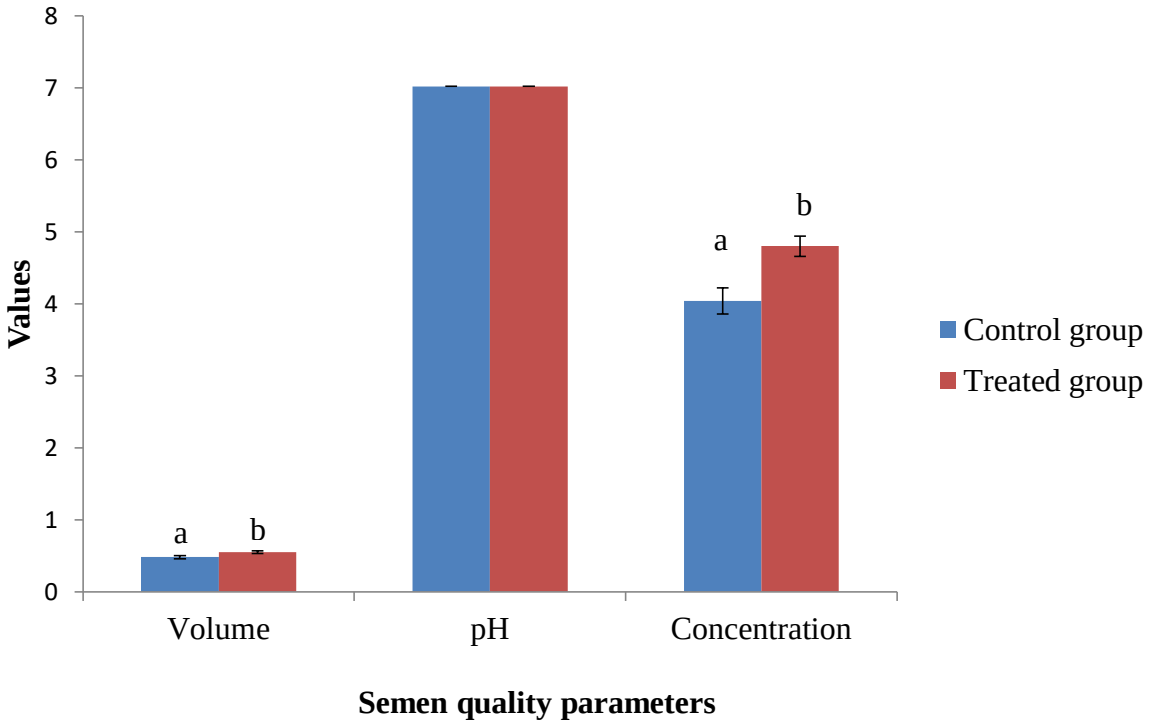

Figure 3.1: Semen volume, pH and sperm concentration (n × 10^9)/mL of Tanzanian local cockerels between the GnRH treated group and the control group. ^{a,b} denotes significant difference between groups.

Sperm motility, proportion of morphological normal and proportion of live spermatozoa increased significantly ($p<0.05$) after GnRH injection (Figure 3.2). Sperm motility, proportion of morphological normal and proportion of live spermatozoa between the control and treated groups varied from 74.90±0.76 to 80.02±0.30%, 87.58±0.43 to 91.25±0.3% and 89.05±0.55 to 91.65±0.31 respectively.

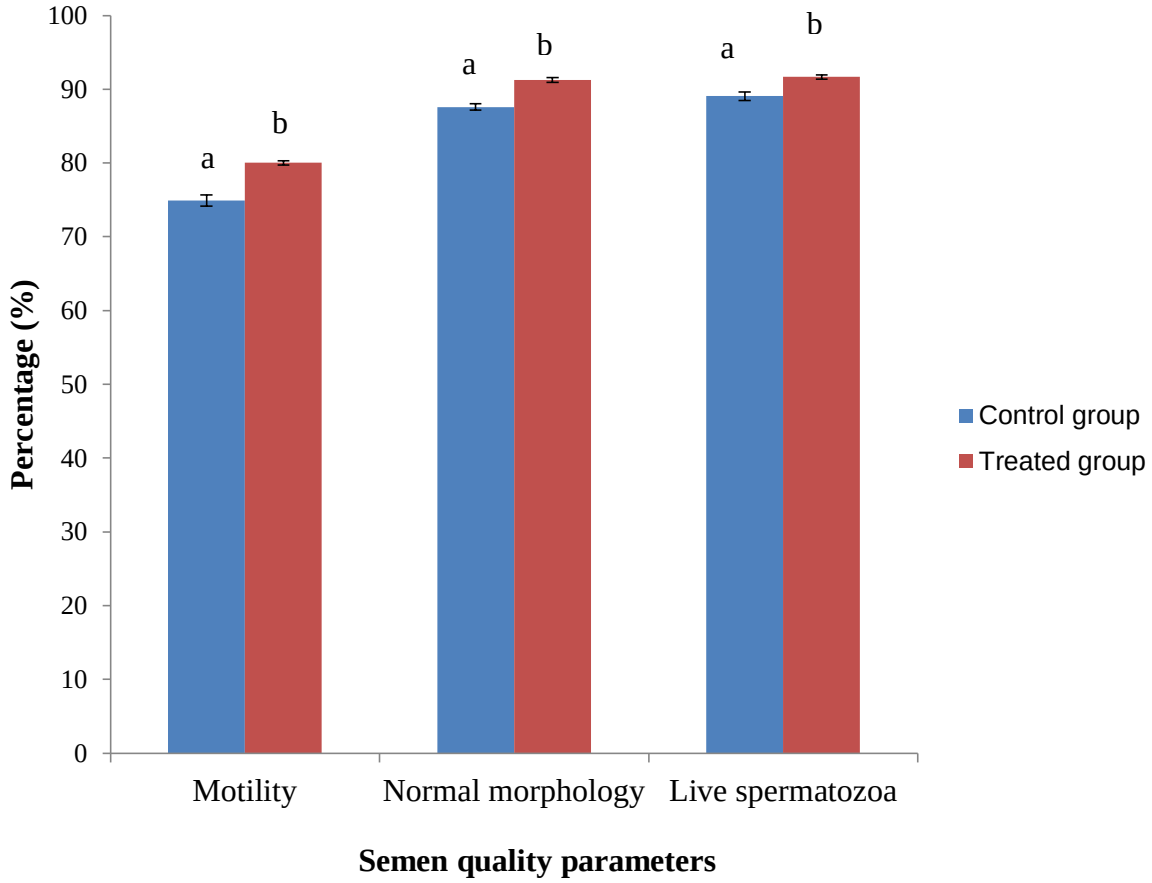

Figure 3.2: Sperm motility, normal morphology and proportion of live spermatozoa of Tanzanian local cockerels between the GnRH treated group and the control group. [a,b] denotes significant difference between groups.

In the treated group, semen volume and sperm motility decreased from week 2 to week 4 of evaluation but the variations were not statistically significant ($p>0.05$) (Figure 3.3 and 3.4). The proportion of morphologically normal spermatozoa started to decrease at week 3 to week 4 and the difference was statistically significant ($p<0.05$) (Figure 3.4). Sperm concentration started to decrease at week 2 of evaluation to week 3 but increased again at week 4 and the variations on sperm concentration within the five weeks were significant ($p<0.05$) (Figure 3.3). The proportion of live spermatozoa decreased at week 3 but it also increased at week 4 of semen evaluation and the variations were statistically significant ($p<0.05$) (Figure 3.4). A higher semen volume and proportion of live spermatozoa was recorded at week 2 of evaluation, while a higher sperm motility, sperm concentration and proportion of morphologically normal spermatozoa was recorded at week 1 of evaluation.

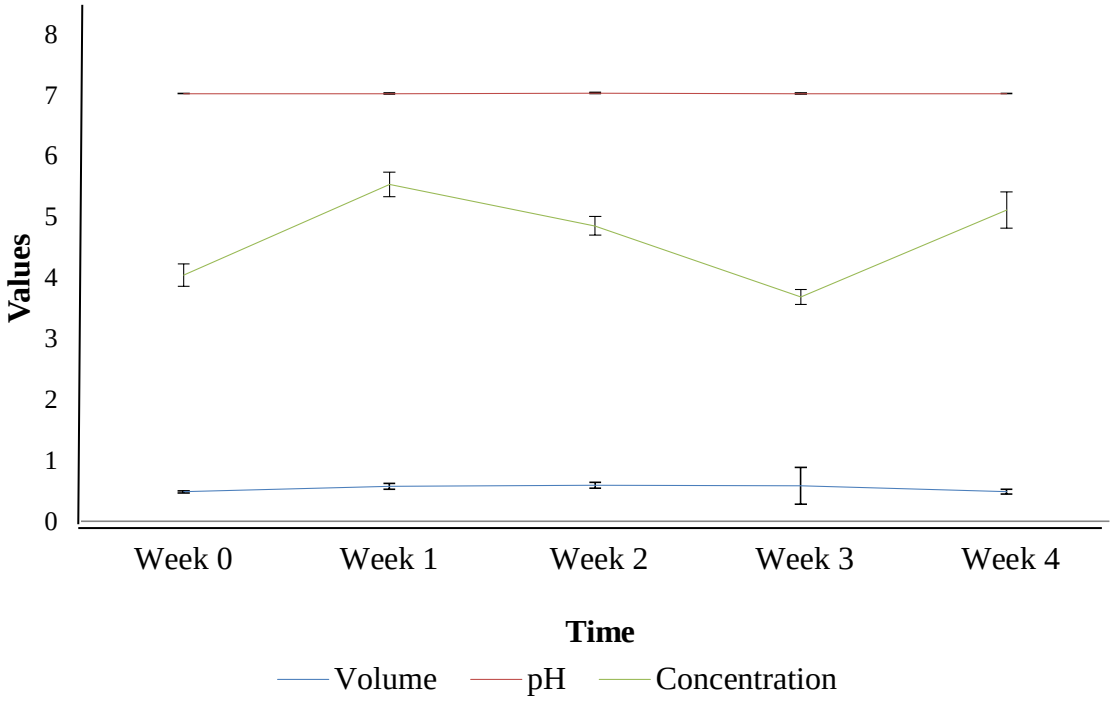

Figure 3.3: A weekly trend of semen volume, pH and sperm concentration (n × 10^9)/mL of Tanzanian local cockerels after GnRH injection. ^{a,b,c} denotes significant difference between collections.

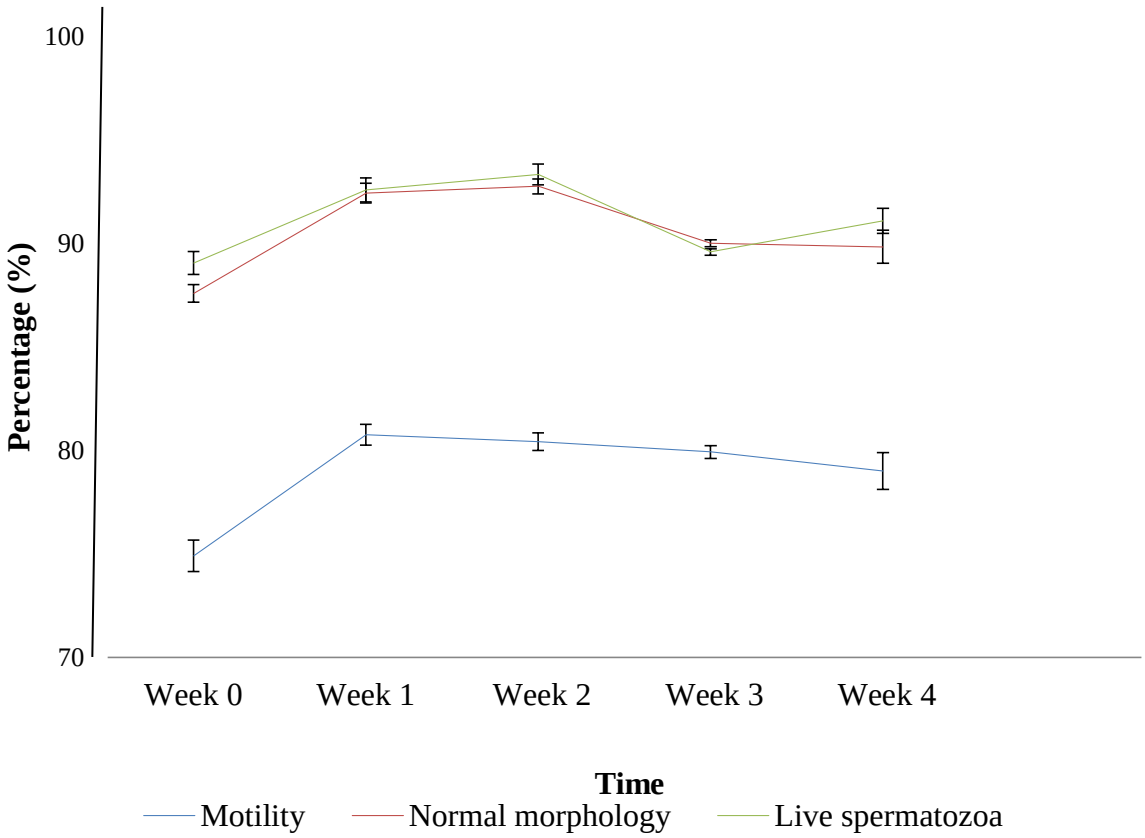

Figure 3.4: A weekly trend of sperm motility, normal morphology and proportion of live spermatozoa of Tanzanian local cockerels after GnRH injection. ^{a,b,c} denotes significant difference between collections.

All semen quality traits except semen pH increased after GnRH injection in all three ecotypes and the difference was only statistically significant with sperm motility, concentration, morphological normal and proportion live spermatozoa ($p<0.05$) (Table 3.1). Semen pH remained unchanged in Ching'wekwe and Kuchi ecotypes even after GnRH injection but it increased in Morogoro medium ecotype but the increase was not significant ($p>0.05$).

Table 3.1: Semen quality parameters among the three ecotypes before and after GnRH injection

Ecotype	Treatment	N	Semen volume (mL)	pH	Sperm motility (%)	Sperm concentration ($n \times 10^9$)/ml	Morphological normal spermatozoa (%)	Live spermatozoa (%)
Ching'wekwe	CG	10	0.42±0.04	7.01±0.00	72.81±1.27	4.11±1.96	86.16±0.55	88.13±0.79
	TG	50	0.51±0.05	7.01±0.01	79.81±0.77	4.78±2.65	90.81±0.73	91.94±0.62
Kuchi	CG	10	0.51±0.03	7.02±0.00	76.63±1.35	3.90±0.98	89.38±0.80	90.97±0.81
	TG	50	0.59±0.04	7.02±0.01	80.75±0.31	4.73±2.12	91.94±0.51	92.38±0.55
Morogoro - medium	CG	10	0.52±0.03	7.02±0.00	75.25±1.26	4.12±0.87	87.22±0.79	88.06±1.13
	TG	50	0.57±0.02	7.03±0.01	79.50±1.27	4.87±2.67	91.00±0.27	90.62±0.38
P value			0.025	0.687	0.000	0.007	0.000	0.001

Values are mean ± SEM. N = Number of ejaculates; CG = Control Group; TG = Treated Group

Discussion

Semen volume increased significantly in a treated group ($p<0.05$) and varied between treated and control cockerels. The increase in semen volume also has been reported in Gimmizah cocks treated with GnRH analogue (Abdo *et al*., 2021). Similar findings were also reported when Alexandria cockerels were given GnRH analogue (Receptal®), (Samar, 2009). Furthermore, a ram study found that GnRH injection increased testosterone concentration and seminal fluid content in rams, resulting in increased semen volume (Ungerfeld and Fila, 2011). Semen volume increased up to week 2 of GnRH injection and then began to decrease; this decrease in semen volume can be explained by the hormone's tendency to reach a threshold level above which it can no longer exert the required effect.

The semen pH was not significantly affected after GnRH injection, this finding agrees with Abdo *et al*. (2021) who also reported that semen pH was not significantly affected among treatments and it appears that GnRH is ineffective in changing semen pH. Semen pH remained fairly constant even during a five week treatment period and this finding was similarly reported by Abdo *et al*. (2021) who stated that semen pH was not significantly affected among treatments groups.

The concentration of spermatozoa in treated cockerels was higher than the control group and it varied from 4.04±0.18 to 4.80±0.14 × 10^9/mL. The increase in sperm concentration was also reported in Gimmizah cockerels treated with GnRH (Abdo *et al*., 2021). Similarly, Fathi *et al*. (2000) stated that GnRH treatment improved spermatozoa concentration of the naked neck cockerels particularly after long time treatment. Abdo *et al*. (2021) also reported that spermatozoa concentration of GnRH treated cockerels

was significantly higher than the control group but concluded that GnRH dose has insignificant effect on spermatozoa concentration. Studies on the injection of three doses of GnRH analogue (gonadorelin diacetate tetrahydrate) every 2 days have been found to increase testosterone concentration and have decreased the semen collection duration and increased the sperm concentration in the ejaculate of camels (Monaco *et al*., 2015).

Sperm motility increased significantly ($p<0.05$) after GnRH injection (figure 3.2). This effect was also reported by Fathi *et al*. (2000) who saw an increase in motility of naked neck cockerels' spermatozoa when treated with GnRH and Samar (2009) who also reported improvement on sperm motility as a consequence of GnRH analogue treatment in cockerels.

Proportion of spermatozoa morphological normalcy and viability increased significantly ($p<0.05$) after GnRH injection. The findings in this study agrees with Abdo *et al*. (2021) who reported that GnRH treatments caused an increase in percentage of normal sperms and a decrease in the percentage of abnormal sperms in all GnRH treated cockerels. Similarly, the percentage of dead spermatozoa was significantly lower in the treated groups than in control group. The administration of a synthetic GnRH result in the continued release of LH from the anterior pituitary gland and the production of Leydig cell enzymes capable of converting cholesterol into testosterone, hence affecting semen quality parameters. Proportion of morphological normal and proportion of live spermatozoa increased up to week 2 of GnRH injection and it started to decrease thereafter, this decrease in proportion of morphological normal and proportion of live spermatozoa can be explained by the fact that the hormone tends to reach a threshold level above it can no longer exert the required effect.

Conclusion

It can be concluded that semen quality traits were improved after GnRH injection but the improvement was only significant with semen volume, sperm concentration, motility, proportion of morphological normal and proportion of live spermatozoa but not with semen pH.

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Conflict of interest

Authors do not have any conflict of interest.

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CHAPTER FOUR

4.0 SUMMARIZING DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Summarizing Discussion

The current study aimed at evaluating the semen quality of three Tanzanian native chicken ecotypes, and it has proved that semen quality can be affected by ecotype, age and hormonal treatment. The generated information will be useful in breeding programs as it will give a picture of semen quality in our local chickens. For the effect of ecotype on semen quality, the findings from the current study indicate that only the proportion of live spermatozoa and proportion of spermatozoa with normal morphology was significantly affected by the ecotype. In this study, the proportion of live spermatozoa in semen sample varied from 88.06% to 90.97%. However, lower proportion of live spermatozoa (72 to 82%) in rooster semen has been stated by Siudzińska and Łukaszewicz (2008). The difference in proportion of live spermatozoa among ecotype in the current study and when compared to other researchers may be due to genetic disparities in tolerance to stains used for processing. However, the proportion of live spermatozoa in our study was good for breeding purposes in poultry.

The proportion of morphologically normal spermatozoa in rooster semen under this study ranged from 86.16 to 89.38% which is similar to the observation made by Tarif *et al.* (2013) who reported 87.2 to 90.1% morphologically normal spermatozoa in rooster semen. Nevertheless, the proportion of spermatozoa with normal morphology reported in this study significantly varied among ecotypes of roosters, this finding agrees with that reported elsewhere (Łukaszewicz *et al*., 2008; Feyisa *et al.,* 2018). However, our findings on sperm morphology mismatch with others (Shanmugam *et al.,* 2012; Tarif *et al.,* 2013; Almahdi *et al.,* 2014; Ameen *et al.,* 2014) who reported insignificant variation in the sperm morphology in different breeds/strains of cockerels. Furthermore, Siudzińska and Łukaszewicz (2008) reported higher (91 to 94%) morphologically normal spermatozoa obtained from 4 breeds of domestic fowl. The common sperm morphological defects recorded in this study included bent midpieces, coiled and bent tails. This findings is similar to Feyisa *et al.* (2018) who also reported that bent midpieces, coiled tails, detached head, broken and knotted tails or heads as common morphological defects in different breeds of cockerels studied. Gradually sperm defects can designate disturbances in the process of sperm formation ascribed to age, nutrition and pollution *(*Bah *et al*., 2001). Additionally, inappropriate handling of semen during processing can lead to higher sperm defects in semen. However, the proportion of morphological normal spermatozoa in our study was good for breeding purposes in poultry.

The effect of synthetic GnRH on semen quality parameters in three ecotypes of Tanzanian native chickens was also assessed. Semen parameters increased significantly ($p<0.05$) between control and treatment group; semen volume (0.48±0.02 mL versus 0.55±0.02 mL), sperm motility (74.90±0.76% against 80.02±0.30%), concentration (4.04±0.18 × 10^9/mL versus 4.80±0.14 × 10^9/mL), proportion of morphological normal spermatozoa (87.58±0.43% versus 91.25±0.3%) and proportion of live spermatozoa (89.05±0.55% against 91.65±0.31%). The increase in semen volume also has been reported in Gimmizah cocks treated with GnRH analogue (Abdo *et al.,* 2021). Similar findings were also reported when Alexandria cockerels were given GnRH analogue (Receptal®), (Samar, 2009). The increase in sperm concentration was

also reported in Gimmizah cockerels treated with GnRH (Abdo *et al.,* 2021). Similarly, Fathi *et al.* (2000) stated that GnRH treatment improved spermatozoa concentration of the naked neck cockerels particularly after long time treatment. The increase in sperm motility was also reported by Fathi *et al.* (2000) who saw an increase in motility of naked neck cockerels' spermatozoa when treated with GnRH. Proportion of spermatozoa morphological normalcy and viability increased significantly ($p<0.05$) after GnRH injection. The findings in this study agrees with Abdo *et al.* (2021) who reported that GnRH treatments caused an increase in percentage of normal sperms and a decrease in the percentage of abnormal sperms in all GnRH treated cockerels. The administration of a synthetic GnRH result in the continued release of LH from the anterior pituitary gland and the production of Leydig cell enzymes capable of converting cholesterol into testosterone, hence affecting semen quality parameters.

4.2 Conclusions

Only the variations in proportion of spermatozoa with normal morphology and proportion of live spermatozoa among the three ecotypes of roosters were significant ($P<0.05$). Age of indigenous roosters had no significant influence on semen quality except for semen volume and only semen volume showed a positive and a significant correlation with increasing body weight of the roosters. The Pearson correlation coefficients between semen volume and other quality characteristics were mostly low to medium with positive values ranging from 0.01-0.51 between semen volume and sperm motility and morphological normal spermatozoa and proportion of live spermatozoa respectively. Semen quality traits such as volume, sperm concentration, motility, proportion of morphological normal and proportion of live spermatozoa were improved after GnRH injection.

4.3 Recommendations

Although there is minimal variation in semen quality among ecotypes and age groups, all the ecotypes might still be used in breeding purposes to maintain native chickens, because the results found were within the reference range for chickens. Semen quality parameters were improved by injecting GnRH to cockerels and therefore GnRH can be incorporated in breeding programmes to increase productivity in the poultry industry and thereby improving the livelihood of the communities at large.

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APPENDICES

Appendix 1: Statement of research ethical approval

STATEMENT OF RESEARCH ETHICAL APPROVAL

1. *This project has been considered and has been **Approved/Not Approved** by the Department/College Research and Publication Committee, Department/College/Unit

   Click here to the name of the unit or delete this guiding text and print and write by hand

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(Chairperson, Research & Publication Committee)

2. This project has been considered and has been **Approved/Not Approved** by the Ethical Committee, DPRTC

Signature ..... Name: Click here to the name. DR. D. G. NDOSSI
Date: Click here to select a date 18/03/2022
(Chairperson, Ethics Committee, DPRTC)

3. This project has been considered and **Approved/Not Approved** by the Senate Research and Publication Committee (SRPC), Sokoine University of Agriculture

Signature: ..... Name: Click here to enter the name. DR. A. W. MWANRI
Date: Click here and down arrow to enter a date. 18/03/2022
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SECTION P : FOR OFFICIAL USE

(i) APPROVAL

Date received : Click here and arrow to enter a date. 18/03/2022	Received by: Click here to type names. LUCIA P. MADALLA
Date of approval: Click here and arrow to enter a date. 18/03/2022 **Name**: DR A.W. MWANRI **Title**: FOR DIRECTOR DPRTC Approving authority in capital letters (example: SRPC, Departmental /College/Centre R&PC Click here to enter the name of approving authority. Director Postgraduate studies, Research, Technology Transfer and Consultancy Sokoine University of Agriculture P. O. Box 3151, Morogoro TANZANIA	Approval reference number: Click here to enter number. DPRTC/R/186/F26 Approval is valid from Click here and arrow to enter a date. 18/03/2022 To: Click here to enter a date.
*All undergraduate studies shall be evaluated and/or approved by the College/Centre R&PC and Reports submitted to the chair Research Ethica Committee, DPRTC	

(ii) **NOT APPROVED**

☐ The applicant is required to revise the application by addressing reviewer's concerns (Reviwer's comments are provided to the applicant)

☐Other reasons (Describe briefly)

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