EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 3 (4): September 2022

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

[1]LUVANGA J D., [1]KASHOMA I P*

[1]Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3020, Chuo Kikuu, Morogoro, Tanzania.

***Corresponding Author**: juliusluvanga@sua.ac.tz

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*

Received: 14/06/22
Accepted: 25/08/22
Published: 29/09/22

Cite as: *Luvanga and Kashoma, (2022)* Effect of ecotype and age on semen characteristics of three Tanzanian native chickens. *East African Journal of Science, Technology and Innovation 3(4).*

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 e*t al.*, 2013), age (Shanmugam e*t 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.

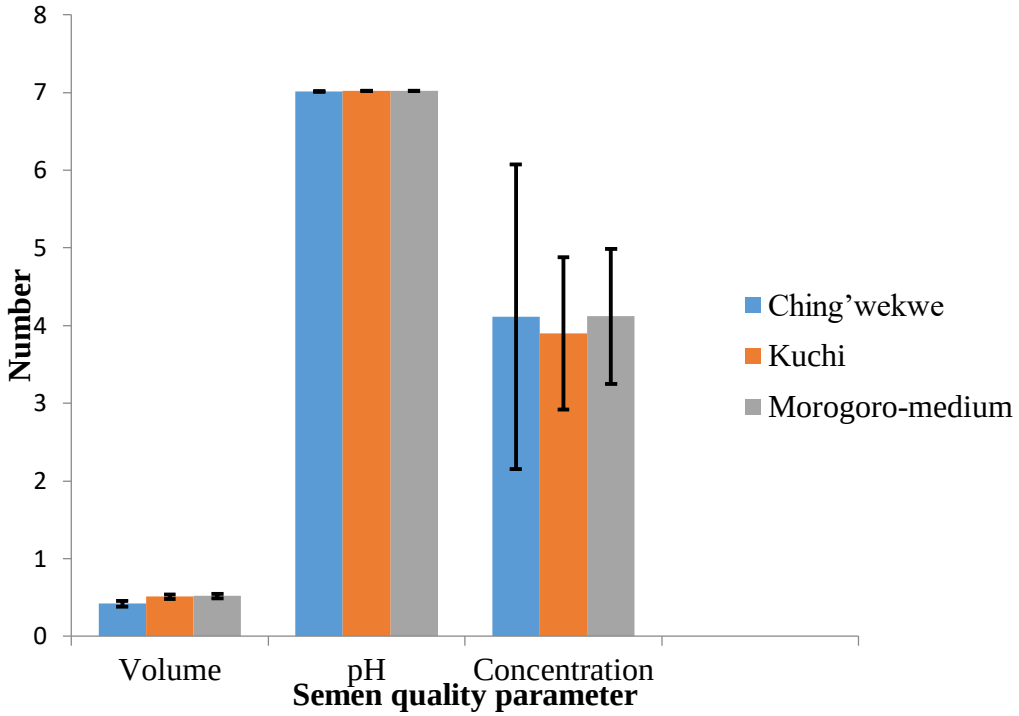

Figure 2. Comparison of ejaculate volume, semen pH and sperm concentration (nx10^9/mL) among the three rooster ecotypes, Morogoro, Tanzania

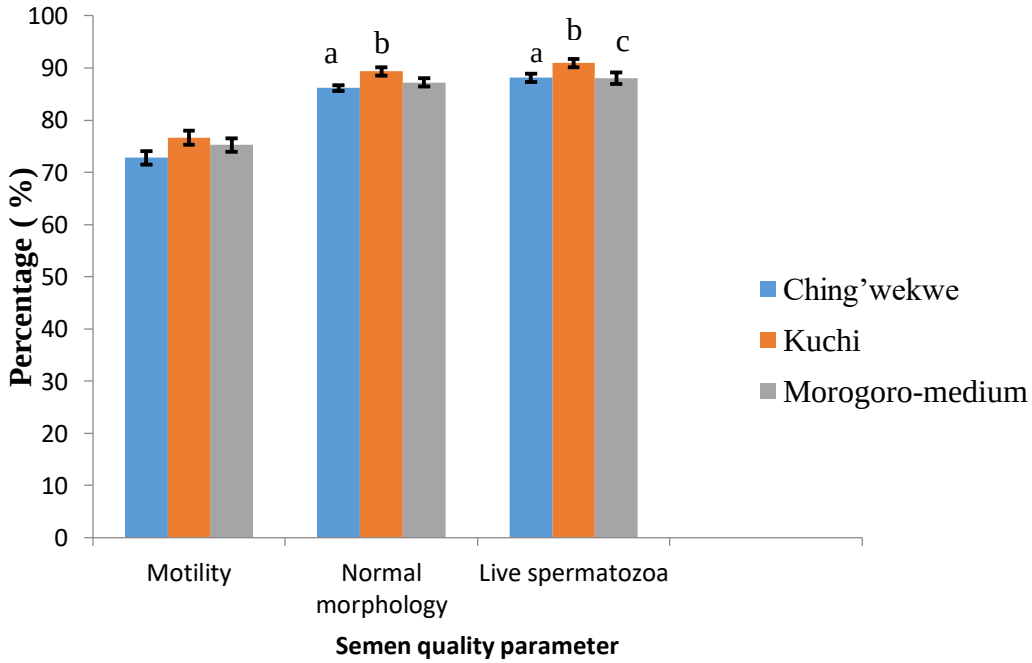

Figure 3. Comparison of sperm cell motility, proportion of morphological normal and of live spermatozoa in fresh semen among three Tanzanian native rooster ecotypes.
[a,b,c] Mean values with dissimilar letters differ significantly ($p<0.05$), and error bars denote SEM

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 ($P > 0.05$).

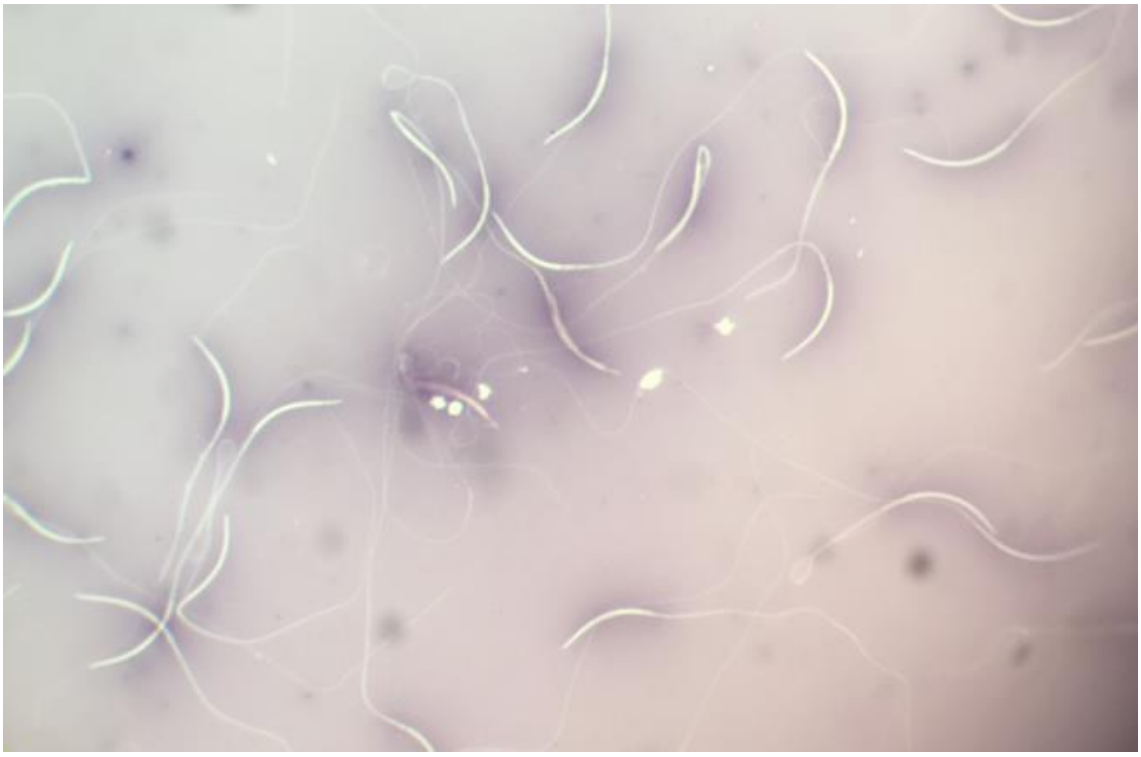

Figure 4. Micrograph of rooster spermatozoa, pink coloured (Eosin stained) considered as dead and colourless (without eosin penetration) considered as live (Eosin Nigrosin Stain, 1000X)

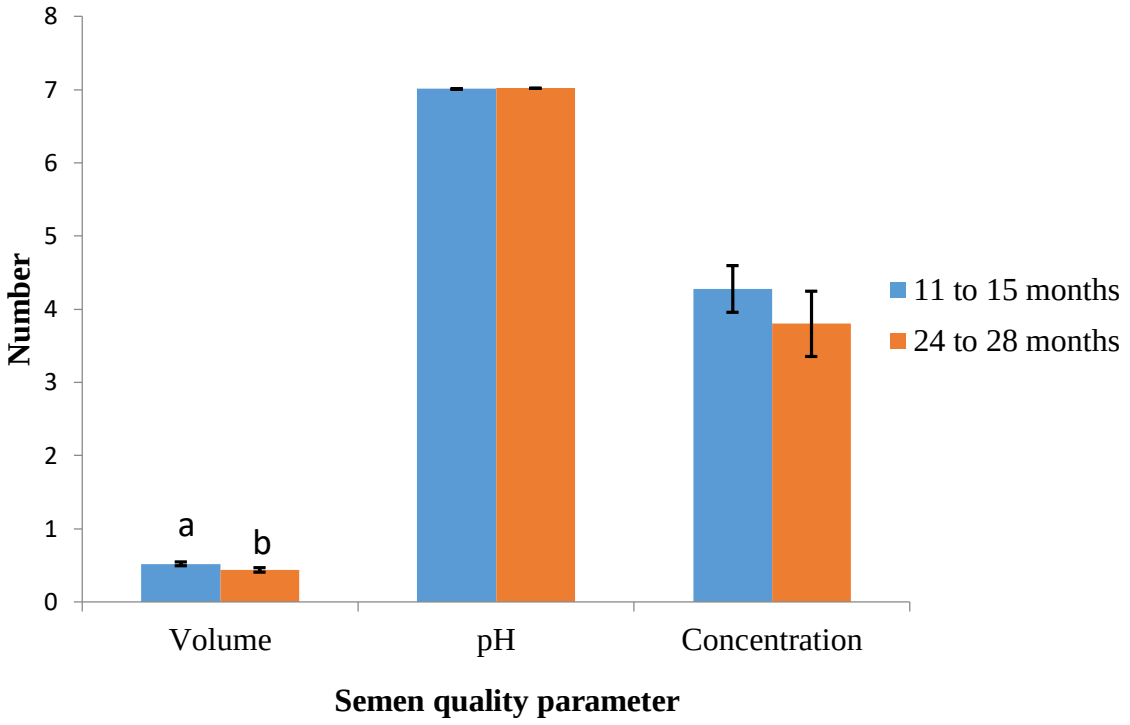

Figure 5. Comparison of ejaculate volume (mL), semen pH and sperm concentration (nx10⁹/mL) among the two age groups of Tanzanian native rooster ecotypes

[a,b,c] Mean values with dissimilar letters differ significantly ($p<0.05$), and error bars denote SEM

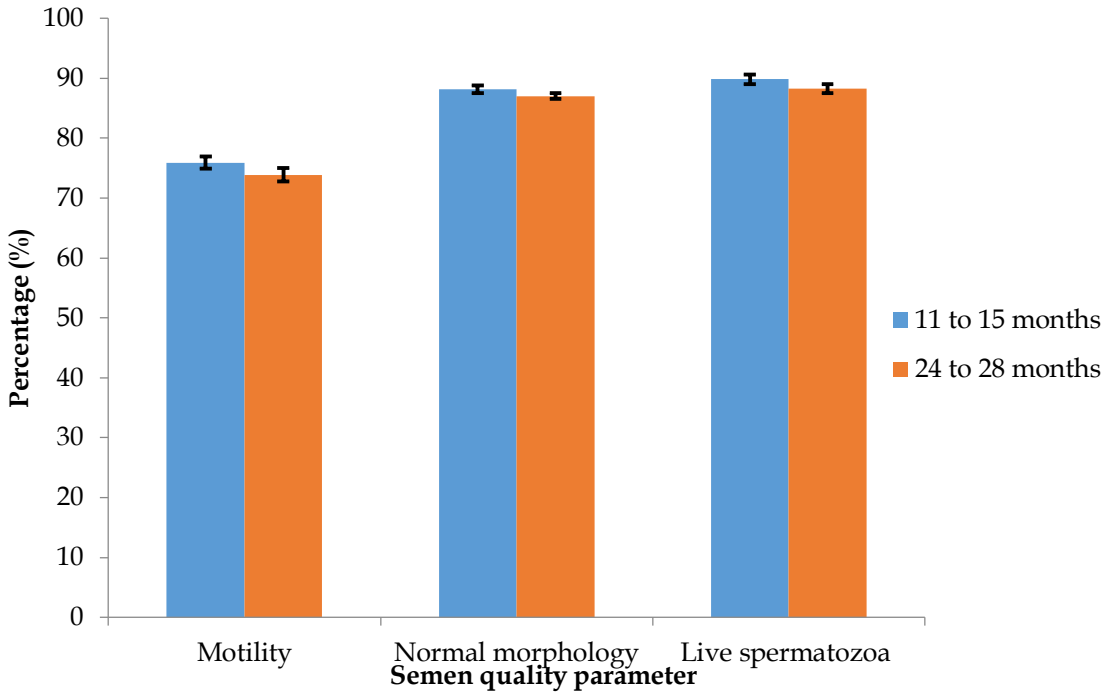

Figure 6. Comparison of sperm motility, proportion of morphological normal and proportion of live spermatozoa among the two age groups of Tanzanian native rooster ecotypes

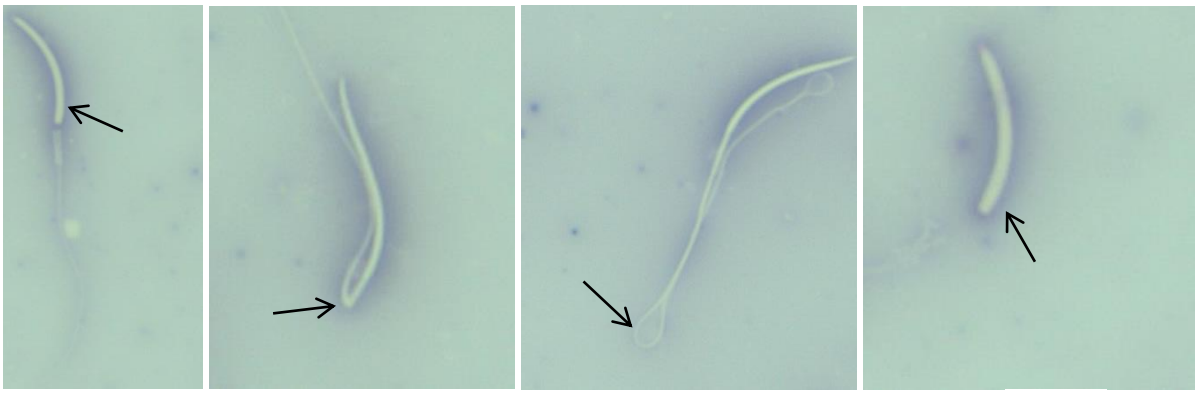

Figure 7. Micrograph of normal and abnormal morphology of Tanzanian native rooster spermatozoa (Eosin Nigrosin stain, 1000X immersion oil) (A) Normal morphology, (B) Bent midpiece, (C) Coiled tail, (D) Loose head/Detached head

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.

Acknowledgement

The authors wish to thank the Deputy Vice Chancellor (Academic) office and the Genomics to Improve Poultry (GIP) project, for their financial support on this study.

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