Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.3, 2016

Effects of Some Toxic Plant Seeds on Semen and Sperm Quality of Adult Cockerels

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Abstract
The toxicological effects of raw jack beans (RJB), bambara groundnuts (RBG) and benne seeds (RBS) on semen and sperm quality of adult cockerels were analyzed in a twelve week feeding trial. Full fat soya was replaced with the RJB, RBG and RBS at 25% and 50% levels. Anti-nutritional factors in the raw pant seeds were determined. Haemagglutinin, canavanine and trypsin inhibitor contents were higher in the RJB, while tannin, oxalate and phytate were higher in the RBS . Feed intake was significantly ($P<0.05$) reduced across the groups. Higher significant reductions ($P<0.05$) were obtained in the semen volume of the birds fed the RJB, RBG and RBS at 50% level respectively. Marked ($P <0.05$) reductions were obtained in the sperm concentration and live sperm cells of birds fed 25% and 50 % RJB, and 50% RBS respectively. Dead sperm cells and abnormal sperm cells were significantly ($P<0.05$) increased across the groups, but more pronounced in birds fed with 50% RJB and 50% RBG respectively. Semen protein was significantly ($P<0.05$) reduced across the groups. Overall, the most striking effects on the semen quality and sperm reserves were obtained in birds fed 50% RJB and 50% RBG respectively. It is therefore concluded in this study that adequate processing be given to the plant seeds upon usage as feed ingredients in poultry feeding.
Keywords : Cockerels, Semen quality, Toxicology

1. INTRODUCTION

Artificial or natural toxic compounds are known to constitute a great danger to the health and optimum performance of livestock animals. Intake of artificial toxic compounds is known to have deleterious effects on biochemical composition of non – ruminant animals. According to Sahin *et al.,* (2001) and Chowdhury *et al*., (2004) , intake of chromium and mycotoxins (two artificial toxic compounds) led to severe damage to body parts and metabolites of livestock animals. Yousef *et al.,* (1995) also reported direct cytotoxic effects on spermatogenesis and/or an indirect effect via hypothalami – pituitary – testis axis controlling the reproductive efficiency of male rabbits fed with two toxic compounds (Carbofuran and glyphosate). In addition to this, the reproductive organs and semen production of some non – ruminant animals have been reported to be affected when fed some artificial toxic compounds. Salem *et al* ., (1988) reported that the inclusion of dimethoate and dehtamethrin (two artificial toxic compounds) in diets of male rabbits reduced their sperm concentration .

Moreover, apart from the artificial toxic compounds, certain plants also contain some natural toxic compounds otherwise known as anti – nutritional factors ,which cause deleterious effects on performance of livestock animals consuming them. For example, the gossypol toxicity for male reproduction was reported to inhibit spermatogenesis, which decreases the sperm count and spermatozoid motility and viability (Randel, *et al* 1992). According to El-Sharaky *et al.,(*,2010), in effective doses, gossypol causes infertility by inhibiting sperm motility, decreasing sperm concentrations, inducing specific mitochondrial injury to the sperm tail, and damaging the germinal epithelium.

Other examples of such plants are the raw grain legumes and oil seeds. Plants of such are a source of dietary protein and energy to livestock animals (Apata, 1990). Moreover, their nutritive elements are masked with the occurrence of anti – nutritional factors such as lectins (haemagglutinins), trypsin inhibitors, canavanine, tannin, oxalate and phytate which lead to significant physiological and biochemical alterations in the body of livestock animals (D'Mello, 1989)..

Hence, in view of the effects of the nutritional and biochemical toxicity associated with anti – nutritional factors in most raw grain legumes and some oil seeds, this study was designed to assess the toxicological effects of raw jack beans , bambara groundnuts and benne seeds on the gonadal sperm reserves and quality of semen produced by exotic adult cockerels.

2. MATERIALS AND METHODS

Dry and raw seeds of jack bean (*Canavalia ensifirmis (L) DC*), bambara groundnuts (*Vigna subterranean (L) Vendc*) and benne seeds (*Sesamum indicum L.)* were obtained from the Department of Agronomy, University of Ibadan, and the National Cereals Research Institute , Moor Plantation, Apata, Ibadan, South Western Nigeria.

2.1 Chemical Analyses

Analytical methods of AOAC (1984) were used to determine the proximate compositions of raw jack beans , bambara groundnuts and benne seeds

The trypsin inhibitor activities in the raw seeds were obtained using the method described by (Van Oort, Hammer and Slager, 1989) The inhibitor activity was evaluated on the basis of the extent to which a portion of an aqueous extract of the seeds inhibited the action of trypsin on benzoyl-ol arginine- p -nitoanilide as substrate.

The heamagglutinin activity was determined by the photometric technique of Valdebouze *et al*, (1980) which measures the ability of heamagglutinin to agglutinate rabbit erythrocytes. Absorbance was read at the end of a three hour incubation period .

Tannin was determined using the method of Hoff and Singleton (1977) .This involved soaking weighed sample(2g) each with a solvent mixture of acetone and glacial acetic acid for 5hrs. The samples were then filtered in order to obtain the filtrate. A set of standard solutions of tanninc acid was prepared ranging from 10ppm to 50ppm.The absorbance of the standard solutions as well as that of the filtrates were read at 500nm on a spectronic .

The phytate contents were determined using the method described by Dye (1956). 2g of each sample was weighed into different 250mls conical flask. 100mls of 2% concentrated HCL(aq) was used to soak each sample for 3hrs. The samples were then filtered. 50mls of each filtrate was placed in 250mls beaker and 100mls of distilled water was added in each case to give proper acidity. 10mls of 0.3% Ammonium thiocyanate solution was added as indicator. This was titrated with standard iron (III) chloride solution which contained 0.00195g iron/ml .

The oxalate content was determined by the method of Wheeler and Ferrell (1971). The extraction was done weighing 5g of each sample into different 250mls conical flasks and soaked with 100mls of distilled water for 3hrs. The samples were then filtered. 10ppm to 50ppm standard solutions of oxalate were prepared and read in the spectrophometer at 420nm for the absorbance.

The canavanine content was determined by the colorimetric method described by Natelson and Bratton (1994)

2.2 Experimental Diets

Seven (7) different diets were formulated for this experiment (Table 1). Raw jackbeans, bambara groundnuts and benne seeds replaced full fat soybean at 25% and 50% levels in diets for adult cockerels. Minor adjustments were made in the ingredients to keep the diets isonitrogenous and isocaloric. All diets were supplemented with 3.0 g/kgDM methionine because the grain legumes are known to be deficient in the amino acid. . In addition, the benne seed – based diets were supplemented with 1.5g/kgDM and 3.0g/kgDM lysine respectively, because the oil seeds are known to be deficient in the amino acid.

Table1: Gross Composition of Diets (g/kgDM

	Control	**25% RJB**	**50% RJB**	**25% RBG**	**50% RBG**	**25% RBS**	**50% RBS**
Maize	422.0	422.0	420.0	420.0	422.0	418.5	419.0
Full fat Soya	180.0	135.0	90.0	135.0	90.0	135.0	90.0
Jack bean	-	45.0	90.0	-	-	-	-
Bambara Groundnuts	-	-	-	45.0	90.0	-	-
Benne Seeds	-	-	-	-	-	45.0	90.0
Fish meal	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wheat bran	345.0	345.0	347.0	347.0	345.0	347.0	345.0
Bone meal	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Oyster shell	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL – methionine	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Lysine	-	-	-	-	-	1.5	3.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated Crude Protein (%)	16.9	16.3	16.8	16..2	16.6	16. 2	16.5
Calculated Metabolizable energy (kcal/kg)	2671.3	2624.9	2.641.1	2631.8	2643.6	2683.4	2685.1

RJB = Raw Jack beans, RBG = Raw bambara groundnuts, RBS = Raw benne seeds

2.3 Experimental Birds and Management

A total of two hundred and ten (210) exotic adult cockerels (Goldmine strain) of twenty four (24) weeks old were randomly distributed into seven dietary groups at the rate of thirty (30) birds per group. Each group was sub-divided into three (3) replicates at ten (10) birds per replicate group. The birds were housed singly in battery cage compartments. The birds were first stabilized for the first two weeks during which they were fed with commercial cockerel ration. After this period, the experimental diets were introduced to the birds for twelve weeks during which the semen was collected for analysis. The birds were adequately dewormed at the onset of the experiment. Food and water were served ad-libitum.

2.4 Semen Collection and Evaluation

Semen was collected on a weekly basis from the adult cockerels using the double handed massage method (Della *et. al*, 2011). Four birds per replicate group were initially made to adapt to the massage method in the second week of the study. Subsequently, semen was collected in the tenth, eleventh and twelfth week of the study at three days per week into 1ml micro centrifuge tubes. The volume of semen per bird was then read to the nearest 0.01ml.

Sperm concentration , sperm motility and proportions of live, dead and abnormal sperm cells were obtained using the improved Neubauer haemocytometer. Undiluted and thoroughly mixed semen was drawn with a red blood diluting pipette. Formal saline (90% physiological saline + 10% formalin) was added to the semen. The mixture was thoroughly shaken, and by capillary action allowed to run under the cover glass of the haemocytometer. The sperm cells were allowed to settle for 3 minutes before counting. Five squares on the diagonal of the ruled area were counted, starting from the upper left to the lower right. The total counts obtained were multiplied by 10 million to give sperm count per ml to give the sperm concentration. The proportions of live, dead and abnormal sperm cells were determined by adding a drop of undiluted semen to the drop of the staining solution, Nigrosin-eosin, already on a clean slide. Rapid drying was carried out with the aid of slide warmer and examination was done under oil immersion. (Saied and Al – Soudi, 1975). The semen protein was determined by the analytical method of AOAC (1984)

2.5 Statistical Analysis

The results were subjected to analysis of variance. Means with significant differences were compared using the Duncan's multiple range test (Steel and Torrie, 1980)..

3. RESULTS

Data on proximate compositions of raw jack bean , bambara groundnuts and benne seeds are shown in Table 2. Raw jack beans contained higher crude protein and fiber contents. Extract content was 8 and 25 times higher in raw benne seeds than raw bambara groundnut and jack beans respectively.

Table 2: Proximate Composition of Raw Jack beans, Bambara groundnut and Benne seeds (Expressed as percentage of dry matter)

	RJB (%)	RBG (%)	RBS (%)
Dry matter	96.00	97.7	96.6
Crude Protein	27.7	20.3	25.2
Crude fibre	9.5	3.2	4.0
Ether extract	2.5	6.7	52.8
Ash	3.5	3.5	5.3
Nitrogen free extract	52.8	64.0	9.3

RJB = Raw Jack beans, RBG = Raw bambara groundnuts, RBS = Raw benne seeds

The values of the anti – nutritional factors obtained in the raw plant seeds are summarized in Table 3. Higher values of Haemagglutinin , canavanine and trypsin inhibitor contents were obtained in raw jack bean than in raw bambara groundnuts . Raw benne seeds however contained higher amounts of tannin, phytate and oxalate

Table 3: Anti – nutritional factors in Raw Jack beans , Bambara groundnuts and Benne seeds.

Raw Plant Seed	CAN (mg/g)	Haemagglutinin (g/seed)	Trypsin inhibitor (g/seed)	Tannin (g/100gDM)	Oxalate (g/100gDM)	Phytate (g/100gDM)
RJB	26.9	13,497.9	1,675.3	0.4	0.4	0.7
RBG	-	941.3	380.4	0.4	0.2	0.3
RBS	-	-	-	2.4	2.4	5.2

RJB = Raw jack beans, RBG = Raw Bambara groundnuts , RBS = Raw Benne seeds, CAN = canavanine

The data showing physical characteristics of semen of adult cockerels are summarized in Table 4.

Table 4: Semen And Sperm Quality of Adult Cockerels Fed Raw Jack bean, Bambara Groundnuts and Benne Seeds

	Control	25% RJB	50% RJB	25% RBG	50% RBG	25% RBS	50% RBS	SEM
Semen volume (ml)	0.31^{a}	0.19^{c}	0.14^{d}	0.21^{b}	0.19^{c}	0.26^{b}	0.23^{bc}	0.08
Sperm motility	79.89^{a}	46.67^{b}	31.00^{d}	48.33^{b}	48.33^{b}	48.33^{b}	41.61^{c}	23.41
Sperm concentration (X 10^{9}/ ml)	0.21^{a}	0.07^{c}	0.02^{d}	0.09^{c}	0.09^{c}	0.16^{b}	0.14^{b}	0.01
Live sperm Cells (%)	87.34^{a}	41.50^{b}	16.17^{d}	41.07^{b}	25.00^{c}	42.00^{b}	40.25^{b}	9.23
Dead sperm Cells (%)	6.23^{c}	25.17^{b}	28.58^{a}	24.50^{b}	25.34^{b}	24.25^{b}	24.35^{b}	2.25
Abnormal Sperm Cells (%)	6.43^{d}	33.33^{c}	55.25^{a}	34.43^{c}	49.66^{b}	33.75^{c}	35.40^{c}	1.34
Semen protein (mg/100ml)	1.33^{a}	0.52^{c}	0.34^{d}	0.82^{b}	0.58^{c}	0.81^{b}	0.60^{c}	0.20

Means with different superscripts in the rows are significantly different
RJB= Raw jack beans, RBG = Raw Bambara groundnuts, RBS = Raw Benne seeds.
SEM = Standard error of mean

Marked ($P< 0.05$) reductions were obtained in semen volume of birds fed with 50% raw jack beans (0.14ml) and 50% raw bambara groundnuts (0.19ml) respectively . Sperm motility was significantly ($P <0.05$) reduced across the groups fed with the raw plant seeds. The sperm motility was however statistically ($P>0.05$) similar in birds fed 25% raw jack bean , 25% raw bambara groundnuts, 50% raw bambara groundnuts and 25% raw benne seeds respectively.

Sperm concentration was markedly ($P<0.05$) reduced in birds fed 50% raw jack beans (0.02 X 10^{9}/ml). Other groups fed with the raw legumes also gave significant ($P <0.05$) reductions in the sperm concentration.

Significant ($P<0.05$) reductions were obtained in the amount of live sperm cells of all groups when compared with that of the control group. However, birds fed 50% raw jack beans and 50% raw bambara groundnuts gave the lowest values of the live sperm cells respectively.

Marked ($P<0.05$) increases were obtained in the dead sperm cells of the birds across the groups, but the highest amount was obtained in birds fed 50% raw jack beans(28.58%). The percentages of abnormal sperm cells were significantly ($P<0.05$) higher in birds fed 50% raw benne seeds (35.40%), 50% raw bambara groundnuts(49.66%) and 50% raw jack beans (55.25%) respectively. Semen protein was significantly reduced in all groups fed with the raw legumes .

4. DISCUSSION

The result of the chemical composition of raw jack beans , bambara groundnuts and benne seeds obtained in this study are similar to the those reported by Oyenuga (1966) and Apata (1990). The higher content of ether extract obtained in the raw benne seeds (52.75%) corroborates the report of Card and Nesheim (1975).. The higher levels of haemagglutinin and trypsin inhibitor obtained in the jack beans used in this study agree the reports of Ologhobo (1981) ,Grant *et al*, (1983) and Jaffe (1980). Raw bambara groundnuts have also been reported to contain lower amounts of haemagglutinin, tannin , oxalate and phytate contents (Apata, 1990), while raw benne seeds according to Mamputu and Buhr, (1991) contain higher amounts of tannin.

Semen volume was significantly reduced in all the birds fed the raw legumes, but more pronounced in those fed 50% jack beans. This observation may be attributed to inhibition in the metabolism of dietary protein. Louis *et al*, (1994) reported a reduction in semen volume of boars when fed with low protein dietary level. Although the dietary protein levels given to the birds in this study were considered adequate, the combined toxic effects of anti – nutritional factors such as the canavanine , haemagglutinin and trypsin inhibitor in raw jack beans and bambara groundnuts , and the combined effects of tannin ,oxalate and phytate in raw benne seeds could have made a substantial part of the ingested protein unavailable for normal semen production. According to Bitto (2007) , gossypol , an anti – nutritional factor in cotton seed, reduced sperm production rates and the efficiency of spermatogenesis in humans and and rabbits.. Hence, in this study, a possible interference with protein metabolism, enzyme activities and hormonal regulation in the adult cockerels by the haemagglutinin contents of the jack bean and bambara groundnut – based diets (Jaffe, 1980; Pusztai, 1989) might have affected the spermatogenesis thereby leading to marked reduction in the semen volume The high tannin content in the raw benne seed based diets might also have led to formation of complexes with dietary proteins (Apata ,1990) , inhibition of a broad spectrum of protein digestive enzymes such as dipeptidase and trypsin (Ahmed *et al*, 1991; Horigorne *et al* (1988), and a consequent reduction in the semen volume of the adult cockerels .

Significant reductions were obtained in the values of sperm motility of all groups when compared with

the control group. The most striking effect was however obtained in birds fed 50% raw jack beans . This observation is similar to the findings of Yousef *et al*, (1995) on feeding diets containing two toxic compounds (carbofuran and glyphosate) to rabbits. Egbunike and Oluyemi (1979) reported that lower seminal fluids caused reduction in nutritive substances needed for normal sperm motility and viability. In essence, the lower semen volume obtained in the birds fed with the raw plant seeds in this study might have contributed significantly to the lower sperm motility .

Results on sperm concentration revealed marked significant reductions in birds fed with higher amounts of the raw plant seeds respectively. The values of semen concentration obtained in this study among the birds fed with the raw plant seeds are much lower than the 0.200 x 10^9 /ml score reported by Dube *et al.,* (1977) for exotic cockerels. The nutritional and biochemical toxicity in raw jack beans, bambara groundnuts and benne seeds is believed to have caused the reduction in the sperm concentration. This is because King and Mclelland, (1975) reported that nutritional toxicity affects spermatogenesis which subsequently reduces sperm concentration. Yousef *et al,* (1995) also reported reductions in sperm concentration of rabbits fed with diets containing carbofuran and glyphosate . This reduction in sperm concentration was attributed to direct cytotoxic effects of the toxic compounds on spermatogenesis and/or indirectly via hypothalami-pituitary-testis-axis, which controls the reproductive efficiency. Salam *et al* (1988) also reported reduction in sperm concentration of male rabbits fed with diets containing toxic compounds known as dimethoate and dehtamethrin..

The percentages of live sperm cells were severely reduced in birds fed 50% raw jack beans, 50% raw bambara groundnuts and 50% raw benne seeds respectively. These observations corroborate the findings of Footh (2002) on infertility of of sperm cells of rabbits fed 5mg/ml ipronidazole . Goldman *et al* (1990) also reported that intake of a toxic compound known as chlordimeform caused reductions in the concentrations of serum gonadotropins , lutenizing hormone and Follicle stimulating hormone and testosterone in rats. Lutenizing hormone is known to promote testosterone synthesis from the leydig cell, which is required for normal spermatogenesis and to maintain the integrity of male accessory glands.

The higher occurrences of abnormal sperm cells in birds fed 50% raw jack beans, 50% raw bambara groundnuts and 50% raw benne seeds respectively are similar with the findings of Benpong and Hall (1983) . According to these authors, male mice fed with diets containing a toxic substance, 1,3 – diphenylguanidine, gave sperm abnormalities. Also, gossypol from cotton seeds has been to inhibit calcium influx (Breitbart, *et al.,* 1989) and Mg-ATPase and Ca-Mg-ATPase activity in spermatozoid plasmatic membranes. Chenoweth, *et al*., (2000) also reported that abnormal spermatozoids are produced because gossypol produces ultrastructural alterations in the nuclear membrane, endoplasmic reticulum, and mitochondria

In this study, haemagglutinin, trypsin inhibitor , tannin , oxalate and phytate were the main toxic principles in the raw plant seeds fed by the cockerels and these could have had adverse effects on the testis, resulting in an increased number of abnormal sperm cells

Semen protein was also significantly reduced in birds fed with the raw plant seeds but the reductions were pronounced in those fed with 50% raw jack beans. The effect of canavanine , a free toxic non-protein amino acid in jack bean, on the alteration of protein synthesis is a likely factor that could be responsible for the reduced semen protein. Canavanine has been reported to hinder metabolism of arginine (D'Mello, 1989). Arginine is an essential amino acid and a major constituent of male reproductive cells (Card and Nesheim, 1975). Apata (1990) showed that the incorporation of canavanine in place of arginine into proteins or protein vital for initiation of a new round of deoxyribonucleic acid (DNA) replication resulted into faulty canavanyl-protein. Accordingly, once canavanyl-protein is formed the organism cannot commence a new round of DNA synthesis, since the protein is faulty. The faulty protein cannot be replaced by a functional arginine-containing protein until a new cycle of DNA synthesis has been completed, which in fact cannot take place. This rigidity stops all ribonucleic acid (RNA) synthesis due to extensive physical disruption on the nuclear region. Moore *et al* (1985) also reported reductions in semen protein, seminal vesicle, caput epididymis, testis weight and plasma testosterone when rats were fed with a diet containing a toxic compound known as 2,3,7,8 – tetrachlorodibenzo – p-dioxin.

6. CONCLUSION

The results obtained in this study revealed the toxicological effects of the anti – nutritional factors , haemagglutinin, trypsin inhibitor , tannins , oxalate and phytate , in raw jack beans, bambara groundnuts and benne seeds on the semen quality and sperm reserves of exotic adult cockerels . More importantly, raw jack bean proved to have more toxicological effects on semen quality and sperm reserves than raw bambara groundnuts and benne seeds. These are indications that unless proper processing methods are employed in treating the raw plant seeds , their full potentials as sources of protein and energy for production of viable sperm cells may not be met. Attempts to enhance the nutritive value of jack beans, bambara groundnuts and benne seeds have been reported. Focus on detoxification of raw jack bean has been on the canavanine and haemagglutinin contents. Udedibie and Carlini (1998) demonstrated the efficiency of dry urea treatment prior to

toasting as a method of improving the nutritive value of jack beans. Obizoba and Obiano (1998) also reported that canavanine solubulizes in water and converts to non-toxic cyclic deamino-canavanine on heating. The trypsin inhibitor and haemagglutinin are heat labile toxic factors in both jack beans and bambara groundnuts which get destroyed under moist heating (Ologhobo *et al*., 1993). Furthermore, since most of the tannin, oxalate and phytate contents of raw benne seeds are located in the hulls, thorough dehulling has much tendency to enhance its nutritive value.

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