Effect of semen extenders, dilution rates and storage periods on spermatozoa quality of Horasi chicken ecotype

Amina Burilo\textsuperscript{ab} | Isaac Kashoma\textsuperscript{b}

\textsuperscript{a}Tanzania Livestock Research Institute (TALIRI), P. O Box 1425, Mtwara, Tanzania. 
\textsuperscript{b}Department of Veterinary Surgery and Theriogenology, P.O. Box3020, Sokoine University of Agriculture, Morogoro, Tanzania.

Abstract Numerous semen extenders have been recommended for storing poultry semen. This study was conducted to assess a suitable extender, storage period and dilution rate for semen collected from the Horasi chicken ecotype. To accomplish the objectives, a 4 x 4 x 4 factorial experiment in a completely randomized design employing four extenders (BPSE, LAKE, CARI and garlic extract mixture), four dilution rates (1:2, 1:3, 1:4 and 1:5) and four storage periods (0, 12, 24 and 48 hours) was used. Semen samples were collected from ten Horasi cockerels using the abdominal massage technique, kept at 4°C and subsequently examined for semen motility, viability and normalcy after extension with specific diluent, each dilution rate and storage period. Sperm motility and viability were significantly (p<0.05) reduced across all extenders and dilution rates examined, while defective spermatozoa increased with storage period. The Beltsville Poultry Semen Extender (BPSE) maintained high spermatozoa motility, viability, and normalcy at all dilution rates and storage periods tested, followed by the CARI and LAKE extenders, whereas the Garlic extender performed worse at all dilution rates and storage periods than the other extenders, especially at a 1:5 dilution rate and 48 hours of storage. Based on our findings, a high percentage of sperm motility, viability, and normalcy for Horasi chicken ecotype semen were observed while using BPSE at a dilution rate of 1:2, and the quality was maintained throughout the storage period tested. Thus, it is concluded that BPSE extender at a 1:2 dilution rate is a suitable extender for Horasi chicken ecotype semen.

Keywords: garlic extracts, semen diluent, semen motility, viability, semen morphology

1. Introduction

Tanzania’s poultry industry is divided into two production systems: traditional and commercial. Recent information indicates that the traditional system, which keeps indigenous chickens, is the largest, accounting for more than 90% of the chicken flock and supplying the majority of meat and eggs consumed in remote regions, as well as approximately 20% of eggs consumed in urban areas (Mushi et al 2020). Indigenous chickens are raised by many families, especially in rural areas, which are mainly kept under extensive production systems and are only left to scavenge with no or very little supplementation (Waktole et al 2018). Furthermore, indigenous chicken rearing provides income through sales of meat and eggs and thus contributes to the livelihood of people in rural settings (Manyelo et al 2020; Akid et al 2018). However, because of their low genetic capacity for meat and egg production, indigenous chickens have not been able to meet the increasing demand for chicken products, which is associated with an increase in the human population as well as income growth (MLDF 2019).

Strategies to increase the output of indigenous chickens through crossbreeding with foreign breeds have failed due to lack of adaptability, disease susceptibility and high production costs of the crossbreeds (Katule 1990; Fulla 2022). However, selective breeding, especially within and between existing indigenous populations to generate breeds with specific features for enhanced production and efficiency, is acknowledged as an effective strategy for increasing the productivity of indigenous chickens (Bett et al 2011; Okeno et al 2013; Wilson et al 2018).

In comparison to other indigenous chicken breeds kept in Tanzania, the Horasi chicken ecotype has demonstrated superiority in terms of growth rate, environmental adaptability, and egg and meat production potential (Guni et al 2013; Mangoka et al 2016). Thus, the Horasi ecotype can be employed in breeding programs aiming to boost indigenous chicken production by tapping the Horasi cocks’ superiority through artificial insemination (AI). Artificial insemination is globally considered a robust method for enhanced productivity and realizes rapid genetic gains in livestock. To achieve the benefits of AI, semen storage and cryopreservation are crucial factors to be considered (Sontakke et al 2004). However, to maintain the fertilizing ability of sperm for a long time and to reach the full potential of AI in poultry, suitable semen extenders are needed (Kubicova et al 2010). For good fertility of frozen semen, an extender that will preserve optimal sperm viability and motility, obstruct routes that are harmful to sperm survival (Oluwatoba et al 2017), provide energy to spermatozoa, and maintain
favorable pH and osmolarity values comparable to those of seminal plasma is highly recommended (Boucif et al 2011; Udeh and Oghenesode 2011; Ogbu et al 2014; Woelders 2021).

For poultry semen, many extenders have been advocated due to breed variances in the storability of semen, and hence, the careful selection of extenders for specific chicken breeds or ecotypes is needed (Dumpala et al 2006; Ogbu et al 2014; Siudzinska and Lukaszewicz 2008; Siudzinska and Lukaszewicz 2008). Sperm motility and the percentage of live and morphologically normal spermatozoa are some of the important metrics to consider when evaluating the suitability of semen extenders (Fischer et al 2014). These factors allow for the prediction of long-term spermatozoa storage and fertilization after artificial insemination (Froman 2000; Blesbois et al 2008). Therefore, the present study aimed to assess the performance of four different semen diluents on liquid stored Horasi chicken semen and to determine the dilution rate and storage time that can maintain spermatozoa quality.

2. Materials and Methods

2.1. Study Area

This study was conducted at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture (SUA). Sokoine University of Agriculture is 3.0 km from the center of Morogoro Municipality, which is approximately 200 km west of Dar es Salaam. Morogoro Municipality is in the eastern part of Tanzania, latitude: 6°49′15″ S, longitude: 37°39′40″ E. The elevation above sea level is 504 m, with an average annual temperature of 24.3°C and an average annual precipitation of 935 mm (www.climatedata.eu).

2.2. Experimental design

This study used a 4 x 4 x 4 factorial experiment with four varieties of extender, four dilution rates, and four storage periods in a completely randomized design (CRD). Four types of extender were used, including Beltsville Poultry Semen Extender (BPSE), LAKE, CARI and Garlic mixture (Garlic extract + Ringer’s solution + Coconut water). Dilution rates tested for each extender were 1:2, 1:3, 1:4, and 1:5. The storage periods tested were 0, 12, 24, and 48 hours. Throughout the experimental periods, the storage temperature was set at 4–5°C (refrigeration temperature).

2.3. Experimental birds and management

In this investigation, ten healthy adult Horasi ecotype cockerels aged between 6 and 8 months were used. The cockerels were intensively reared individually in a breeder cage system (40 × 40 × 60 cm) in an open-sided building with 12 hours of daylight. Each cock was fitted with a numbered wing band for individual identification. Chickens were vaccinated against common viral diseases (Gumboro, Newcastle disease, and fowl pox), regularly dewormed and given normal managerial practices, including the provision of commercial chicken breeder’s feeds (containing 17% crude protein) according to body requirements (100 – 120 gm per day) and clean water throughout the day. Cages were cleaned and disinfected weekly.

2.4. Semen collection

Cockerels were given two weeks of adaptation to the new environment before beginning semen collection and subsequently trained for two weeks to adapt to the adopted semen collection procedure. The abdominal massage method described by Burrows and Quinn (1935) was used to collect semen. Semen collection was carried out by the same person throughout the experiment twice a week, always between 8.00 and 9.00 AM. Ejaculates were collected in graduated collection tubes, macroscopically evaluated for volume (milliliter) and color, sealed with stoppers and finally stored at 5°C until processed.

2.5. Extender composition and preparation

Semen extenders used in this study were prepared as described elsewhere: CARI (Mohan et al 2000), BPSE (Sexton 1977), Lake’s semen diluents (Lake 1960) and Garlic diluent (Esqueria et al 2020). Briefly, BPSE was prepared by dissolving potassium diphosphate (trihydrate) (1.27 g), sodium glutamate (monohydrate) (0.867 g), fructose (0.5 g), sodium acetate (anhydrous) (0.43 g), tris (0.195 g), potassium monophosphate (0.065 g), potassium citrate (monohydrate) (0.064 g), and magnesium chloride (0.034 g) into 100.00 mL of double distilled water. This extender’s pH was set to 7.5, and the osmotic pressure was 333.00 mOsmol/kg. LAKE diluent preparation involved the addition of sodium glutamate (monohydrate) (1.92 g), sodium acetate (anhydrous) (0.51 g), potassium citrate (monohydrate) (0.128 g), glucose (0.80 g), magnesium acetate (0.08 g), potassium acetate (0.5 g), and polyvinylpyrrolidone (0.3 g) into double distilled water (100 ml). A pH of 7.08 and osmotic pressure of 344 mOsmol/kg were maintained in this type of diluent. The CARI diluent was prepared by dissolving sodium glutamate (0.85 g), fructose (0.600 g), sodium acetate (anhydrous) (0.400 g), tris (0.200 g), magnesium chloride (0.050 g), dipotassium hydrogen phosphate (0.634 g), potassium hydrogen phosphate (0.472 g), tripotassium citrate (0.100 g)
g), and sodium bicarbonate (0.002 g) in 100.00 mL of double distilled water. For this diluent, osmotic pressure and pH were maintained at 320 mOsmol/kg and 7.20, respectively, throughout the experiment. Garlic extender composition consisted of coconut water (0.2 g), garlic extract (0.02 g), sodium phosphate (0.04 g), and Ringer’s solution (100 mL). A sterile white cloth was used to filter the water from a young coconut before being collected in a sterile flask. Garlic extract was obtained by mixing garlic meat and double-distilled water and then blending using a blender. The ratio of garlic to water used to make the garlic extract was 1:4 (20 g of peeled garlic meat to 80 mL of double-distilled water). The combination was left to sit for a period of 72 hours (24.7-25°C) before being placed in a refrigerator (4-5°C) for 30 minutes; a white cloth that had been sterilized was then used to filter the mixture, and a garlic extract was obtained. Throughout the experiment, fresh extenders were prepared in each semen evaluation.

2.6. Semen quality assessment

In the laboratory, collected semen samples were assessed microscopically for motility, vitality and morphological normalcy. Semen samples with good quality (> 5.5 x 10⁹ sperm per ml, >70% motility, >80% viability and > 80% normalcy) were then pooled to avoid the effect of individual variability between the cocks. Thereafter, the pooled semen was diluted with specific extenders (CARI, BPSE, Lake and Garlic diluents). Four dilution rates (semen: extender) of 1:2, 1:3, 1:4, and 1:5 were used. Samples were divided into twelve (12) graduated tubes with equal volumes for every kind of extender and dilution rate and stored at refrigeration temperature (4°C) for four periods (0, 12, 24 and 48 hours). After each specific time of storage, three graduated tubes containing extended semen samples were thawed individually at 37°C for 30 seconds in a water bath and examined for spermatozoa quality (motility, viability and morphology).

2.7. Sperm motility

The thawed liquid stored semen was then examined under a light microscope to determine sperm motility (Hafez and Hafez 2000). Progressive sperm motility was examined at room temperature by placing a drop of semen sample on a prewarmed, spotless, and grease-free glass slide (37°C) with a cover slip over it and observed under a light microscope (Olympus, Japan). A scale from 0 to 100% was used to subjectively rate the proportion of motile sperm in five different areas of the sample on each slide.

2.8. Semen viability and morphology

Liquid stored semen was thawed and evaluated to obtain the percentage of live spermatozoa and percentage of morphologically normal spermatozoa. Assessment of live and dead spermatozoa and spermatozoa normalcy was performed using Eosin-Nigrosin staining (Blesbois 2007; Bakst and Dymond 2013). In brief, two drops of eosin-nigrosin stain were combined with one drop of semen sample, smeared on a microscopic glass slide, and fixed by air-drying at room temperature before observation under a microscope. In each slide, at least two hundred (200) spermatozoa were examined under an oil immersion (1000×) microscope. The number of live and dead spermatozoa was counted. Sperm that completely or partially absorbed the stain were counted as dead, while those that did not were considered to be alive. Furthermore, the same slides were also utilized to estimate the percentage of defective spermatozoa based on observable abnormalities in the spermatozoa’s head, neck, mid piece, and tail area.

2.9. Statistical analysis

Statistical analysis of the data was performed by using Statistical Package for the Social Sciences (SPSS) 25.0 software (Univariate Analysis of Variance). The proportions of sperm motility, viability, and morphology between extenders, storage periods, and dilution rates were compared by Tukey’s post hoc multiple comparisons. The variations in sperm motility, viability, and morphological normal spermatozoa among extenders, storage periods, and dilution rates were regarded as significant at the level P<0.05.

3. Results

3.1. Effect of semen extenders on spermatozoa motility, viability and morphology

Spermatozoa motility, proportions of spermatozoa with normal morphology and living spermatozoa in BPSE, LAKE, CARI, and Garlic diluents are presented in Table 1. Sperm motility, sperm viability, and proportion of morphologically normal spermatozoa differed significantly (P<0.05) between the four semen extenders (Table 1).

Nevertheless, the percentage of spermatozoa motility, viability and morphologically normal spermatozoa were higher in BPSE, followed by CARI and LAKE diluents, but the parameters were lower in Garlic extender (Table 1). The proportion of living spermatozoa and morphologically normal spermatozoa differed between CARI and LAKE diluents; however, the difference was not statistically significant (P>0.05).

3.2. The influence of storage times on spermatozoa motility, viability, and morphology
Spermatozoa motility, percentage of live-dead sperm cells and percentage of morphologically normal spermatozoa at 0 hours, 12 hours, 24 hours and 48 hours of storage are shown in Table 1. Significant (P<0.05) variations were detected in semen motility, viability and morphologically normal spermatozoa between the four storage periods (Table 1). Spermatozoa motility, live sperm cells, and morphologically normal spermatozoa decreased with storage time (Table 1).

3.3. Effect of dilution rates on spermatozoa motility, viability and morphology

The sperm motility, percentage of live spermatozoa and percentage of spermatozoa with normal morphology at 1:2, 1:3, 1:4, and 1:5 dilution rates are presented in Table 1. Sperm motility, viability and percentage of morphologically normal spermatozoa varied significantly at P<0.05 among the four dilution rates (Table 1). However, higher spermatozoa motility, percentage of live sperm cells, and percentage of morphologically normal spermatozoa were observed in 1:2, followed by 1:3, 1:4 and 1:5 (Table 1).

Table 1 Effects of semen extenders, storage periods, and dilution rates on sperm motility, viability and proportion of spermatozoa with normal morphology (mean ± SEM) of Horiasi chicken ecotype semen.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
<th>Parameters</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
<th>Morphological normal spermatozoa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extender</td>
<td>BPSE</td>
<td>86.94 ± 1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.35 ± 0.87&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.27 ± 1.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LAKE</td>
<td>77.42 ± 2.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.25 ± 1.67&lt;sup&gt;h&lt;/sup&gt;</td>
<td>67.40 ± 1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td>CARI</td>
<td>85.04 ± 1.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.83 ± 1.16&lt;sup&gt;h&lt;/sup&gt;</td>
<td>68.60 ± 1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td>Garlic extender</td>
<td>44.85 ± 3.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>52.92 ± 2.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.13 ± 2.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Storage period</td>
<td>0</td>
<td>91.02 ± 1.16&lt;sup&gt;h&lt;/sup&gt;</td>
<td>85.60 ± 1.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.69 ± 1.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>79.25 ± 2.51&lt;sup&gt;h&lt;/sup&gt;</td>
<td>79.33 ± 1.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.02 ± 1.86&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>24</td>
<td>68.9 ± 3.39&lt;sup&gt;h&lt;/sup&gt;</td>
<td>72.04 ± 2.57&lt;sup&gt;h&lt;/sup&gt;</td>
<td>62.29 ± 2.39&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>55.04 ± 3.50&lt;sup&gt;h&lt;/sup&gt;</td>
<td>63.38 ± 3.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.40 ± 2.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dilution rate</td>
<td>1:2</td>
<td>79.00 ± 3.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.27 ± 1.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>74.94 ± 1.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>75.23 ± 3.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.54 ± 2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.27 ± 2.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>72.29 ± 3.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.52 ± 2.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.50 ± 2.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>67.73 ± 3.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.02 ± 2.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.69 ± 2.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a, b</sup> Values with different letters within same column differ significantly from each other

3.4. Interaction effects of semen diluents, dilution rates and storage times on spermatozoa motility

In both extenders tested, the percentage of motile sperm cells decreased as the storage hours and dilution rates increased, whereby a large number of motile sperm (98.67 ± 1.16) were observed in semen extended using CARI diluent at 0 hours of storage and at a dilution rate of 1:2, and the lowest number of motile sperm (10.00 ± 5.00) was recorded in semen extended using Garlic diluent at 48 hours of storage at a dilution rate of 1:5 (Table 2). In the LAKE diluent, spermatozoa motility decreased as the storage hours and dilution rates increased, except at 0 hours at dilution rates of 1:2 and 1:3, sperm motility was maintained (Table 2). The maximum sperm motility (86.33 ± 1.53) for the Horasi chicken ecotype at the highest storage time was seen in semen diluted using BPSE extender at 1:2 dilution rates.

Table 2 Effect of BPSE, LAKE, CARI and Garlic extender on the motility of sperm cells of the Horasi chicken ecotype (mean ± SD) at different storage times and dilution rates.

<table>
<thead>
<tr>
<th>Extender</th>
<th>Dilution rate</th>
<th>Sperm motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Hour</td>
<td>12 Hours</td>
</tr>
<tr>
<td>BPSE</td>
<td>98.33 ± 0.58</td>
<td>93.6 ± 3.22</td>
</tr>
<tr>
<td></td>
<td>97.00 ± 1.73</td>
<td>88.32 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>96.00 ± 1.73</td>
<td>86.00 ± 5.29</td>
</tr>
<tr>
<td></td>
<td>95.33 ± 0.58</td>
<td>87.67 ± 2.52</td>
</tr>
<tr>
<td>LAKE</td>
<td>91.67 ± 2.89</td>
<td>88.3 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>91.67 ± 2.89</td>
<td>88.0 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>90.33 ± 4.04</td>
<td>85.0 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>86.67 ± 2.89</td>
<td>84.3 ± 4.04</td>
</tr>
<tr>
<td>CARI</td>
<td>98.67 ± 1.16</td>
<td>94.67 ± 4.16</td>
</tr>
<tr>
<td></td>
<td>97.00 ± 1.73</td>
<td>88.67 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>96.33 ± 1.53</td>
<td>88.6 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>94.33 ± 4.04</td>
<td>86.00 ± 1.73</td>
</tr>
<tr>
<td>Garlic extender</td>
<td>82.33 ± 2.52</td>
<td>65.0 ± 5.00</td>
</tr>
<tr>
<td></td>
<td>79.00 ± 3.61</td>
<td>61.6 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>80.00 ± 5.00</td>
<td>45.0 ± 5.00</td>
</tr>
<tr>
<td></td>
<td>75.00 ± 5.00</td>
<td>36.6 ± 7.64</td>
</tr>
</tbody>
</table>
3.5. Interaction effects of semen extenders, dilution rates and storage times on sperm viability

As the storage times and dilution rates increased, the proportion of spermatozoa that were alive decreased in both diluents (Table 3). However, in semen extended using BPSE, similar sperm viability was maintained after 0 hours and 12 hours of storage at dilution rates of 1:2 and 1:3 (P˂0.05). The highest proportion of live spermatozoa (91.67 ± 2.89) was observed at 0 hours of storage at dilution rates of 1:2 and 1:3 in semen diluted using BPSE, and the lowest sperm viability (17.67 ± 2.52) was observed at 48 hours of storage at a 1:5 dilution rate in semen diluted using Garlic extender (Table 3). The maximum sperm viability (87.00 ± 2.65) for the Horasi chicken ecotype at the highest storage time was seen in semen diluted using BPSE extender at 1:2 dilution rates.

Table 3 Effect of BPSE, LAKE, CARI and Garlic extender on the viability of sperm cells (mean ± SD) at different storage times and dilution rates.

<table>
<thead>
<tr>
<th>Extender</th>
<th>Dilution rate</th>
<th>0 Hour</th>
<th>12 Hours</th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPSE</td>
<td>1:2</td>
<td>91.67 ± 2.89</td>
<td>91.0 ± 3.61</td>
<td>88.6 ± 1.16</td>
<td>87.0 ± 2.65</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>91.67 ± 2.89</td>
<td>91.0 ± 3.61</td>
<td>83.33 ± 2.89</td>
<td>78.3 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>89.33 ± 1.16</td>
<td>86.6 ± 2.89</td>
<td>84.3 ± 4.04</td>
<td>82.0 ± 3.46</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>86.67 ± 2.89</td>
<td>82.3 ± 2.52</td>
<td>76.6 ± 7.64</td>
<td>75.0 ± 5.00</td>
</tr>
<tr>
<td>LAKE</td>
<td>1:2</td>
<td>88.33 ± 2.89</td>
<td>84.3 ± 4.04</td>
<td>76.6 ± 2.89</td>
<td>73.3 ± 5.77</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>87.67 ± 2.52</td>
<td>83.3 ± 7.64</td>
<td>81.6 ± 2.89</td>
<td>75.0 ± 5.00</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>86.67 ± 2.89</td>
<td>83.3 ± 5.77</td>
<td>76.6 ± 7.64</td>
<td>70.0 ± 5.00</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>86.00 ± 1.73</td>
<td>78.3 ± 2.89</td>
<td>75.0 ± 5.00</td>
<td>61.6 ± 2.89</td>
</tr>
<tr>
<td>CARI</td>
<td>1:2</td>
<td>90.00 ± 0.00</td>
<td>88.3 ± 2.89</td>
<td>84.3 ± 4.04</td>
<td>80.0 ± 0.00</td>
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<tr>
<td></td>
<td>1:3</td>
<td>88.67 ± 1.16</td>
<td>87.6 ± 2.52</td>
<td>81.6 ± 2.89</td>
<td>70.0 ± 5.00</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>88.33 ± 2.89</td>
<td>86.6 ± 2.31</td>
<td>83.3 ± 2.89</td>
<td>73.0 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>89.33 ± 1.16</td>
<td>85.0 ± 5.00</td>
<td>83.3 ± 5.77</td>
<td>63.3 ± 2.89</td>
</tr>
<tr>
<td>Garlic extender</td>
<td>1:2</td>
<td>88.67 ± 1.16</td>
<td>75.0 ± 5.00</td>
<td>61.6 ± 2.89</td>
<td>51.3 ± 4.16</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>80.00 ± 5.00</td>
<td>65.0 ± 5.00</td>
<td>45.3 ± 0.58</td>
<td>34.3 ± 4.04</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>70.00 ± 5.00</td>
<td>54.3 ± 4.04</td>
<td>37.6 ± 2.52</td>
<td>21.6 ± 7.64</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>66.67 ± 2.89</td>
<td>45.0 ± 5.00</td>
<td>32.3 ± 2.52</td>
<td>17.6 ± 2.52</td>
</tr>
</tbody>
</table>

3.6. Interaction effects of semen extenders, dilution rates and storage times on spermatozoa normalcy

The proportion of morphologically normal spermatozoa decreased over the storage period, and as the dilution rates increased in BPSE, CARI, and Garlic diluents, although in the CARI diluent, the proportion of morphologically normal spermatozoa was maintained at 0 hours at 1:3 to 1:5 dilution rates (Table 4). In semen extended using LAKE diluent, the percentage of spermatozoa with normal morphology was higher at 0 hours of storage at the 1:4 dilution rate than at the 1:2, 1:3 and 1:5 dilution rates (Table 4). Additionally, at 0 hours of storage at 1:2 and 1:5 dilution rates, the percentage of morphologically normal sperm cells was maintained, as well as after 24 hours of storage at 1:3 and 1:4 dilution rates (Table 4). The highest proportion of spermatozoa with normal morphology (88.33 ± 2.89) was recorded in semen extended using BPSE at 0 hours of storage and a 1:2 dilution rate, and the lowest proportion of spermatozoa with normal morphology (14.33

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± 4.04) was observed in semen extended using Garlic extender at 48 hours of storage and a 1:5 dilution rate. The maximum proportion of morphologically normal spermatozoa (81.67 ± 2.89) for the Horasi chicken ecotype at the highest storage time was seen in semen diluted using BPSE extender at 1:2 dilution rates.

4. Discussion

To fully utilize artificial insemination (AI) in poultry, semen extension is a very helpful procedure. Semen extenders are used to dilute the semen, keep it viable and fertile in vitro, and increase the number of hens that can be inseminated (Vasicek et al. 2015). The current study examined the impact of BPSE, CARI, LAKE, and garlic extract extenders on semen quality at different dilution rates and storage times. The BPSE, CARI and LAKE diluents had good performance in preserving Horasi chicken semen at 4°C. However, the BPSE extender was found to be superior to CARI and LAKE diluents in maintaining the motility, viability and normal morphology of spermatozoa under liquid storage for 48 hours at 4°C. These findings were in line with those reported in past research in Turkey and guinea fowl spermatozoa (Venkatesh 2005; Laffaldano 2010; Keerthy 2016). Furthermore, good reproductive rates (74%) were obtained in turkey sperm diluted with BPSE extender, inseminated after twenty-four (24) hours of storage (Burrows and Quinn 1937), which could also be due to preserved higher spermatozoa motility (Keerthy 2016). In contrast to the current study, CARI diluent was reported to be superior to BPSE, LSE and normal saline, leading to higher fertility rates (58.80 ± 9.36) in guinea fowl inseminated after 24 hours of storage (Mohan et al. 2015). These variations in performance among different poultry semen extenders could be due to a difference in the content of the diluents, pH, and metabolites produced during storage (Keerthy 2016).

The motility, viability, and morphological normal spermatozoa of Horasi chicken semen had a decreasing trend over the storage period regardless of diluents or dilution rates. These observations are in agreement with those reported in guinea fowl semen diluted using Lake Semen Extender (LSE), Modified Beltsville Poultry Semen Extender (MBPSE) and Beltsville Poultry Semen Extender (BPSE) (Keerthy 2016); Helmeted guinea fowl semen diluted using powdered coconut water (Lavor et al. 2012); Philippine local chicken semen diluted using AU and Lake’s Low Temperature (LLT) extenders (Antalan et al. 2015); and Bangladeshi native chicken diluted using Lake A and Lake B diluents (Das et al. 2016). In addition, similar outcomes were also reported elsewhere (Mohan et al. 2013; Hudson 2015). The decrease in spermatozoa motility, percentage of live spermatozoa, and morphologically normal spermatozoa over the storage period may be because sperm metabolites are released, changing the nutritional composition of the medium (Keerthy 2016). It is also documented that the osmolarity of the medium changes as sperm is stored (Clark 1984); this causes numerous sperm defects, which further lowers the sperm cells’ progressive motility (Keerthy 2016).

The present study, irrespective of extenders and storage periods used, observed a significant influence of dilution rates on the sperm motility, viability and morphology of Horasi cockerels. These semen parameters decreased as dilution rates increased. Comparable observations were reported by Hudson (2015), who reported superior sperm motility at lower dilution rates (1:2 and 1:3) in semen extended using BPSE and IMV diluents. Furthermore, Bonato et al. (2021) found that as the dilution rate increased, the proportion of normal and abnormal living spermatozoa decreased. Subsequently, the proportion of dead sperm cells increased as the dilution increased, as reported elsewhere (Bonato et al. 2021). Similarly, a comprehensive study conducted by Mohan et al. (2017) for preserving chicken semen using a CARI extender for 24 hours at various dilution rates of 1:1, 1:2, 1:3, 1:4 and 1:5 showed higher fertility (91.07 ± 1.91%) at a 1:2 dilution rate, inferior fertility (76.93± 2.54%) at a dilution of 1:1 and fertility of 49.40 ± 2.545 at a dilution rate of 1:5 during the fertile period of up to five days following artificial insemination. Furthermore, it has been noted that semen diluent types, dilution rates and preservation have a major effect on sperm motility, ionic balance, gas exchange and energy utilization (Saeki 1960; Parker and McDaniel 2003; Mohan et al. 2018). A dilution rate above fivefold decreases the life span of sperm cells, whereas semen diluted below 1:1 spermatozoa will not endure low temperatures (Hayden et al. 2015; Mohan et al. 2018). This is because malformed cells are the natural constituents of any cell population, and avian sperm are particularly concentrated; at low dilutions (below 1:1), normal cells remain in close contact with malformed cells (Mohan et al. 2018). Through mutual contact, morphologically abnormal cells could mechanically injure normal sperm cells. Thus, a low dilution rate (below 1:1) of avian sperm is not practically recommended (Mohan et al. 2018). Additionally, excessive dilution rates should be avoided because they reduce the amount of spermatozoa per insemination dose (Parker and McDaniel 2003; Mohan et al. 2018).

5. Conclusions

We can conclude from the foregoing discussion that the suitable semen extender for liquid storage (4°C) of the Horasi chicken ecotype semen is the BPSE extender at a 1:2 dilution rate. The above-stated combination maintained higher sperm motility, viability, and morphologically normal spermatozoa at a maximum storage period of 48 hours. Thus, this combination can be used for liquid storage of Horasi cockerel semen.

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Ethical Considerations

This study was carried out following the approval of the Sokoine University of Agriculture Ethical Committee (SUA/DPRTC/R/186 VOL III).

Conflict of Interest

The authors declare no competing interest.

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