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SPERM CHARACTERISTICS AND REPRODUCTIVE HORMONES OF NOILER COCKS ON HIGH SALT DIET

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Abstract

Poultry birds are prone to high salt consumption resulting from poor feed milling or technical error. The role of high salt diet in reproductive dysfunction, inflammation and oxidative stress in humans and experimental rats is well reported. Sixteen noiler cocks (34 weeks old) were randomly divided into two groups – A (control) and B each containing eight birds and reared for eight weeks. Group A received control diet (0.36 % salt) while group B was placed on high salt diet (0.54% salt) for eight weeks. Blood and semen samples were collected at the end of 8th week to evaluate sperm characteristics and serum level of testosterone, follicle stimulating hormone and luteinizing hormone. Mean $\pm$ SEM values were calculated and compared for significant differences with independent T-test at $p < 0.05$. The sperm livability decreased significantly in group B when compared with group A. The semen volume observed in group B was significantly higher than semen volume recorded in group A. The serum concentrations of testosterone and luteinizing hormone decreased significantly in group B when compared with group A. The mass activity, sperm motility and serum concentrations of follicle stimulating hormone in both groups were not significantly different when compared.

The above results revealed that high salt diet improved semen volume but impaired reproductive capacity of the birds by reducing sperm viability and the circulating level of reproductive hormones.

Keywords: Noiler cocks, Salt, Sperm characteristics, Reproductive hormones

Introduction

The management of the reproduction efficiency of the hen and rooster is the basis of breeder production. The increased poultry production is dependent on the fertility of the parent flocks, male and female. The fertility of male parent flock is more important as few number of males are used for insemination of large number of hens and production of a huge number of fertilized eggs (Lagares *et al.*, 2017). Various factors such as oxidative stress, stressors (Fouad *et al.*, 2019), diet and minerals have been reported to impair the fertility of cocks. Testis of cocks is naturally prone to oxidative stress due to their high metabolic rate (Faure *et al.*, 2017) and this can become aggravated in the presence of oxidative stress-causing agents. Study has shown that oxidative stress is a common feature in male infertility (Hamid *et al.*, 2017) and caused poor sperm quality (Agrawal *et al.*, 2014). Minerals including sodium chloride are essential component of feed formulation and important for health of poultry birds. The deficiency and excesses of minerals and micronutrients has been reported to impair reproductive functions (Smith and Akinbamijo., 2000). High dietary salt intake in birds is commonly associated with poor feed milling. There is a report of salt intoxication due to excessive salt intake in broilers (Perelman *et al.*, 2016). The role of

high salt diet in oxidative stress has also been shown (Huang *et al.*, 2017). Furthermore, pro-inflammatory role of high salt concentration on immune cells has been reported (Barbaro *et al.*, 2017).

Despite the reported effects of high salt diet on the reproductive performance of mammals and experimental rats, there is insufficient information regarding the effects of high salt diet on reproduction of poultry birds. Therefore, this study was carried out to examine sperm characteristics and reproductive hormone level of noiler cocks on high salt diet.

Materials and Methods

This experiment was carried out at Oyo State College of Agriculture and Technology, Igboora

Source of birds and feed formulation

Sixteen male noiler breeders (34 weeks old) were sourced from a reputable farm in Ibadan. Commercial feed (Top feed) containing 0.36% (3.6 kg/1 tonne of feed) of salt was bought and divided into two portions. One portion served as the control diet while the second portion served as high salt diet. High salt diet (0.54%) was achieved by adding 18 g to every 10 kg of feed.

Experimental design and birds' management

The birds were randomly divided into two groups (A and B) each containing eight birds and reared for eight weeks. Group A received control diet (0.36 % salt) while group B was placed on high salt diet (0.54% salt). The birds were reared in a demarcated pen with good ventilation. Feed and water were given *ad libitum* to the birds throughout the period of the experiment.

Birds sacrifice and sample collection

At the end of eighth week of rearing, the birds in each group were bled via the wing vein puncture into plain bottles. The blood samples collected were separated by centrifuge at 2000-3000 RPM for 20 minutes to obtain serum for analysis of reproductive hormone. Semen samples were collected into plain sample bottles by abdominal massage technique which involved manual massage of cloacal region to achieve phallic tumescence and cloacal stroke.

Blood and Semen Analysis

Serum concentrations of testosterone was analysed using ELISA-TE187S (Bioassay Technology Laboratory, USA). Serum concentrations of follicle stimulating hormone and luteinizing hormone were determined using ELISA kit-FS232F (Calbiotech), ELISA kit-LH231F (Calbiotech) and ELISA kit-respectively.

Semen volume for each birds was measured using tuberculin syringe to the nearest 0.1ml. Mass motility was determined by placing a drop of fresh semen on a clean glass slide and examined with a microscope under x 10 objective lens. The mass motility was scored subjectively according to the intensity of the wave motion seen in the medium by collective activities of spermatozoa from the absence of wave motion (+) to very turbulent motions (+++). Sperm motility was determined by adding 2 drops of warm 2.9 % sodium citrate to the semen on the slide. The viability and motility were assessed by staining of sperm smear with eosin-nigrosin staining technique (WHO, 2010)

Data analysis

The data collected from this experiment were analyzed with SPSS (Version 23). Means and standard deviations were calculated (Mean $\pm$ SD) and the differences between the means were tested with independent T-test at $P < 0.05$.

Results

The sperm livability decreased significantly in group B when compared with group A as shown in table 1. There were no significant differences in sperm membrane integrity and sperm motility in both groups. Similarly, the mass activities recorded in groups A and B were not significantly different. The semen volume observed in group B was significantly higher than semen volume recorded in group A. The serum concentrations of testosterone and luteinizing hormone decreased significantly in group B when compared with group A. The serum concentrations of follicle stimulating hormone in both groups were not significantly different when compared.

Table 1: Sperm Characteristics and Serum Level of Reproductive Hormones in Control and Treatment Groups

Parameters	Group A	Group B
Sperm livability (%)	99.67 $\pm$ 0.82	95.55 $\pm$ 3.01*
Membrane integrity (%)	87.83 $\pm$ 8.23	89.00 $\pm$ 6.42
Sperm motility (%)	80.83 $\pm$ 13.93	85.83 $\pm$ 5.84
Semen volume	0.13 $\pm$ 0.06	0.28 $\pm$ 0.07*
Mass activity (%)	73.33 $\pm$ 24.22	86.67 $\pm$ 24.22
Testosterone (ng/ml)	5.72 $\pm$ 0.56	3.85 $\pm$ 0.58*
LH (miu/ml)	0.68 $\pm$ 0.03	0.42 $\pm$ 0.09*
FSH (miu/ml)	0.48 $\pm$ 0.05	0.47 $\pm$ 0.06

* indicates group B is significantly different from group A

Discussion

Sperm characteristics is widely used to assess the reproductive potential or fertility of male animals including roosters. The significant elevation in semen volume in birds on high salt diet might be related to increased presence of mineral ions which have to potential to improve reproductive efficiency in animals. The recent study that reported reduced ejaculate volume in animals fed with high salt diet contradicts the finding in this present study (Fang *et al.*, 2018). The significant reduction in sperm viability in birds on high salt diet was as a result of lower gonadotropin level recorded in the group. In line with the observation in this present study is the finding that reported low sperm viability in rats on high salt diet (Adekunbi *et al.*, 2016).

The reduced testosterone level in group on high salt diet could be linked to low level of luteinizing hormone recorded in the group. LH is known to be key hormones that stimulates production of steroid hormones such as testosterone in the testis (Dunn et al., 2003), hence its decreased level in the circulation can be associated with reduction in testosterone synthesis in the gonad. The reports showing reduction in plasma concentration of follicle stimulating hormone, luteinizing hormone and testosterone in animals on high salt diet supported the observation in this present study (Evenson and Jost, 2000; Fang *et al.*, 2018).

In conclusion, high salt diet improved semen volume but impaired reproductive capacity of the birds by reducing sperm viability and the level of reproductive hormone in the circulation.

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