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# The Reproductive Performance of Breeder Cocks Fed Cottonseed Cake-based Diets

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**Abstract:** Histology of the testes of breeder cocks fed replacement levels of 0, 25, 50, 75, or 100% of cottonseed meal (CSM) for soybean meal (SBM) in five different breeder diets for fifteen weeks were investigated in a completely randomized design. Chemical analyses were carried out to determine the crude protein (CP) and gossypol content of CSM. The 23 week-old breeder cocks were fed the experimental diets for 15 weeks, euthanized and their testes removed for histological studies. The determined CP and gossypol contents of CSM were 35.11% and 570 g/ton respectively. The daily sperm production (DSP) by the breeder cocks was depressed when CSM dietary inclusion was above 50% with DSP values ranging from $1.5\times10^{9}$ to $2.9\times10^{9}$/mL. The investigation showed that replacing 75% SBM with CSM was not injurious to the non-reproductive health of the breeder cocks. However, CSM may not replace more than 50% of SBM for breeder cocks because of the depressing effect of gossypol on sperm production when this level is exceeded.

**Key words:** Breeder cocks, cottonseed meal, daily sperm production, histology, reproduction

## Introduction

In the late 1930's and early 1940's not a single childbirth was reported during a period of ten years in one area of China. It was found that for economic reasons villagers had switched from cooking with soybean oil to crude cottonseed oil (Liu, 1957). Women suffered from amenorrhea and men were impotent. These epidemiological findings led to clinical studies and further animal investigations. Several investigators have reported the antifertility effect of gossypol in males and females of non-ruminant species (Wu *et al*., 1981; Anderson, 1985; Bender *et al*., 1988). It was collectively indicated that gossypol acetic acid treatment of female rodents disrupts the normal pattern of estrous cycles through effect on pituitary and ovarian hormone secretion. Post implantation development disruption was further reported by the same set of researchers.

It has been reported that at effective doses, gossypol causes males to be infertile because of sperm immotility and depressed sperm counts (Randels *et al*., 1992). Specific mitochondrial damage in the tails of spermatozoa seems to render them immotile and extensive damage to germinal epithelium may be responsible for depressions in spermatogenesis.

Findings have also shown that there are marked differences in species susceptibility to gossypol toxicity (Kalla *et al*., 1982; Weinbauer *et al*., 1982; Wong *et al*., 1984). It has been reported that anti-fertility responses are both dose and time dependent (Saksena and Salmonsen, 1982). Gossypol treatment at pubertal time upwards markedly affected the reproductive performances of test animals depending on the doses applied. The objective of this research therefore was to determine the effect of CSC on reproductive performance of breeder cocks and the extent to which it can be incorporated into their diets without depressing their fertility.

## Materials and Methods

**Experimental diets and bird management:** Five isonitrogenous and isocaloric diets were formulated as shown in Table 1. The control treatment (diet 1) contained no CSM, while diets 2 to 5 were formulated by substituting CSM at 25, 50, 75, and 100% for SBM protein.

Thirty 23 week-old egg laying leghorn breeder cocks were randomly allotted to five different dietary treatments with three replicates, each having 2 cocks per replicate. The birds were moved into the experimental room at 21 weeks of age and allowed to acclimatize for two weeks on the deep litter pens, after which they were weighed on the day the feeding trial started. Subsequent weighing was recorded at weekly intervals. Water and feed were provided *ad libitum* and from these a record of weekly feed intake was taken. From the records of daily feed intake and weight gain the feed conversion ratio was calculated. The feeding trial was carried out for twelve weeks. All animal care procedures were as approved by the Animal Care and Use Unit of the University of Ibadan.

**Testicular histology:** Testicular density was calculated from the weight and volume of the testes. The density was expressed in g/cm$^3$ centimeter. Samples from the left and right testis of cocks from each treatment were fixed in about 10 times their volume in aqueous Bouin's solution for 6 h. Histological sections of 7 microns

Table 1: Composition of experimental cotton seed cake based diets fed to breeder Cocks Treatments

| Ingredients (Kg) | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Maize | 57.38 | 55.45 | 54.50 | 54.48 | 55.60 |
| Soybean meal | 21.72 | 16.20 | 10.85 | 5.42 | 0.00 |
| Cottonseed cake | 0.00 | 6.50 | 13.05 | 19.50 | 26.05 |
| Wheat offal | 14.36 | 14.80 | 14.66 | 13.06 | 10.81 |
| Fish meal | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Blood meal | 0.50 | 1.00 | 1.00 | 1.50 | 1.50 |
| Lysine | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Methionine | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Oyster shell | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Bone meal | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| *Breeder premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Total Calculated | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| CP (%) | 18.50 | 18.45 | 18.48 | 18.47 | 18.40 |
| ME (Kcal/Kg) | 2,806 | 2,809 | 2,801 | 2,800 | 2,804 |

*Premix supplied per kg of diet: Vit A, 10,000 IU; Vit D3, 2,800 IU; Vit E, 35,000 IU; Vit K, 1,900mg; Vit B12 19mg; Riboflavin, 7,000mg; Pyridoxine, 3,800mg; Thiamine, 2,200mg; D-Pantothenic acid, 11,000mg; Nicotinic acid, 45,000 mg; Folic acid, 1,400mg; Biotin, 113mg; Cu, 8,000mg; Mn, 64,000 mg; Zn, 40,000mg Fe, 32,000mg; Se, 160mg; Iodine, 800mg; Cobalt, 400mg; Choline, 475,000mg; Methionine, 50,000mg; BHT, 5,000mg; Spiramycin, 5,000mg

Table 2: Chemical composition of experimental breeder diets

| Parameters (%) | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 |
|---|---|---|---|---|---|
| Dry matter | 90.00 | 91.79 | 91.63 | 91.04 | 91.80 |
| Crude protein | 18.40 | 18.14 | 18.47 | 17.79 | 17.52 |
| Crude fibre | 2.94 | 3.48 | 3.08 | 3.79 | 4.39 |
| Ether extract | 6.50 | 7.30 | 7.20 | 6.60 | 6.50 |
| Ash | 14.00 | 13.00 | 11.50 | 12.00 | 13.53 |
| Nitrogen free extract | 58.16 | 58.08 | 59.75 | 59.82 | 58.06 |
| Moisture | 10.00 | 8.21 | 8.37 | 8.96 | 8.20 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

thick were floated and flattened out on 40°C water and then picked up with clean slides smeared with Mayer's egg albumin. The slides made were incubated for 30 min before staining. Two sets of slides were prepared for each cock. One set was stained according to the Period-Acid-Schiff (PAS) technique and counter stained with haematoxylin. The second set was stained with haematoxylin-eosin (H and E).

The stages of development of round spermatids to spermatozoa were identified by a combined method of acrosome development assessment as stated by PAS technique (Leblond and Clermont, 1952) and by nuclear morphology shown by haematoxylin-eosin stained paraffin embedded tissue (DeReviers, 1971). The volumetric proportions of spermatogenic elements in the seminiferous epithelium were determined using the methods of (Chalkley, 1943). A twenty-five point ocular graticule was used in the estimation. The daily sperm production (DSP) was determined by quantitative testicular histology.

**Determination of daily sperm production (DSP) by quantitative testicular histology:** The DSP can be calculated from information based on the morphometric analysis of histological sections. A method was developed in 1970 which utilized the volumetric proportions and life span of round spermatids in the tissue cross section (Amann, 1970). The accuracy of this method was ensured by correcting for the shrinkage of the testis volume during histological processes. The time divisor calculated from the volume percent of the round spermatid is reported to be more accurate than that for mature spermatids (Amann, 1981).

The DSP was determined from the histological analysis using the equation below:

DSP = (CTV) (vol % of round spermatid nuclei in the testis%) / (Ave. vol per round spermatid nucleus in testis) (Life span of round spermatids in days).

The corrected testicular volume (CTV) was obtained by the formula

CTV = (Gross testis weight-tunica albuginea wt-volume, of mediatinum%) × (Shrinkage correction)

Shrinkage = Initial volume-final volume / Initial correction factor volume

This formula has the advantage of being adaptable for DSP calculations in various species, if the life span of round spermatids, average volume of a round nucleus, shrinkage value and volumetric proportion of the species are known.

**Statistical analysis:** Data obtained from the parameters considered were subjected to descriptive statistics and analysis of variance using (SAS, 1999). Differences between means were detected using Duncan's multiple range tests.

## Results

**Identification of spermatogenic elements and cellular associations:** Sperm cells were identified at their different stages of development following their reactions to the staining techniques used. Spermatogonia A line the lamina of the seminiferous tubules and were characterized by a large pale ovoid nucleus ranging from 2.82 to 2.99 microns in diameter. Spermatogonia B were characterized by a smaller and dark nucleus having a diameter range of 1.34 to 1.39 microns, and they were more nearly spherical in shape.

The sertoli cells were easily identified by their characteristic irregular shapes with deep invaginations of nuclear membrane. They were seen to support the spermatogenic epithelium. The size of the sertoli cells ranged from 0.71 to 0.95 microns.

Table 3: Average seminiferous tubular diameter and volumetric proportion of testicular elements of cocks fed cottonseed cake based-diets

| Cell type (%) | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | SEM |
|---|---|---|---|---|---|---|
| Spermatogonia A | 2.99 | 2.99 | 2.98 | 2.83 | 2.82 | 0.07 |
| Spermatogonia B | 1.38 | 1.39 | 1.38 | 1.36 | 1.34 | 0.02 |
| $1^0$ Spermatocyte | 11.97[a] | 11.94[a] | 11.48[ab] | 11.15[b] | 10.50[c] | 0.07 |
| $2^0$ Spermatocyte | 0.38[a] | 0.29[b] | 0.24[c] | 0.22[c] | 0.22[c] | 0.02 |
| Round Spermatid | 8.32[a] | 7.23[b] | 7.30[b] | 7.23[b] | 7.30[b] | 0.04 |
| Elongated Spermatid | 10.46[a] | 10.26[ab] | 10.02[ab] | 9.78[b] | 9.76[b] | 0.12 |
| Spermatozoa | 5.13[a] | 4.72[ab] | 4.41[b] | 3.77[c] | 3.53[c] | 0.13 |
| Sertoli cells | 0.95[a] | 0.88[ab] | 0.88[ab] | 0.83[b] | 0.71[c] | 0.03 |
| Interstitial cells | 0.79[a] | 0.68[b] | 0.71[b] | 0.65[b] | 0.66[b] | 0.02 |
| Cytoplasm | 49.24[b] | 49.73[ab] | 50.52[ab] | 50.39[b] | 51.22[a] | 0.44 |
| Tubular Diameter (μ) | 237.93[a] | 234.81[a] | 236.04[a] | 239.43[a] | 221.30[b] | 3.36 |

[1]Means within the same row with different superscripts are significantly different ($p<0.05$)

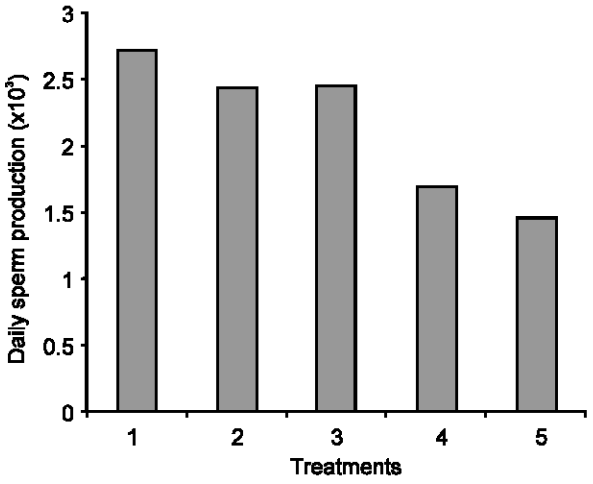


Fig 1: Daily sperm production ($x10^3$) values of breeder cocks fed CSC-based diets

Primary spermatocytes looked like spermatogonia B but with a more visible nucleoli and chromosome. The primary ($1^0$) spermatocytes range from 10.50 to 11.97 microns in diameter. Secondary spermatocytes have spherical nuclei with a network of chromatin granules like the type observed in the first stage of spermatid development, but larger than the round spermatid. Their diameter range in microns varies from 0.22 to 0.38.

**Volumetric proportions of testicular elements:** The results of the volumetric proportions of testicular elements in breeder cocks fed cottonseed cake based diet are presented in Table 3. Differences ($p<0.05$) were observed in most of the testicular elements measured. Spermatogonia A and B did not show a significant difference. Interstitial cell was highest in the control (0.79%) and lowest in treatment 4 (0.65%).The defining cell of testicular activities, the sertoli cells was highest in the control diet (0.95%) and lowest in treatment 5 (0.71%).

**Tubular diameter of seminiferous tubules:** The mean tubular diameter is also shown in Table 3. The significant differences recorded existed between only treatment 5 and the other treatments.

**Daily sperm production (DSP) in breeder cocks fed cottonseed cake-based diets:** The results of DSP were based on histometric analysis and presented in Fig. 1 below. The highest DSP value was recorded for treatment 1 (2.72) while the lowest value observed was from treatment 5 (1.48). There were differences ($p<0.05$) between the dietary treatments. Treatments 4 and 5 were lower ($p<0.05$) than the other treatments. Daily Sperm Production values fell sharply after treatment 3 with the inclusion level of CSC at or greater than 13%.

## Discussion

The results obtained in this study are similar to the work of (Nkanga, 1989) and (Nkanga and Egbunike, 1990) who identified two categories of spermatogonia based on their nuclear size, chromatin configuration and the relative position of these cells in the seminiferous tubules. This identification and similarity of the arrangement of cells is also corroborated by earlier authors, (Leblond and Clermont, 1952; Clermont, 1960; Maldjian *et al.*, 2002) who have morphologically differentiated between spermatogonia A and B.

The five categories of primary spermatocytes reported by (DeReviers, 1971; Nkanga and Egbunike, 1990) in cocks were observed in the testicular sections made from testis of the breeder cocks used for this experiment. Though similarities were observed in the five categories of cells in all the dietary treatments, significant differences were recorded as dietary levels of cottonseed cake protein replacement for soybean exceeded 50%.

Every species has its capacity for sperm production which is determined genetically but it has been clearly observed that other factors like nutrition, disease and stress influence which portion of the germinal epithelium enters into spermatogenesis (Garner and Hafez, 1993; Etchu and Egbunike, 2002) reported that poultry exudates are very sensitive to dietary changes

and that the slightest imbalance in feed composition goes a long way in influencing its physiology and subsequently its performance. Significant differences were noted in some of the elements considered like primary spermatocytes, round spermatids, elongated spermatids, seminiferous tubules, sertoli cells and spermatozoa.

The values obtained in this work agree with the report of (DeReviers, 1971; Nkanga and Egbunike, 1990) and are close enough for comparison. From the results of this work it was glaring that any diet that increases the level of anti-nutritive factors particularly free gossypol beyond the amount in diet 3 (0.01 g/ton) would discourage the production of germ cells and subsequently spermatogonia. Dietary treatments that had higher proportions of spermatozoa were also observed to have a high corresponding proportion of sertoli cells. The implication of this is that any substance that limits the activity of sertoli cells will adversely affect sperm production.

Dietary treatments that had higher than 50% cottonseed cake (represented by any free dietary gossypol greater than 0.001%) replacement for soyabean cake had a significantly lower sertoli cells values than those with lower levels of cottonseed cake. The relationship observed between sertoli cells and spermatozoa can be explained in relation to the function of the sertoli cells whose acronym is "nurse cell" incapacitation of the nurse cell by anti-nutrient activities means the nourishment provided during the morphological transformation of spermatids during spermiogenesis is impeded which subsequently lowers sperm production.

The volume in (%) occupied by the seminiferous tubules showed significant treatment effect, and the figures obtained were lower than those observed by (DeReviers, 1971) for adult chickens. The volume (%) of seminiferous tubules in the testis has been reported to be species specific (Nkanga, 1989). The seminiferous tubule diameters for the different diets follow the proportion of sertoli cells in their tubules. It can be inferred from the observed values that any diet that influences the number of sertoli cells will influence the size of the seminiferous tubules.

Significant differences were observed in DSP by the histometric method employed for this estimation. These results, though slightly different, agree with the work of (Maldjian *et al*., 2002; Garner and Hafez, 1993; Egbunike and Oluyemi, 1979). The slight variations in figures are probably due to differences in species which agrees with the report of (Garner and Hafez, 1993), that every species has it's capacity for sperm production which is genetically determined, though environmental influences cannot be ruled out, which determines what proportion of the germinal epithelium enters into spermatogenesis.

The control diet (diet 1) and diets 2 and 3 had higher DSP values than diets 4 and 5 and subsequently, higher testicular weights were observed in birds on diets 1 to 3 than diets 4 and 5. Testicular weights have been reported to have a high correlation with sperm reserves in the testis or epididymis and therefore a reflection of sperm production. Thus the higher the testicular weight, the more the sperm production. Additionally, the testes weights were higher in the control diets and diets 2 and 3 because they had a higher proportion of their testicular mass occupied by the seminiferous epithelium while the tunica albuginea formed a higher proportion of the testicular mass in diets 4 and 5. This influenced the overall weight of the testis which was reflected in the amount of sperm produced daily by the breeders.

From the results observed, CSC may not replace more than 50% of SBC for breeder cocks because of its depressing effect on cock fertility. Thus CSC can effectively substitute SBC up to 50% by providing the needed protein and quality feed essential in the production performance of breeder cocks.

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