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# Testicular biochemicals, sperm reserves and daily sperm production of West African dwarf bucks fed varied levels of dietary aflatoxin

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ABSTRACT

An experiment was conducted with twenty West African dwarf (WAD) bucks (5–6 months old) to assess reproductive potentials of growing WAD bucks to varied dietary aflatoxin of 0 μg/kg, 50 μg/kg, 100 μg/kg and 150 μg/kg containing in diets 1 (control) 2, 3 and 4 respectively, for a period of 12 weeks. At the end of the 12th week, the reproductive tracts of bucks were excised and homogenised in physiological saline for assessment of glucose, total protein and testosterone concentration, gonadal and extra gonadal sperm reserves. Results showed that gonadal and extra-gonadal sperm reserves of goats fed control diet ($2.71 \times 10^9$ and $3.07 \times 10^9$ spermatozoa respectively) were superior ($p < 0.05$) to those fed 50 μg/kg, 100 μg/kg and 150 μg/kg [($1.59 \times 10^9$ and $2.33 \times 10^9$), ($1.09 \times 10^9$ and $2.45 \times 10^9$) and ($1.00 \times 10^9$ and $1.41 \times 10^9$) spermatozoa respectively]. Daily sperm production of bucks fed the control diet was significantly ($p < 0.05$) higher ($7.60 \times 10^8$ spermatozoa/testis) than those fed 50 μg/kg ($4.47 \times 10^8$), 100 μg/kg ($3.07 \times 10^8$) and 150 μg/kg ($2.80 \times 10^8$ spermatozoa/testis). Sperm production efficiency also follows the same trend as daily sperm production. Glucose and total protein concentration in the testes declined significantly as the aflatoxin level increases in the diets. Testosterone level was significantly lower in goats fed 100 μg/kg than others. The study suggest that exposure of male goats to dietary aflatoxin up to 50 μg/kg diet will reduced testicular biochemical and testosterone with resultant depression in sperm storage capability and daily sperm production in the animals.

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## 1. Introduction

The success of any animal breeding programme lies on optimisation of extrinsic factors such as nutrition and environmental temperature (Lewis et al., 2009). Steaming up and flushing are nutritional strategies of improving animal performance potential prior to reproductive processes (Steele, 1996) such as semen collection, super ovulation, early and late gestation foetal growth. Concentrate supplementation in ruminant diet is essential for improve performance of the animals, by increase availability of essential nutrients.

Diet is the major way through which humans as well as animals are exposed to aflatoxins (Whitlow and Hagler, 2005). Because ruminants consume forages, by-product feeds, and wet feeds, they are exposed to a broader range of mycotoxin at concentrations that are perhaps higher than those that are found in dry grain mixtures (Agag, 2004; Ewuola et al., 2013). Aflatoxin occurrence is a cross-cutting

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issue that is undermining public health and development efforts. Unless aflatoxin levels in crops and livestock are effectively managed, international development efforts to achieve greater agricultural development, food security and improve health will be undermined, particularly in sub-Saharan Africa where contamination is widespread and often acute (Marin et al., 2002). Because of its toxicity, Aflatoxin contamination is both a food safety and public health issues worldwide most especially in Africa (Atanda et al., 2013).

Chronic aflatoxin exposure in animals can result in impaired reproductive efficiency. Hussein and Brasel (2001) observed that production (milk, beef, wool), reproduction and growth can be altered when ruminants consumed mycotoxin-contaminated feed for extended periods of time. Aflatoxicosis has also been shown to cause decreased fertility, abortion, lowered birth weights and disturbances in hormonal metabolism (Raisbeck et al., 1991) in sheep. There is an imminent link between chronic exposure of animals to aflatoxin and male reproductive health (Ibeh, 2000). Pregnant and growing animals are less susceptible than young animals, but more susceptible than mature animals (Cassel et al., 1988).

In light of these, there is need for the evaluation of reproductive response of Nigerian indigenous bucks to dietary aflatoxin exposure as information obtained will help to highlight consequences and inform livestock breeders of economic impact of aflatoxin on breeding programmes in Nigeria under the tropical humid environment.

## 2. Materials and methods

### 2.1. Experimental site

The experiment was carried out at the Teaching and Research Farm, University of Ibadan, Nigeria.

### 2.2. Experimental materials and feeding trial

This study was approved by our institutional committee on the care and use of animals for experiment. *Aspergillus flavus* cultured maize grains containing aflatoxin was generated at the Plant Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan and Aflatoxins were quantified using scanning densitometer, CAMAG TLC Scanner 3 with win – CATS 1.4.2 software (Camag AG, Muttenz, Switzerland), as described by Suhagia et al. (2006). Aflatoxin contaminated maize was used with uncontaminated maize to vary the level of aflatoxin concentration to be 0 μg/kg, 50 μg/kg, 100 μg/kg and 150 μg/kg in diets 1, 2, 3 and 4 respectively.

Twenty male goats of 5–6 months old with average weight of 8.4 ± 0.2 kg were procured from the same location in Oyo town, Nigeria and acclimatised for 3–4 weeks at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The animals were treated against ecto- and endo-parasites and also vaccinated against *Peste des pestis ruminants* (*PPR*) disease during the physiological adjustment period. The animals were randomly allotted to the four dietary treatments such that there were 5 animals, housed individually per treatment in a completely randomised designed experiment that lasted for 12 weeks.

Animals were fed concentrate as supplement to *Gliricidia sepium* (40:60). Dietary treatments were offered to the respective animals twice daily. Feed supply was adequate and responsive to the bucks' weight changes, since feed consumption would be expected to change with body weight. The gross composition of the diets (concentrates) is shown in Table 1. All diets were isonitrogenous and isocaloric. At the end of the feeding trial, the animals were sacrificed and their reproductive tracts were dissected for assessment of gonadal and extra gonadal sperm reserves.

### 2.3. Estimation of gonadal sperm reserves

The right and left testes of each goat were carefully excised after slaughter, trimmed and weighed. The left testis and the right testis were homogenised separately, in physiological saline (0.154 M NaCl) at 200 mg/ml and the volume recorded. The suspension was mixed and filtered through a double layer of sterile gauze into graduated test tubes and analysed the same day. A gonadal sperm reserve was estimated as the total number of late spermatids and spermatozoa in the homogenised testicular tissues. Suitable adjustments were made for the dilution and the sperm concentration in the left and right testis by weight as outlined in Ewuola and Egbunike (2010). All sperm reserves were expressed in millions.

### 2.4. Estimation of extra gonadal sperm reserves (ESR)

The intact left and right epididymis were carefully removed from the testis, separated into the three main regions of epididymis (caput, corpus and cauda) on the basis of external morphology and completely macerated in normal saline at 100 mg/ml. The volume was recorded and the suspension filtered through two layers of cheese cloth into graduated test tubes and analysed immediately. Extra gonadal sperm reserves were determined by direct counting of sperm cells in haemocytometer after 1:2 (v/v) dilutions as outlined in Ewuola and Egbunike (2010). The extra gonadal sperm reserve was the total number of spermatozoa in all the sections of epididymal tissues expressed in millions.

### 2.5. Daily sperm production (DSP)

The daily sperm production was determined by direct measurement from testicular sperm reserves. The numbers of late spermatids and spermatozoa in homogenised testes were determined by haemocytometeric counts using time divisor of 3.56 proposed by Amann (1970) as estimated from the 48 days duration of one cycle of the seminiferous epithelium. The daily sperm production was then calculated with the formula as outlined in Ewuola and Egbunike (2010).

$$\text{DSP} = \frac{\text{Testis sperm count}}{\text{Time divisor}}$$

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