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

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Abstract

The effect of hormone treatment on semen quality characteristics and reproductive performance in male animals has been studied extensively. However, limited information is available on effect of Gonadotropin releasing hormone (GnRH) treatment on semen characteristics in galliform species. The aim of this study was therefore to examine the effect of synthetic GnRH on semen characteristics in three ecotypes of Tanzanian native chickens. A total of thirty-six mature cockerels (Ching'wekwe, Morogoro-medium and Kuchi ecotypes) were used in this study. Thirty cockerels (ten from each ecotype) were intramuscularly injected with 10 mcg (0.2 mL) of GnRH (Factrel®) once a week for five consecutive weeks. Six cockerels (two from each ecotype) were used for control purposes and they were given 0.2 mL of normal saline solution. Semen was manually collected at weekly interval by abdominal massage technique immediately after last GnRH injection for five consecutive weeks. Results showed that respective semen quality characteristics including semen volume, sperm motility, sperm concentration, proportion of morphologically normal and live spermatozoa increased significantly ($p<0.05$) in the treatment group (0.55±0.02 mL, 80.02±0.30%, 4.80±0.14 × 10^9 sperm cells/mL, 91.25±0.3%, 91.65±0.31%) when compared to the control group (0.48±0.02 mL, 74.90±0.76%, 4.04±0.18 × 10^9 sperm cells/mL, 87.58±0.43%, 89.05±0.55%). Variations in semen pH between treated and control group was not significant. In conclusion this study indicates that semen quality characteristics can be improved by administration of GnRH to cockerels for increased semen quality characteristics and therefore increasing productivity in the poultry industry.

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

Introduction

Tanzania has an estimated 87.7 million chicken population, of which 75.1 million are on small and 12.5 million on large scale farms (URT, 2022), with a constant yearly growth rate of 3.9% (BFAP and SUA, 2018). However, poultry industry outputs in terms of meat and egg production is still below potential due to various elements such as the low genetic potential of native chickens, disease and diet (MLDF, 2015). Chicken native to Tanzania show a broad range of genetic and phenotypic diversity, including plumage colour and type, body shape and size, and productivity (Msoffe *et al.,* 2001; Minga *et al.,* 2004; Msoffe *et al.,* 2004). Among the approximately 200 native chicken ecotypes found in Tanzania, Ching`wekwe, Kuchi, and Morogoro Medium have potential for productivity and disease resistance (Msoffe *et al.,* 2002).

Spermatogenesis is a complex reproductive process involving the split of spermatogonial stem cells and the eventual production of spermatozoa while keeping the stem cell population (Thurston and Korn, 2000). This complex event occurs within the seminiferous epithelium and relies on the neurosecretory action of the hypothalamic nucleus with the secretion

of gonadotropin-releasing hormone (GnRH) (Hezarjaribi *et al.,* 2016). GnRH is primarily involved in the development and function of the reproductive axis in mammals, including birds. GnRH regulates gonadotropin secretion in mammals by stimulating gonadotropin cells in the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Pawson and McNeilly, 2005). FSH works directly on the Sertoli cells which are the cells responsible in providing nutrients and physical support to the developing spermatozoa and LH regulate germ cell development, release androgens by Leydig cells required for the production of mature spermatozoa, and stimulate interstitial cells to produce testosterone to support spermatogenesis. Thus, administration of synthetic GnRH may result in continuous release of LH from the anterior pituitary gland and production of Leydig cell enzymes that can convert cholesterol to testosterone, thereby affecting semen quality characteristics.

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

Materials and Methods

Study area

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

Experimental birds

A total of thirty-six Tanzanian native chicken ecotypes namely; Ching'wekwe, Morogoro-medium and Kuchi were used in this study. Twelve cockerels aged 11 months and of nearly the same body weight (2.8 Kg on average) from each ecotype were randomly selected from a heterogeneous native chicken population kept separately at the experimental poultry farm. The selected cockerels were matured (11 months old), apparently healthy and without any physical abnormalities. All cockerels were handled in accordance with the standard guidelines for animal experimentation approved by Research and Ethical Committee of Sokoine University of Agriculture (DPRTC/R/186/F26).

Figure 1: Photographs A, B and C are Ching'wekwe, Morogoro-medium and Kuchi cock ecotypes respectively

Management of experimental cockerels

The experimental cockerels used in this study were kept in separate breeder cages (40 × 40 × 60 cm) in an open-sided house with natural light hours. The cockerels were given home-made feed (18% crude protein and 2800 Kcal Kg^{-1} metabolizable energy) and fresh water ad libitum throughout the experimental duration. All cockerels were routinely vaccinated against Newcastle Disease, Fowl pox and Infectious bursal disease and were dewormed after every three months using Ivermectin oral solution (Promectine®).

Treatments

Six cockerels (two from each ecotype)

were used as a control group while thirty cockerels (ten from each ecotype) were used as treatment group. The treatment group received 10 mcg (0.2 mL) per kg body weight of GnRH (Factrel® - 50 mcg gonadorelin hydrochloride per ml Zoetis Animal Health, New York, NY, USA) intramuscularly as previously described (Hezarjaribi *et al.,* 2016) once a week for five consecutive weeks and semen collection started immediately after the last injection of hormone (Week 0). The control group received (0.2 mL) normal saline solution intramuscularly.

Semen collection

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

Semen evaluation

Semen volume and pH

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 based on the percentage of sperm showing frontward motion as previously described (Tadondjou *et al.,* 2013). Immediately after one minute of semen collection, 2 µL of semen was mixed with 100 µL of phosphate-buffered saline on a clean; grease free and warmed glass slide (37°C), and a prewarmed cover slip was put on top before examination under light microscope at 400x magnification. The proportion of motile sperm was subjectively estimated on a scale of 0 to 100% and at least 3 microscopic fields were observed and motility was stated as the percentage of spermatozoa with moderate to rapid progressive movement.

Sperm concentration

Sperm concentration (billions per millilitre) in the semen was assessed by the direct cell count technique using Neubauer counting chamber (Haemocytometer) as previously described (Salisbury *et al.,* 1978). 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 previously described (Ax *et al.,* 2000).

Sperm viability and morphology

The proportion of live and dead sperm cells was assessed by differential staining method using Eosin–Nigrosin stain (5% eosin, 10% Nigrosin) as previously described (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 at room temperature. For each particular slide, about 200 spermatozoa were examined using oil immersion at a magnification of 1000X. To determine the percentage of live spermatozoa, 10 fields per slide (at least 100 sperms per field) were directly counted using light microscope. The spermatozoa which appeared pink in colour (totally or partially stained with eosin) were regarded as dead while those 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 defects on the acrosome, head, mid-piece and tail of the spermatozoa were examined and at least 200 spermatozoa were observed from each sample.

Statistical Analysis

Statistical Product and Service Solutions (SPSS version: 20.0.0) software was used to

analyse the data. Analysis of variance (ANOVA) was used to evaluate the overall variation in cockerel semen quality characteristics between the control and treated groups. The data were presented as Mean± SEM and the differences in parameter values were regarded as significant at p value less than 0.05.

Results

A total of 180 semen samples (150 treated and 30 controls) were analysed. Semen volume and sperm concentration increased significantly ($p<0.05$) in a treated group (Fig. 2). Specifically, semen volume and sperm concentration between the control and treated groups significantly increased from 0.48±0.02 to 0.55±0.02 mL, and

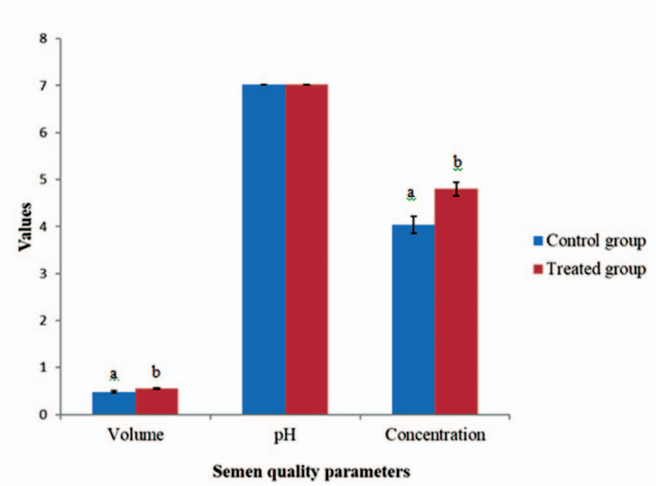

Figure 2: Comparison of semen volume, pH and sperm concentration (n × 10^9 sperm cells/mL) of Tanzanian local cockerels between the GnRH treated group and the control group. Significant differences was observed in semen volume and spermatozoa concentration (a,b=p<0.05)

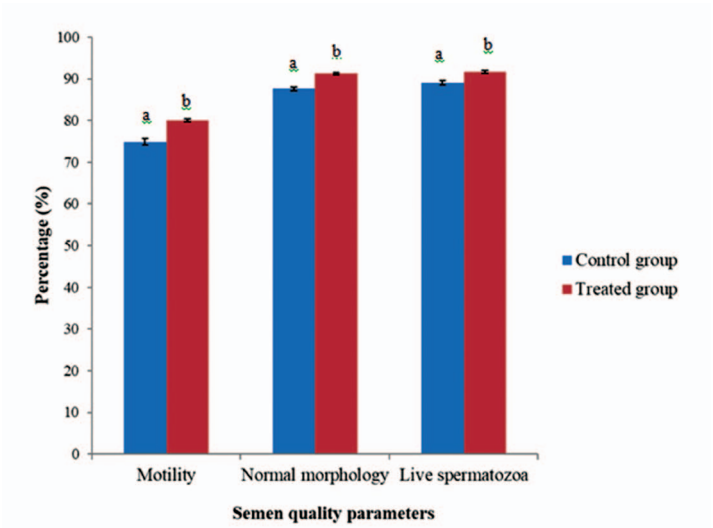

Figure 3: Comparison of sperm motility, normal morphology and proportion of live spermatozoa of Tanzanian local cockerels between the GnRH treated group and the control group. Significant differences are observed in semen motility, normal morphology and live spermatozoa. [a,b]denotes significant difference between collections

4.04±0.18 to 4.80±0.14 × 10^9 sperm cells/mL respectively. However, semen pH remained the same between the control and the treated group (7.02±0.00).

Sperm motility, proportion of morphologically normal and proportion of live spermatozoa increased significantly ($p<0.05$) after GnRH injection (Fig. 3). Sperm motility, proportion of morphologically normal and proportion of live spermatozoa between the control and treated groups varied from 74.90±0.76 to 80.02±0.30%, 87.58±0.43 to 91.25±0.3% and 89.05±0.55 to 91.65±0.31 respectively.

In the treated group, semen volume and sperm motility decreased from Week 2 to Week 4 of study period but the variations were not statistically significant ($p>0.05$) (Fig. 4&5).

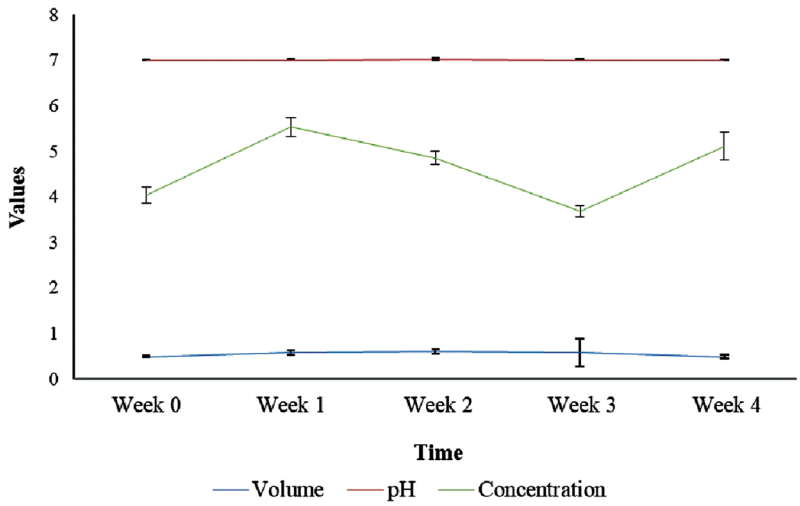

Figure 4: A weekly trend of semen volume (mL), pH and sperm concentration (n × 10^9 sperm cells/mL) of Tanzanian local cockerels after GnRH injection

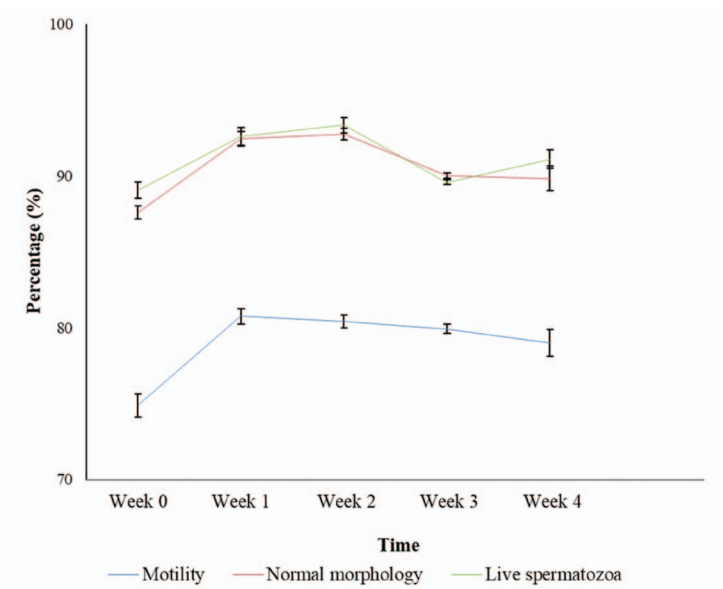

Figure 5: A weekly trend of sperm motility, normal morphology and proportion of live spermatozoa of Tanzanian local cockerels after GnRH injection

Table 1: Semen quality parameters among the three local Tanzanian chicken ecotypes before and after GnRH injection

Ecotype	Treatment	N	Semen volume (mL)	pH	Sperm motility (%)	Sperm concentration ($n\times10^9$)/mL	Morphological normal spermatozoa (%)	Live spermatozoa (%)
Ching'wekwe	CG	10	0.42±0.04	7.01±0.00	72.81±1.27	4.11±1.96	86.16±0.55	88.13±0.79
	TG	50	0.51±0.05	7.01±0.01	79.81±0.77	4.78±2.65	90.81±0.73	91.94±0.62
Kuchi	CG	10	0.51±0.03	7.02±0.00	76.63±1.35	3.90±0.98	89.38±0.80	90.97±0.81
	TG	50	0.59±0.04	7.02±0.01	80.75±0.31	4.73±2.12	91.94±0.51	92.38±0.55
Morogoro- medium	CG	10	0.52±0.03	7.02±0.00	75.25±1.26	4.12±0.87	87.22±0.79	88.06±1.13
	TG	50	0.57±0.02	7.03±0.01	79.50±1.27	4.87±2.67	91.00±0.27	90.62±0.38
P value			0.025	0.687	0.000	0.007	0.000	0.001

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

The proportion of morphologically normal spermatozoa started to decrease at Week 3 to Week 4 and the difference was statistically significant ($p<0.05$) (Fig. 5). Sperm concentration started to decrease at Week 2 of evaluation to Week 3 but increased again at Week 4 and the variations on sperm concentration within the five weeks were significant ($p<0.05$) (Fig. 4). The proportion of live spermatozoa decreased at Week 3 but increased at Week 4 of semen evaluation and the variations were statistically significant ($p<0.05$) (Fig. 5). A higher semen volume and proportion of live spermatozoa was recorded at Week 2 of evaluation, while a higher sperm motility, sperm concentration and proportion of morphologically normal spermatozoa was recorded at Week 1 of study period.

All semen quality characteristics except semen pH increased after GnRH injection in all three ecotypes and the difference was statistically significant with semen volume, sperm motility, concentration, morphologically normal and proportion of live spermatozoa ($p<0.05$) (Table 1). Semen pH remained unchanged in Ching'wekwe and Kuchi ecotypes even after GnRH injection but it increased in Morogoro medium ecotype but the increase was not significant ($p>0.05$).

Discussion

The objective of this study was to evaluate the effect of synthetic GnRH on semen quality characteristics of three ecotypes of Tanzanian native chickens so as to enable poultry breeders to incorporate the use of hormones in breeding programs and achieve high productivity. Results obtained show that, semen volume increased significantly in a treated group ($p<0.05$) and varied between treated and control cockerels. The increase in semen volume also has been reported in Gimmizah cocks treated with GnRH analogue (Abdo *et al.,* 2021). Similar findings were also reported when Alexandria cockerels were given GnRH analogue (Receptal®), (Samar, 2009). Furthermore, a ram study found that GnRH injection increased testosterone concentration and seminal fluid content in rams, resulting in increased semen volume (Ungerfeld and Fila, 2011). Semen volume increased up to week 2 of GnRH injection and then began to

decrease; this decrease in semen volume can be explained by the hormone's tendency to reach a threshold level above which it can no longer exert the required effect, which could be due to GnRH receptor down-regulation.

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

The concentration of spermatozoa in treated cockerels was higher than the control group and it varied from 4.04±0.18 to 4.80±0.14 $\times 10^9$ sperm cells/mL. The increase in sperm concentration was also reported in Gimmizah cockerels treated with GnRH (Abdo *et al.,* 2021). Similarly, Fathi *et al.* (2000) stated that GnRH treatment improved spermatozoa concentration of the naked neck cockerels particularly after long time treatment. Abdo *et al.* (2021) also reported that spermatozoa concentration of GnRH treated cockerels was significantly higher than the control group but concluded that GnRH dose has insignificant effect on spermatozoa concentration. Studies on the injection of three doses of GnRH analogue (gonadorelin diacetate tetrahydrate) every 2 days have been found to increase testosterone concentration and have decreased the semen collection duration and increased the sperm concentration in the ejaculate of camels (Monaco *et al.,* 2015).

Sperm motility increased significantly ($p<0.05$) after GnRH injection (Fig. 3). This effect was also reported by Fathi *et al.* (2000) who saw an increase in motility of naked neck cockerels' spermatozoa when treated with GnRH and Samar (2009) who also reported improvement on sperm motility as a consequence of GnRH analogue treatment in cockerels.

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

Conclusion

It can be concluded that semen quality characteristics were improved after GnRH injection but the improvement was only significant with semen volume, sperm concentration, motility, proportion of morphological normal and proportion of live spermatozoa. Poulty breeders are therefore encouraged to incorporate the use of GnRH hormone to improve semen quality and hence improving productivity in the poultry sector.

Acknowledgements

The authors would wish to thank the office of the Deputy Vice Chancellor (Academic) and the whole management of SUA for the financial support. Special thanks go to the project of Genomics to Improve Poultry (GIP), for a permission granted to use their chickens as semen donors. Special appreciations to Mr. Jackson Baisi Muganyizi and Dr. Benjamin John of the Genomics to Improve Poultry Project for their assistance during sample collection.

Conflict of interest

Authors do not have any conflict of interest.

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