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Semen quality parameters of the Tanzanian Horasi chicken ecotype

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Abstract

Predicting potential fertility and eventual egg hatchability in chickens can be done by evaluating the quality of the semen. This study's objective was to describe the semen characteristics of three different age groups of Horasi chicken ecotype cocks (24-32, 28-36, and 32-40 weeks). From 20 Horasi cocks; 6 cocks (24-32 weeks), 6 cocks (28-36 weeks), and 8 cocks (32-40 weeks) over the course of four weeks (5-29th Dec 2022), 80 semen samples in total were taken. Sperm was harvested from cocks using the abdominal massage technique. The amount of semen, pH, sperm motility, sperm concentration, live spermatozoa percentage, and spermatozoa with normal morphology percentage were all measured. Between the three age groups, the semen volume, pH, sperm motility, sperm concentration, percentage of live spermatozoa, and percentage of spermatozoa with normal morphology ranged from 0.52 ± 0.03mL to 0.68 ± 0.03mL, 7.24 ± 0.01 to 7.31 ± 0.01, 75.16 ± 0.91% to 76.67 ± 1.07%, 4.31 ± 2.27 to 4.56 ± 3.34 × 10^9 sperm cells/mL, 90.00 ± 0.55% to 91.04 ± 0.85% and 87.78 ± 0.51 to 89.71 ± 0.68% respectively. Only differences in ejaculate volume between the three age groups of Horasi chicken ecotype cocks were significant at $p<0.05$. The semen quality parameters had extremely low to moderate Pearson correlation coefficients, with values ranging from - 0.22 to 0.38 between sperm concentration and sperm motility, and between the percentage of morphologically normal sperm cells and the percentage of spermatozoa that are alive respectively. The Horasi chicken ecotype's semen parameters are within the normal range, notwithstanding the possibility that individual cocks may differ from one another in some semen characteristics as a result of environmental influences. Using artificial insemination, breeding programs can therefore exploit the Horasi chicken ecotype to increase the productivity of indigenous chickens.

Keywords: *fertility; hatchability; Horasi chicken ecotype; Indigenous chickens; reproductive efficiency; sperm concentration; sperm motility*

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Introduction

Artificial insemination (AI), a type of assisted reproduction technology, is recognized as a crucial breeding method for genetic improvement and helps to increase chicken production and productivity (Tarif *et al*., 2013; Getachew, 2016). The success of artificial insemination in chicken, as with other livestock species, can be considerably impacted by the level of sperm quality after processing, hence prior to usage examination of sperm quality is crucial (Tarif *et al*., 2013). Assessment of semen quality parameters in poultry has been demonstrated to be an excellent predictor of reproductive capacity and a primary factor of fertility and eventual egg hatchability (Peters *et al*., 2004). Additionally, the examination of the quality of the semen ensures that only high-quality sperm-producing cocks are retained on the farm, which helps to preserve optimum fertility and reduce embryo motality (Taye and Esatu, 2022).

In order to assess and forecast male fertility in poultry, semen properties such volume, sperm concentration, the quantity of living and dead sperms, the morphological normality and abnormality of the sperms, and progressive motility are examined (Sun *et al*., 2019). The potential to fertilize an egg increases only when a significant number of live, increasingly motile, and anatomically normal spermatozoa are inseminated (Lukaszewicz *et al*., 2020). Sperm morphology is assumed to represent both the survivability of sperm storage in the gonadal ducts and the health functions of the male for sperm production. Semen volume and colour, on the other hand, are assessed to identify male teasing and various lesions or diseases that may be present in the reproductive system (Tarif *et al*., 2013)

The quality of sperm in roosters varies with different factors including breed, ecotypes, age, body weight of cocks, climate, nutrition, time and frequency of collection, (Oke and Ihemeson, 2010; Shanmugam *et al*., 2012; Tarif *et al*., 2013; Fouad *et al*., 2019). Additionally, factors such as feed, season, endocrine disruptors, photoperiod length, pH, ion concentration, and temperature have an impact on all stages of semen generation, sperm metabolism, quality, and motility (Riaz *et al*., 2004; Elagib *et al*., 2012; Tadondjou *et al*., 2013; Almahdi *et al*., 2014).

Horasi chickens are among of the indigenous chicken ecotypes that are retained by regional tribes in traditional farming practices in Tanzania's Lake Zone. Compared to other ecotypes in the nation, Horasi ecotype has demonstrated superior growth and performance for both meat and egg production (Guni *et al*., 2013; Mogonka *et al*., 2016). The ecotype is distinguished by its huge and tall body size (body length 39.8 cm, chest circumference 26 cm), multi-coloured plume (white, brown, and black dominating), rose-colored combs, long legs (shank length 6.4 cm, thigh length 13.5 cm), and its rapid growth rate (Guni *et al*., 2013). Comprehensive investigations indicated that Horasi chicks had an average hatch weight of 32 grams, a growth rate of 7.6 to 12.4 grams per day, and a weight of 1186.8 grams for females and 2040 grams for males at the age of 16 weeks (Guni *et al*., 2013; Mpemba *et al*., 2021). Horasi chickens' average daily feed consumption of 56.9 g demonstrates a substantial correlation with daily development rate (Mpemba *et al*., 2021).

Horasi chicken ecotype can be utilized to increase the productivity and performance of indigenous chickens due to its superior performance, and high adaptation compared to other indigenous chicken ecotypes present in the country. Therefore, there is necessity of increasing its production potentials by employing methods like selective breeding, and assisted reproductive technologies like artificial insemination (AI). However, paucity information is available regarding semen quality of Horasi chicken ecotype. Therefore, this research aimed to investigate the semen quality traits of the Horasi chicken ecotype.

Materials and methods

Study area

The research was carried out at the College of Veterinary Medicine and Biomedical Sciences of Sokoine University of Agriculture (SUA). The college is located on the South part about 3 km from the center of Morogoro Municipal lying on the slopes of the Uluguru Mountains. Morogoro Municipality is located in the eastern part of Tanzania and lies at 504 meters above sea level within latitude 6°49′15″ S and longitude 37°39′40″ E. The area receives an average annual ambient temperature of 24.3°C and about 935 millimetres of precipitation (www.climatedata.eu).

Experimental birds and their management

Twenty healthy (20) mature Horasi chicken ecotype cockerels of three age groups (24-32, 28-36 and 32-40 weeks) were used in this study. The cockerels were selected from the population basing on their peculiar characteristics including physical appearance, health status, age, and body weight (not less than 1.5kg). The roosters were housed in individual cages (40 x 40 x 60 cm), identified (numbered wing band), and attended by one person throughout the experiment. The roosters were maintained in a stress-free environment receiving 12 hours of daily natural light, fed with commercial chicken layer's feed (17% crude protein content and 2850 kcal/kg and given100–120g as individual daily feed) and offered with water *ad- libitum*. Additionally, the chickens received routine treatments such as deworming, vaccinations against common viral infections such as Gumboro, Newcastle Disease, and Fowl pox.

Figure 1

Image of Tanzanian Horasi Chickens

Collection of sperm

Semen was obtained from cocks utilizing an abdominal massage technique, as previously described (Burrows and Quinn, 1935; Bakst and Dymond, 2013; Luvanga and Kashoma, 2022). Before experiment, the cockerels were given a preliminary period of four weeks of environmental adaptation and initial training to the abdominal massaging technique. Briefly, the procedure involved massaging the cloaca region followed by compressing the area around the anal canal to release the sperm. The sperm was harvested in graduated collection tubes, immediately the tubes were closed with a stopper to prevent evaporation and placed in a water bath maintained at 36 - 38^{0}C until further analysis. Semen samples were collected once a week from each cock between 09: 00 and 10:00 AM for four consecutive weeks.

Semen quality assessment

After collection, semen volume was read directly from the graduated collection tubes and the volume was measured to the closest 0.1 mL. Semen pH was measured using a calibrated pH meter. Semen colour was visually observed and scored (where 1= white creamy; 2=milky; and 3=opaque). Furthermore, presence of other materials in semen such as blood cells, dust or debris was observed using visual examination.

Sperm motility

Sperm motility was assessed using a light microscope at room temperature according to Hafez and Hafez (2000) and Bakst and Dymond (2013). Briefly, 2µl of semen was mixed with 100µl of phosphate-buffered saline on a clean, grease free, heated glass slide (36-38^{0}C) with a cover slip and examined with a light microscope (Olympus, Japan) at 40x magnification. The percentage of motile sperm was subjectively evaluated by one individual and expressed as the percentage (ranging from 0 to 100) of cells that move progressively on their own. For each sperm sample, five different areas of views were made and average taken as the percentage of a particular sample.

Semen viability and morphology

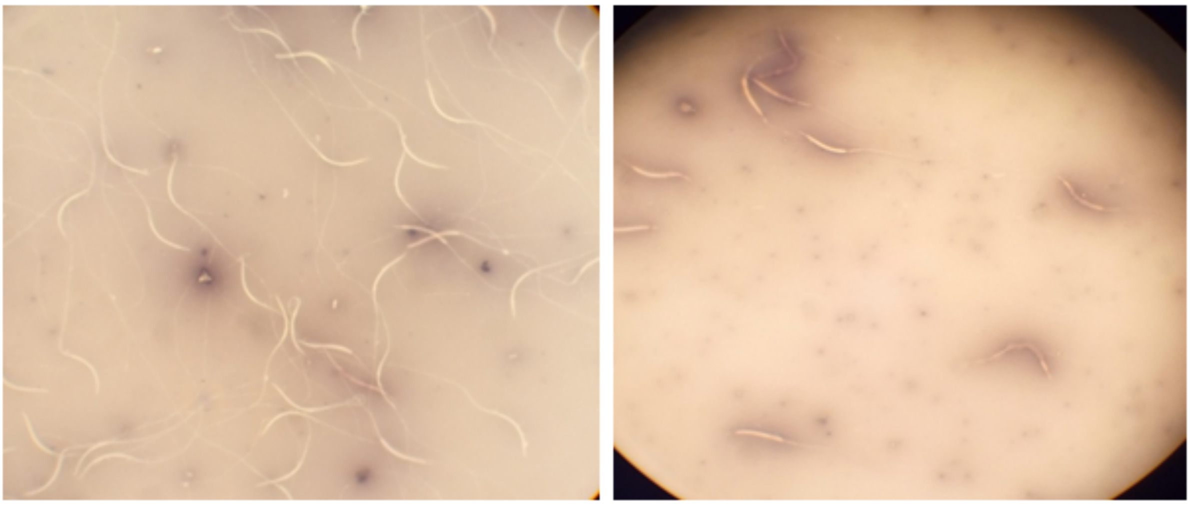
Assessment of semen viability and morphology was done using Eosin-Nigrosin staining approach as demonstrated by Agarwal *et al*., (2016). To summarise, a drop of semen was placed on a clean, grease-free glass slide, mixed with two drops of 1% aqueous Eosin Y for 15 seconds using a wooden stirrer, and then two drops of 10% aqueous Nigrosin were added and properly mixed to obtain a satisfactory mixture. From the final mixture, about 10 µl was pippetted onto clean-grease-free microscopic glass slide and fixed by air drying before observation under microscope. In each slide, at least 100 sperm cells were analysed under oil immersion (1000×) microscope. Spermatozoa that have been totally or partially dyed (appeared pink in colour) counted as dead while none stained (appeared colourless) spermatozoa counted alive. Furthermore, the Eosin-Nigrosin stained semen smears were also used for assessment of spermatozoa abnormalities. A light microscope was used to examine sperm abnormalities in the head, neck, midpiece, and tail area at 1000x magnification. For each sample, at least 200 sperm cells were evaluated. Morphologically normal spermatozoa were thought to be free from any defects of the head, neck, midpiece, and tail area.

Sperm concentration

The Grey Beckman Spectrophotometer (maker) was used to determine the concentration of sperm cells (millions per millilitre) in the sperm. In a square cuvette, 15µl of semen was mixed with 3.0mL of a 2.9% sodium citrate solution (pH 7.0) and inserted in the first of four slots of the cuvette holder in the spectrophotometer. Absorbance was read as soon as the reading stabilizes within 5 to10 seconds. To get sperm concentrations, the absorbance was subjected to a formula; C = (11,170 × Absorbance) – 90. Whereby C = sperm concentration

Figure 2

Cock Spermatozoa Micrograph; Pink coloured (Eosin Stained) is regarded dead and colourless (without Eosin Penetration) is considered alive (Eosin Nigrosin Stain, 1000×).



Statistical Analysis

The collected data were stored into an excel sheet and analysed using the software package SPSS (SPSS 20.0.0). The semen quality parameters across age groups were compared by one way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons. The data were presented as mean ± SEM. The variations in parameters among cocks of different age were regarded as significant at the level $P<0.05$. Relationships between cock's body weight and semen quality parameters were established using estimates of correlation coefficients.

Ethical clearance

This study was conducted after getting ethical clearance by the Directorate of Research, Technology Transfer and Consultancy of Sokoine University of Agriculture and Ethical Approval Reference Number; SUA/DPRTC/R/186 VOL III was given.

Results

Semen quality parameters of Horasi chicken ecotype cocks

The values for semen volume, pH, sperm motility, sperm count, percentage of live sperm cells, and percentage of sperm cells with normal morphology ranged from 0.20 to 1.00mL, 7.06to 7.45, 60 to 80%, 1.55 to 7.57× 10^9sperm cells/mL, 80 to 95%, and 80 to 95% respectively, and semen colour was generally white creamy as shown in Table 1.

Table 1

Fresh semen parameters of Horasi chicken ecotype cocks aged 24 to 40 weeks

Semen parameters	**Ranges**	**Mean** ± SEM
Volume (mL)	0.20 to 1.00	0.62 ± 0.02
pH	7.06 to 7.45	7.29 ± 0.00
Motility (%)	60 to 80	75.63 ± 0.59
Concentration (× 10^9sperm cells/mL)	1.55 to 7.57× 10^9	4.48 ±1.55
Viability (%)	80 to 95	90.33 ±0.40
Morphological normal spermatozoa (%)	80 to 95	87.25 ± 0.37
Colour	White creamy	White creamy

Semen quality parameters among cocks of three age groups

The average semen volume, pH, sperm motility, sperm count, viability and percentage of morphologically normal sperm cells in 24-32 weeks, 28-36 weeks and 32-40 weeks age groups were ranging from 0.30 to 0.90mL, 7.16 to 7.45, 60 to 80%, 1.55 to 6.54× 10^9sperm cells/mL, 80 to 95%, and 80 to 90% ; 0.20 to 1.00mL, 7.06 to 7.37, 60 to 80%, 1.89 to 7.57× 10^9sperm cells/mL, 85 to 95%, 80 to 95%; 0.20 to 1.00mL, 7.15 to 7.43, 60 to 80%, 2.25 to 7.57× 10^9sperm cells/mL, 85 to 95%, and 80 to 95% respectively as presented in Table 2 and 3.

There was a significant variation in the volume of semen taken from cocks of three age groups ($p<0.05$), (Table 2). Cocks aged 32-40 weeks had the highest semen volume (0.68±0.39mL), followed by cocks aged 28-36 weeks (0.63±0.42mL), and the lowest semen volume was observed in 24-32 weeks cocks (0.52±0.32mL). Also, age had significant influence on semen pH of Horasi cocks ($p< 0.05$), (Table 2), whereby cocks aged 24 weeks showed a highest semen pH (7.31±0.01) and the lowest semen pH (7.24±0.01) was observed in cocks aged 28 weeks. Sperm motility, sperm count, percentage of spermatozoa that are alive and percentage of morphologically normal spermatozoa decreased with age of the cocks; However, the differences were not significant ($p<0.05$), as shown in Table 2 and Table 3.

Proportion of spermatozoa morphological abnormalities recorded in Horasi chicken ecotype

The proportion of different spermatozoa morphological defects found in this study of Horasi chicken ecotype semen is presented in Table 4. More than 50% of abnormalities were bent midpieces, followed by coiled tails and bent tails in all age groups. Other abnormalities such as swollen heads, detached heads, and knotted heads were very minimal. However, there was no significant variation in sperm defects between cocks of different age groups, although to some extent they were much higher in cocks aged 28-36 weeks and 32-40 weeks compared to 24-32 weeks. The normal and some of the abnormalities of Horasi chicken ecotype spermatozoa are presented in Figure 3.

Table 2

Comparison of Semen Volume (mL), semen pH and semen motility among the three age groups of Horasi chicken ecotype cocks. [a, b] Values with different letters within same column differ significantly from each other (P<0.05). Values in the table are means ± SEM

Parameters				
Age	**Sample size**	**Volume (mL)**	**pH**	**Motility (%)**
24-32 weeks	24	0.52 ± 0.03^{b}	7.31 ± 0.01	76.67 ± 1.07
28-36 weeks	24	0.63 ± 0.04^{a}	7.24 ± 0.01	75.21 ± 1.14
32-40 weeks	32	0.68 ± 0.03^{a}	7.30 ± 0.01	75.16 ± 0.91

Table 3

Comparison of Sperm Concentration (× 10^9/ml), Proportion of Live Spermatozoa and Proportion of Spermatozoa with Normal Morphology among the three age groups of Horasi chicken ecotype cocks. Values in the table are means ± SEM

Parameters				
Age	**Sample size**	**Concentration (×10^9sperm cells/mL)**	**Viability (%)**	**Morphological normal spermatozoa (%)**
24-32 weeks	24	4.56 ± 3.34	91.04 ± 0.85	89.71 ± 0.68
28-36 weeks	24	4.34 ± 2.63	90.04 ± 0.77	87.08 ± 0.81
32-40 weeks	32	4.31 ± 2.27	90.00 ± 0.55	87.78 ± 0.51

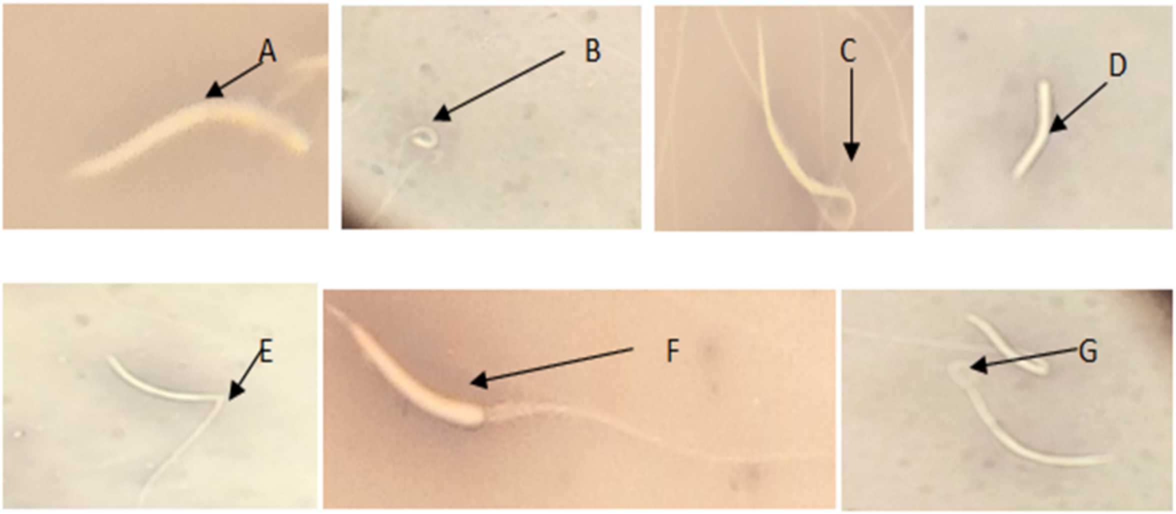
Table 4

Proportion of different Spermatozoa Abnormalities of the three age groups of Horasi chicken ecotype

	Sperm cell abnormalities (%)						
Age	**Sample size**	**Bent midpieces**	**Coiled tails**	**Bent tails**	**Swollen heads**	**Detached heads**	**Knotted heads**
24-32 weeks	24	15	5	4	1	1	1
28-36 weeks	24	19	8	6	2	1	1
32-40 weeks	32	18	7	6	3	1	1
Total	80	52	20	16	6	3	3

Figure 3

A micrograph illustrating the morphology of normal and defective Horasi cock spermatozoa (Eosin Nigrosin stain, 1000× immersion oil). (A) Swollen head, (B) Knotted head, (C) Bent tails, (D) Detached head, (E) Bent midpiece, (F) Normal morphology, and (G) Coiled tail.



Correlation between cock's body weight and semen quality

As the body weight of cocks increased the volume of semen collected also increased, indicating a strong association between the volume of semen produced and the body weight of cocks (Pearson correlation coefficient (r)=0.52). The correlation between these parameters was statistically significant ($p<0.05$). Also, sperm concentration increased as the cocks' body weight increased (r = 0.13). There was no statistically significant association between sperm concentration and body weight ($p<0.05$). A slight positive connection was found between the proportion of spermatozoa with normal morphology and the body weight of cocks (r = 0.03). Sperm motility and the proportion of viable spermatozoa demonstrated a negative connection with cocks' body weight (r = -0.03 and r = -0.04) respectively. Meaning that as body weight of cocks increased, semen motility and viability decreased.

Correlation coefficients of semen quality parameters of Horasi chicken ecotype

Pearson coefficients of sperm quality indicators ranged from very low to medium, with negative and positive values ranging from -0.22 to 0.38 as shown in Table 5. Positive and significant associations were found between sperm concentration and semen volume (r = 0.32), the volume of semen and proportion of spermatozoa that are alive (r = 0.11), sperm motility and morphologically normal spermatozoa (r=0.27), percentage of morphologically normal spermatozoa and proportion of spermatozoa that are alive (r= 0.38). Low but significant associations were observed between semen motility and percentage of sperm cells that are alive (r=0.07), sperm concentration and proportion of live spermatozoa (r = 0.09), and semen volume and sperm motility (r=0.03). Negative associations were observed between sperm motility and sperm concentration (r=-0.22), semen volume and proportion of morphologically normal spermatozoa (r=-0.06), sperm concentration and proportion of morphologically normal spermatozoa (r = -0.05).

Table 5

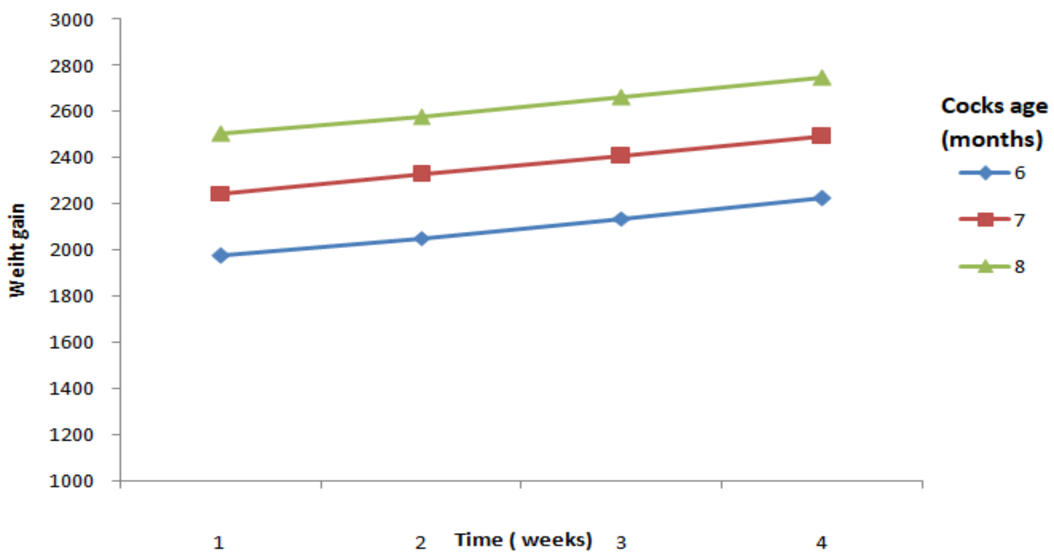
Correlation coefficient matrix of semen quality parameters of Horasi chicken ecotype

Parameter	Volume	pH	Motility	Viability	Concentration	Morphological normal spermatozoa
Volume	1.00	0.23*	0.03*	0.11*	0.32*	-0.06
pH	0.23*	1.00	0.00	0.17*	0.11*	-0.14
Motility	0.03*	0.00	1.00	0.07*	-0.22	0.27*
Viability	0.11*	0.17*	0.07*	1.00	0.09*	0.38*
Concentration	0.32*	0.11*	-0.22	0.09*	1.00	-0.05
Morphological normal spermatozoa	-0.06	-0.14	0.27*	0.38*	-0.05	1.00

*P < 0.05

Figure 4

The trend of Body Weight (grams) Increasement of Horasi cocks over the experimental period.



Discussion

Semen quality parameters of Horasi chicken ecotype were found to be good comparing to the findings reported in other indigenous chicken kept under humid tropical environment. The Volume of fresh semen collected differed significantly between individual cocks of Horasi ranging from 0.20 to 1.00 mL. These results were in line with those reported by Cole and Cupps (1977), indicating an average semen ejaculate of about 0.6 mL, with the cockerel producing between 0.1 and 1.5 mL per ejaculation. However, the observed semen volume of 0.6 mL by average produced by Horasi cocks was higher than those reported in other Tanzanian native chicken ecotypes (Luvanga and Kashoma, 2022). Similarly, our findings are in agreement with what has been documented in heavy breed (0.1- 0.9 mL) but superior than 0.05-0.50 mL produced by light strains of chicken ecotypes (Lake and Stewart, 1978). Hence, Horasi chicken ecotype has expressed its superiority in terms of semen production compared to other chicken ecotypes found in the country. Other traits attributable to Horasi ecotype includes higher hatch weight, fast growth rate and are heavier compared to other indigenous chicken ecotypes. Further researches have revealed that larger testes produce more spermatozoa during spermatogenesis, resulting in a higher amount of semen in roosters with larger body weights. (Adeyemo *et al*., 2007).

The variance in semen volume between individual cocks could be attributable to the fact that, cocks were of different age groups (24-32 weeks, 28-36 weeks and 32-40 weeks) with different body weight. Similar results have been documented regarding the effects of collection periods and ages of cockerels on the quantity of semen (Anderson, 2001). Also, the impact of nutrition and weight on controlling spermatogenesis, as well as responsiveness to the massaging technique during sperm collection may vary the volume of sperm produced among individual birds (Maule, 1962; Tarif *et al*., 2013). In the current study, the body weight of Horasi cocks varied from 2.4kg to 3kg, and the collected semen volume correlated positively with body weight of cocks. These observations were in line with those documented elsewhere (Tarif *et al*., 2013; Luvanga and Kashoma, 2022).

Semen colour is used to determine the density of the ejaculate. In this study, the semen colour was generally white creamy, and there was no any variation in colour of semen produced by cocks of different age. The colour of semen produced by Horasi cocks was normal and free from contaminations. An excellent sperm sample is thick and pearly white in colour (Cole and Cupps, 1977), and any other colour suggests contamination; for example, yellow and green-coloured sperm indicate faecal or urine contamination (Lake, 1983). Blood is frequently indicated by a brownish red pigment or a reddish colour (Etches, 1996), which might be caused by too much pressure during the collecting procedure or damage. Although the colour of the semen can vary according to the species of bird being utilized, it should typically be creamy to suggest a high sperm concentration (Yadav *et al*., 2019).

Different breeds of avian species have a modest variation in the pH of their semen. However, the ideal pH for chicken sperm is between 7.0 and 7.4. (Yadav *et al*., 2019). When the pH is between 7.0 and 7.4 (slightly alkaline), sperm motility is often high. This increases the ability to fertilize, as opposed to a pH of 6.4 (acidic), which could harm the sperm cell's plasma membrane (Yadav *et al*., 2019). In the current investigation, individual cocks' Horasi semen pH varied greatly, ranging from 7.06 to 7.45. Nevertheless, semen pH of 7.01 to 7.02 from indigenous chicken ecotypes range has been reported (Luvanga and Kashoma, 2022). This change in semen pH could be brought on by environmental and genetic influences. Large volumes of fluid from the auxiliary glands are typically present in low-quality semen, which raises the pH (Yadav *et al*., 2019). Also, the longer it takes between collection and measurement, the lower the pH of the semen is likely to be, and thin sperm collecting tubes may encourage sperm to produce lactic acid under anaerobic conditions by converting the fructose in the semen to lactate (Yadav *et al*., 2019). Additionally, ammonia may form in samples of sperm with a high concentration of dead sperm, raising the pH (Yadav *et al*., 2019). Sperm motility is a marker of both living sperm and the quality of the sperm sample (Taye and Esatu, 2022). In domestic fowls, sperm motility is one of the most important sperm characteristics and is a major factor of fertility because it indicates the ability of sperm to swim from the site of deposition to the storage tubules of the hen (Donoghue *et al*., 1998; Luvanga and Kashoma, 2022). Semen motility for Horasi chicken ecotype ranged from 60 to 80%, which is within the documented normal range of 60 to 80% reported elsewhere (Getachew, 2016; Luvanga and Kashoma, 2022). However, there was no significant variation in sperm motility between cocks of different age groups.

The percentage of viable spermatozoa in semen samples collected from Horasi cocks varied from 80 to 95%, which is suitable for routine artificial insemination in poultry breeding. These observations were in line with those reported by Luvanga and Kashoma (2022); where by the proportion of live spermatozoa of indigenous chicken ranged from 88.06 to 90.97%. Contrasting to the current findings, several studies have reported lower proportion of live spermatozoa in cocks' semen.

Siudzinska and Lukaszewicz (2008) reported percentage of spermatozoa that are alive ranging from 82.2 to 87.3%; Tabatabaei*et al*. (2009) reported proportion of live spermatozoa ranging from 82 to 89%. The variation in the quantity of living spermatozoa in cock's semen may be attributable to genetic differences in tolerance to processing stains (Luvanga and Kashoma, 2022).

Estimates for the optimum dilution to create an ideal number of spermatozoa (80-100 million) per insemination dosage can be made using semen concentration determination. Chicken sperm is modest in volume per ejaculation but high in concentration, with an average sperm concentration of 4-6 billion spermatozoa per millilitre (Donoghue and Wishart, 2000). In the present study, sperm concentration ranged from 1.55× 10^9sperm cells/mL to 7.57× 10^9sperm cells/mL. In contrast to the current observations, the concentration of spermatozoa in semen of local indigenous cocks in Nigeria was 2.26 × 10^9/ml (Bah and Chaughari, 2001). Gordon (2005) reported a typical sperm concentration in domestic cockerel sperm of 5 billion sperm cells per millilitre, whereas Hafez and Hafez (2000) reported sperm concentration of 3-7 billion sperm cells per millilitre similar to the current observations. The variance in sperm concentration can be attributed to a variety of factors, including generation, individual performance, and stimulation (Tarif*et al*., 2013). The percentage of spermatozoa with normal morphology in Horasi cock's semen varied from 80 to 95% and is within the normal range of 85 to 90%. The findings from this study are almost similar to those reported by Luvanga and Kashoma (2022), who reported the proportion of spermatozoa with normal morphology ranging from 86.16 to 89.38%, and Tarif *et al*., (2013) who reported the proportion of spermatozoa with normal morphology ranging from 87.2 to 90.1%.

The influence of age on semen parameters of Horasi chicken ecotype cocks was not significant except for semen volume. A significant higher semen volume was produced by cocks aging between 32-40 weeks, followed by cocks aged 28-36 weeks, and the lowest semen volume was produced by cocks aging 24-32 weeks. These findings are different from those reported by Luvanga and Kashoma (2022), and Long *et al.,* (2010). Variations of our findings with those reported elsewhere (Luvanga and Kashoma, 2022; Long *et al*., 2010) might be due to age differences of cockerels used as the cocks used in those studies were much older than those in the current study. This has been pointed out that normal physiological processes regulating spermatogenesis tend to decrease with age as cocks aged over 45 weeks have decreased testicular weight, testosterone hormone secretion and semen volume and may be followed by testicular regression at 55 weeks of age (Fragosa *et al*., 2013). Other semen parameters (sperm motility, sperm concentration, viability, and proportion of spermatozoa with normal morphology) decreased with age of the cocks. The current findings are in agreement with findings of Luvanga and Kashoma (2022) and Long *et al.* (2013) who documented a decrease in semen quality characteristics with age of the cocks. Moreover, complementary observations were reported by Tabatabaei *et al*. (2009), indicating an increase in the proportion of sperm morphological defects in Iranian indigenous chicken with age of cocks. Also, motility, viability and mass movement have been reported to slower in older birds than younger birds (Wishart, 2009).

The correlation between semen quality characteristics (semen volume, pH, sperm motility, sperm concentration, proportion of live spermatozoa, and proportion of spermatozoa with normal morphology) is thought to be important because it mostly determines the ejaculate's capacity to fertilize. The positive association (r = 0.07) between sperm motility and proportion of live spermatozoa indicates that only live spermatozoa have the ability to move, hence semen motility is employed as an indicator of semen viability.

Live and morphologically normal spermatozoa have an intact plasma membrane and acrosome, which prevents the penetration of eosin stains during the evaluation of sperm viability and morphological defects. However, injured and dead sperm cells have a porous plasma membrane that allows eosin to stain interior organelles pink by penetration (Bakst and Cecil, 1997). This is demonstrated by a positive correlation (r=0.38) between the proportion of live spermatozoa and the proportion of spermatozoa with normal morphology. Additionally, sperm cells with normal morphology are the only ones with the capacity to swim properly from the site of deposition to the site of storage and fertilization. This is indicated by a positive association (r=0.27) between sperm motility and the percentage of spermatozoa with normal morphology (Bakst, 2009). The proportion of live spermatozoa and sperm concentration also were found to be positively correlated (r = 0.09), similar findings were reported by Luvanga and Kashoma (2022).

In contrast to the findings of Peters *et al* (2008), Luvanga and Kashoma (2022), a negative association (r=-0.22) between sperm concentration and sperm motility was found. Indicating that, semen motility reduces as sperm concentration rises and vice versa. Deformed cells are recognized to be a normal component of every cell population; in highly concentrated semen, normal cells continue to coexist closely with deformed cells (Mohan *et al*., 2018). Therefore, by coming into touch, physically morphologically abnormal cells may mechanically harm healthy sperm cells, and hence lowering semen motility (Mohan et al., 2018).

Conclusion

In conclusion, Horasi chicken ecotype has higher hatch weight, fast growth rate, higher body weight and cockerels produce semen of higher quality compared to other Tanzanian indigenous chicken ecotypes. Body weight and age of Horasi chicken positively correlated with semen volume, sperm concentration, and sperm motility. Therefore, selection of cockerels basing on semen quality traits especially concentration and sperm kinematics parameters may improve fertility rate. Nevertheless, semen extension and preservation (refrigeration or cryopreservation) are suggested methods for successful artificial insemination. Thus, further studies are needed to elucidate the extension rate, preservation protocols in order to improve and encourage artificial insemination in poultry.

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