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Semen Characteristics and Hematological Indices of Nigerian Local Cocks Subjected to Different Semen Collection Frequencies

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Abstract

This study sought to determine the semen quality and haematology of Nigerian light-ecotype (Normal feather) local chicken subjected to varying semen collection frequencies. Eighteen mature cocks (weighing 1.35 ± 0.15 kg) were randomly assigned to 3 groups of once (T_1), twice (T_2) and thrice (T_3) weekly ejaculation with 6 cocks in each and replicated thrice in completely randomized design. Semen collections were done by abdominal massage method for a period of 8 weeks and were evaluated for physical characteristics. At the 8th week, blood samples were collected for haematology. From the results, the progressive sperm motility and total sperm count did not differ ($p \geq 0.05$) among the treatment groups. Semen volume, sperm concentration ($\times 10^9/\text{ml}$), percentage live and normal sperm were affected by varying ejaculation frequencies. Cocks in T_2 had sperm concentration value (2.43) which was higher ($p < 0.05$) than that of T_1 (2.29). The sperm concentration increased while the percentage live and normal sperm decreased as the frequency of ejaculation increased from once to thrice weekly. Only the haematological parameters of Total white blood cell (WBC), lymphocytes, heterophil and eosinophil levels were observed to be affected by varying ejaculation frequencies. The WBC was observed to lower ($p < 0.05$) when the frequency increased to thrice weekly. The heterophil/lymphocyte ratio decreased with the increase in the ejaculation frequency. It was concluded that subjecting cocks to thrice weekly ejaculation yielded good quality semen while greatly reducing their stress levels and hence recommended for use in local chicken in view of improving their welfare.

Keywords: Artificial insemination, semen quality, hematology, cocks

Introduction

The use of Artificial Insemination (AI) in poultry breeding and hatchery operations has recently become a very common practice in Nigeria. Recently, AI in poultry has been established to express better fertility than natural mating having the ability to speed up the rate of genetic improvement by increasing selection differential, wherein one highly selected sire is mated with thousands of females (Verma *et al.*, 2012). However, selection for specific traits and accelerated genetic improvement using AI can only be achieved if high quality, fertile ejaculates could be acquired from males and inseminated in a stress-free manner into receptive females (Bonatoo *et al.*, 2011). Hence, the successful application of the AI to commercial hatchery operations depends largely on the ability to collect semen, as well as on the quantity and quality of sperm obtained from the breeder male (Zhaug, 2006). Maximizing sperm output, the absence of adequate procedures to handle poultry semen over long periods and the need to use the best genetic potential available from male line sires form the major reasons to optimize semen collection frequency in commercial AI programmes (Noirault and Brillard, 1999).

Despite the fact that several research efforts (Fan *et al.*, 2004; Zhaug, 2006; Mkpughe and Bratte, 2015) have been directed towards obtaining the optimal ejaculation frequency level which will produce the highest sperm output with the least negative impact on the semen quality indices, necessary attentions have not been paid to the physiological implications of these exercises.

Keeping this in view, the present study sought to evaluate the effect of varying ejaculation frequencies on the seminal characteristics and haematology of Nigerian light ecotype local chicken.

Materials and Methods

The study was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Nigeria. Eighteen (18) sexually mature light ecotype (Normal feather) Nigerian local cocks, about 10 months of age and with an average body weight of 1.35 ± 0.15 kg were used for the study. The birds were sourced from the Local Chicken Breeding Unit of the Department of Animal

Science Teaching and Research Farm, University of Nigeria, Nsukka. The birds were then randomly assigned to three treatment groups of 6 birds each in a Completely Randomized Designed (CRD). Each group was replicated thrice with 2 cocks each. The birds were housed individually in battery cages and allowed a pre-experimental period of 1 week for acclimatization and observations. All cocks were fed commercial breeder pellets containing approximately 16% crude protein (CP) and 2400 kcal/kg metabolizable energy (ME) while cool clean water was provided *ad libitum*. Routine management practices including vaccination and drug administration where necessary were observed.

The cocks in treatments 1, 2, and 3 (T₁, T₂, and T₃ respectively) were subjected to semen collection frequencies of once a week (Sundays), twice a week (Sundays and Wednesdays) and thrice a week (Sundays, Wednesdays, and Fridays) respectively. Semen collection was done in the mornings between 8.00am and 10.00am Nigerian time (GMT + 1) using the abdominal massage technique of Burrows and Quinn (1937). Clean graduated tubes were used to carefully collect the ejaculates and the semen volumes were read off directly to the nearest 0.01 ml. The tubes were then placed in a water bath maintained at 37°C prior to evaluation. Semen evaluation was done as described by Peters *et al.* (2008).

Haematological analysis was carried out at the end of the 8th week. Three birds were randomly selected from each treatment group making a total of nine samples. Blood samples were collected from the neck vein using a 5ml syringe and 23-gauge needle. They were immediately placed in sterilized bottles containing Ethylene Diamine tetra-acetic Acid (EDTA) anticoagulant for determining the haematological values. The haematological parameters were determined using Mindray Haematology Analyser (Mindray BC-2300, Guangzhou Shihai Medical Equipment Co., Ltd, China).

Data collected were subjected to analysis of variance (ANOVA) using SPSS software (IBM® version 20.0) and significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955) and accepted 5 or 1% level of probability.

Results and Discussion

The result of the physical semen characteristics of Nigerian light ecotype local cocks subjected to varying frequencies of ejaculation are presented in Table 1. Significant differences ($p < 0.05$) were observed in the semen quality characteristics of Semen volume, concentration, percentage live and dead sperm cells and percentage normal and abnormal sperm cells among the different treatment groups. However, the progressive sperm motility and the total spermatozoa per ejaculate were not affected ($p \geq 0.05$) by ejaculation frequency. Semen volume was similar for the cocks in T₁ and T₂ (0.36 and 0.37 ml respectively) but appeared to lower ($p < 0.05$) when ejaculation frequency was increased to thrice weekly collection (0.31 ml). The sperm concentration was observed to progressively increase as the frequency of ejaculation increased in the study (2.29, 2.36. and $2.43 \times 10^9/\text{ml}$ for cocks in T₁, T₂, and T₃ respectively). And the percentage live and normal sperm cells were observed to decrease as the ejaculation frequency increased from once to thrice weekly collection.

Table 1: Mean ($\pm$ SEM) Semen characteristics of Nigerian local cocks subjected to varying ejaculation frequencies

Parameters	T1(Once/Week)	T2(Twice/Week)	T3(Thrice/Week)	P value
Volume (ml)	0.36 ^a $\pm$ 0.02	0.37 ^a $\pm$ 0.02	0.31 ^b $\pm$ 0.01	0.006*
Prog. Sperm. Mot. (%)	78.75 $\pm$ 1.65	77.26 $\pm$ 1.10	76.49 $\pm$ 0.91	0.464 ^{NS}
Sperm Conc. ($\times 10^9/\text{ml}$)	2.29 ^b $\pm$ 0.05	2.36 ^{ab} $\pm$ 0.03	2.43 ^a $\pm$ 0.03	0.051*
Live Sperm cells (%)	90.40 ^a $\pm$ 0.85	88.34 ^{ab} $\pm$ 0.67	87.14 ^b $\pm$ 0.61	0.031*
Dead Sperm cells (%)	9.60 ^b $\pm$ 0.85	12.10 ^{ab} $\pm$ 0.92	12.86 ^a $\pm$ 0.61	0.097*
Normal Sperm cells (%)	88.75 ^a $\pm$ 0.81	85.76 ^b $\pm$ 0.59	85.26 ^b $\pm$ 0.52	0.004*
Abnormal Sperm cell (%)	11.25 ^b $\pm$ 0.81	14.71 ^a $\pm$ 0.83	14.62 ^a $\pm$ 0.52	0.022*
Total sperm cells ($\times 10^9/\text{ejac.}$)	0.83 $\pm$ 0.06	0.88 $\pm$ 0.04	0.76 $\pm$ 0.04	0.121 ^{NS}

a, b, c Row means with different superscripts differ significantly ($p < 0.05$); ^{NS}: Non significant; *: Significant; Prog. Sperm. Mot: Progressive sperm motility; Sperm Conc.: Sperm concentration; ejac.: ejaculation

Sperm reserves in birds are located in the distal halves of the ductus deferens and, depending on the rate of replenishment; time is needed to refill them with semen (Bakst and Cecil, 1981). In view of this, McDaniel and Sexton (1977) demonstrated that a smaller interval between two successive daily semen collections yielded

less sperm in chickens. The progressive decline in the semen volume as well as the progressive increase in the sperm concentration associated with increasing semen collection frequency as reported in the present study may suggest a possible decrease in seminal fluids secretion, but not in rate of spermatogenesis, with increasing ejaculation frequency.

Mayorga-Torres *et al.* (2015) argued that frequent ejaculations over an extended period of time could lead to insufficient time for the maturation of spermatozoa. Consequently, this will negatively influence the chromatin quality which is associated with immature spermatozoa that are highly susceptible to DNA fragmentation. Thus, it is possible that higher frequent ejaculation may have drained the vas deferens at a higher rate than can allow enough time for full sperm maturation, hence the increase in the amount of abnormal and dead sperm cells observed in the present study.

The effect of varying frequencies of semen collection on the haematological indices of Nigerian light ecotype local cocks are shown in Table 2

Table 2: Effect of semen collection frequencies on the haematological indices of Nigerian Local cocks

Parameters	Frequency of semen collection (Weekly)			P-value
	Once (T ₁)	Twice (T ₂)	Thrice (T ₃)	
PCV (%)	32.25	36.83	36.33	0.363 ^{NS}
HB. Conc (g/dl)	11.39	10.50	13.13	0.125 ^{NS}
RBC. Count(×10 ⁶ /ml)	2.24	2.77	2.78	0.241 ^{NS}
Total WBC (×10 ³ /ml)	90.50 ^a	100.33 ^a	68.17 ^b	0.001 ^{**}
Lymphocytes (×10 ³ /ml)	46.02 ^{ab}	50.21 ^a	35.14 ^b	0.056 ^{**}
Heterophil (×10 ³ /ml)	36.33 ^a	36.66 ^a	23.53 ^b	0.028 ^{**}
Eosinophil (×10 ³ /ml)	2.79 ^{ab}	5.05 ^a	2.55 ^b	0.078 ^{**}
Monocyte (×10 ³ /ml)	2.70	3.35	3.15	0.818 ^{NS}
Basophil (×10 ³ /ml)	3.66	5.06	3.80	0.247 ^{NS}
H/L ratio	0.79	0.73	0.67	

a, b, c Row means with different superscripts differ significantly ($p < 0.05$); ^{NS}: Non significant; ^{*}: Significant; PCV: HB: haemoglobin concentration; Packed cell volume; RBC: Red blood cell; WBC: White blood cell;

The results of the haematology revealed that the packed cell volume, mean haemoglobin concentration, red blood cells count, monocyte and basophil level did not differ ($p > 0.05$) among the various ejaculation frequency groups. The total white blood cell count (WBC), lymphocytes (lymph), heterophil, and eosinophil counts however, were observed to differ ($p < 0.05$) among the treatment groups. The cocks in the twice-weekly semen collection group had the highest WBC count and Heterophil value which were similar to those of the once weekly collections. The least WBC and heterophil values were observed in the thrice-weekly collection which differed ($p < 0.05$) from those of the other groups. Similarly, the highest blood lymphocytes and eosinophil counts were observed in T₂ group which were higher ($p < 0.05$) than the values obtained for the T₃ group which had the least value. The blood lymphocyte and eosinophil of the T₁ cocks were however observed to be similar ($p \geq 0.05$) to those of the T₂ and T₃. The heterophil/lymphocytes values were highest in the once weekly semen collection and progressively declined as the frequency of ejaculation increased to thrice weekly.

The blood leucocytes in avian have been reported by Shaniko (2003) to serve a phagocytotic role and are responsible for defending the body against infections and as such are routinely used as indicators of stress responses and sensitive biomarkers of immune functions. The lower WBC and heterophil count observed for the birds in T₃ indicates that they were less stressed compared to birds in the once and twice weekly ejaculation groups. Increase in H/L ratio has been used as a reliable and steady indicator of stress in birds and other animals (Altan *et al.*, 2000), suggesting that increasing ejaculation frequency of birds in this study reduced their stress levels. Sexual activities have been known to greatly relieve stress and boost immunity by the release some pituitary glands compounds such as endorphins and oxytocin (Rokade, 2011). The increased frequency of ejaculation in this study may have resulted to more release and longer body circulations of these endogenous opioids (endorphins), leading to observed reduced stress and improved immunity in thrice weekly ejaculated cocks.

Conclusion

It was concluded from the study that subjecting Nigerian local cocks to an ejaculation frequency of thrice weekly yielded optimal semen of appreciably quality when compared to lower frequencies of once and twice weekly with a tremendous influence in reducing the stress levels and improving the immune state of the birds.

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