

Histopathology of Organs of Cockerels Fed Raw Jack Bean, Bambara Groundnut And Benne Seed Based Diets .

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Abstract

The study was carried out to determine the toxicological effects of raw jack beans (RJB), bambara groundnuts (RBG) and benne seeds (RBS) – based diets on organs of cockerels (Goldmine strain) after eight weeks of feeding trial. Full fat soya was replaced with RJB, RBG and RBS at 25% and 50% levels. Anti – nutritional factors such as canavanine, haemagglutinin (Hg), trypsin inhibitor (TI), tannin and phytate contents were determined in the plant seeds. High amount of canavanine was obtained in RJB and none in RBG and RBS respectively. High amounts Hg and TI activities were obtained in both RJB and RBG. The tannin, phytate and oxalate contents were higher in RBS than RJB and RBG respectively. At the termination of the experiment, the birds were killed by cervical dislocation before the liver, kidney, pancreas and testis were dissected out and preserved in 10% formalin solution. The tissues of the organs were trimmed, fixed in Bouin's fixative for 24h, embedded in wax, sectioned at 6µm with microtome and finally stained with haematoxylin and eosin for histopathological examination. The livers of the birds fed 25% and 50% RJB based diets showed extensive multifocal and lymphocytic infiltration with necrosis of hepatocytes. Moreover, the pancreas of birds fed RBG and RJB based diets showed necrosis of glandular cells, lymphoid aggregates, thickening of the supporting stroma and multifocal interstitial lymphocytic infiltration. In the testes of birds fed 25% and 50% RJB, there was extensive degeneration of the seminiferous tubular epithelia cells with widespread interstitial lymphocytic infiltrations of the stroma. The liver, pancreas, kidney and testes of birds fed RBS however showed moderate lesions. It is concluded in this study that RJB showed more toxicity RBG and RBS with respect to lesions in the organs of the birds, thus depicting a severe negative effect when fed to non – ruminant animals over a long period of time. There is therefore the need to detoxify the raw plant seeds with a view to enhancing their nutritive value.

Key words: Histopathology, organs, cockerels, feeds

1. Introduction

Legumes and oil seeds are important sources of protein and energy for farm animals respectively. Several underutilized grain legumes and oil seeds abound in the sub Saharan Africa, and examples include the jack beans, bambara groundnuts and the benne seeds.

Jack bean (*Canavalia ensiformis*) is a tropical leguminous plant with a high yield of seeds and foliage (Eke et al., 2007). According to Sridha (2005), the seed of the jack bean is a relatively good source of protein and apparent metabolizable energy for the young chicks. Bambara groundnut has also been reported to be a balance food as it contains sufficient quantities of carbohydrate (63%), protein (16.25%) and fats (6.3%) with relatively high proportions of lysine and methionine as percentage of the protein (6.6 and 1.3% respectively) (Brough and Azam- Ali 1992). Benne seed is however reported to contain higher oil content, but with low amount of lysine (Akanji, 2002).

Moreover, despite the nutritional significance of jack bean, bambara groundnut and benne seed, they are known to contain anti- nutritional factors (ANFs) in the raw form. Bulk of the ANFs in the raw jack beans and bambara groundnuts are known to be present in the seed cotyledons, while those of the raw benne seeds are in the hulls (Akanji, 2002). Previous reports on the effects of raw grain legumes on chickens showed growth depression, decreased protein digestibility and pancreatic enlargement (Essien and Udedibie, 2007). Studies have shown that feeding of raw lima beans to albino rats caused marked alterations in the normal activities of several hepatic and extra – hepatic enzymes and disruption intestinal microvilli (Aletor and Fetuga, 1988). Furthermore, Olkowski *et al.*, (2001), reported an interference of lectins with the glycoproteins and glycolipids of the digestive tract coupled with several side effects on organs controlling immune functions, protein metabolism, enzyme activities and hormonal regulations.

The ANFs in grain legumes have been linked with disruption in normal metabolic activities in the body of an animal. Grant *et al.*, (1991) were of the opinion that changes in metabolic activities and nutrient absorption in rats were from structural damage, malformations and visible lesions of organs. Concanavanine A, has been found to enhance shedding of the brush border membranes and decrease in villus length in rats (Russell *et al.*, 2008). Also, canavanine has been reported to form a bond with proteins (L-canavanil proteins) which lacks the

capacity to form crucial ionic interactions, resulting in altered protein structure and function, which leads to cellular death (Bence and Crooks 2003)

Apart from the natural toxic components of raw grain legumes, the intake of some artificial toxic compounds such as chromium (Sahin *et al.*, 2001) and mycotoxins (Chowdhury *et al.*, 2004) have been reported to cause damage to body parts and metabolites of livestock animals. In addition to negative effects of some of these toxic artificial compounds on growth of animals, the reproductive organs and semen production were also reported to be affected

Hence, this present investigation was therefore designed to determine the toxicological effects of raw jack beans, bambara groundnuts and benne seeds based diets on liver, kidney, pancreas and testes of cockerels.

1.1 Materials and Methods

Dry and raw seeds of jack bean (*Canavalia ensiformis* (L) DC), bambara groundnuts (*Vigna subterranean* (L) Vende) 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.

1.1.1 Chemical Analyses

Concentrations of lectins and trypsin inhibitor were determined using the haemagglutinin assay (Valdebouze *et al.*, 1980) and a modified Kakade method (Van Oort, Hammer and Slager, 1989) respectively. Tannin, oxalate and phytate contents were determined by methods described by Apata (1990) respectively. Canavanine content was determined by method described by Natelson and Bratton (1994)

1.1.2 Experimental Diets

Seven (7) different diets were formulated for this experiment (Table 1). Raw jack beans, bambara groundnuts and benne seeds replaced full fat soybean meal 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 1.5g/kgDM methionine because soybeans, jack beans and bambara groundnuts are known to be deficient in methionine. In addition, the diets containing the benne seeds were supplemented with 2.0g/kgDM of lysine to ensure that the amino acid was not limiting.

1.1.3 Experimental Birds and Management

A total of one hundred and forty (140) cockerels (Goldmine strain) of twenty four (24) weeks old were randomly distributed into seven dietary groups at the rate of twenty (20) birds per group. Each group was further divided into five (5) birds per replicate group. The birds were housed singly in battery cage compartments. Foods and water were served ad-libitum, and the experiment ran for 8 weeks.

1.1.4 Histopathological Examination

At the end of the eighth week of the experiment, three birds per replicate group were fasted over – night and killed by cervical dislocation. The internal organs were dissected out and the liver, kidney, pancreas and testis were preserved in 10% formalin solution for histopathological examination. The tissues were trimmed and fixed in Bouin's fixative for 24h, embedded in wax and sectioned at 6µm with microtome (Leitz and Weszlar) and finally stained with haematoxylin and eosin. The lesions of the organs were carefully observed under a microscope.

Table1: Composition of diets for cockerels (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	418.0	420.0
Full fat Soya	180.0	135.0	90.0	135.0	90.0	135.0	90.0
Jack bean	-	45.0	90.0	-	-	-	-
Bambara	-	-	-	45.0	90.0	-	-
Benne	-	-	-	-	-	45.0	90.0
Fish meal	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wheat bran	346.5	346.5	348.5	348.5	346.5	348.5	346.5
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	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lysine	-	-	-	-	-	2.0	2.0
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/g)	2.62	2.61	2.59	2.61	2.58	2.61	2.62

RJB = Raw Jack beans, RBG = Raw bambara groundnuts, RBS = Raw benne seeds
 2.5g premix supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2,000 IU; vitamin E, 50 IU; vitamin B1, 1 mg; vitamin B2, 3 mg; vitamin B6, 1 mg; vitamin B12, 10 µg; vitamin K, 2 mg; copper (cupric sulphate), 75 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40mg; zinc, 90 mg; iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; sodium, 10 g

1.2 Results And Discussion

The results obtained on the anti-nutritional factors are summarized in Table 2. Haemagglutinin and trypsin inhibitor were higher in jack beans than the bambara groundnuts, while no activity of the two anti – nutrients was obtained in the raw benne seeds in this study. A canavanine content of 26.9mg/g was obtained in the raw jack beans while no activity of the anti-nutrient was detected in the raw bambara groundnuts and benne seeds respectively. No trypsin inhibitor activity was obtained in the raw benne seeds. Tannin, phytate and oxalate contents were higher in the raw benne seeds. These observations are consistent with the findings of Essien and Udedibie (2007), Lutz and Prytulski (2008), Mamputu and Buhr (1994) and Rubio, *et al.*, (2003) respectively. The presence of high amount of Haemagglutinin in jack beans signifies poor nutritional value as well as high toxicity when fed to non- ruminant animals in the raw form. Haemagglutinins have been reported to induce anorexia, prostration and even deaths in rats (Akanji *et al.*, 2008). The relatively high trypsin inhibitor activity obtained in raw jack beans limit its usefulness as a source of protein for livestock when fed in the raw form to monogastrics. Tannin, phytate and oxalate contents obtained in the raw benne seeds in this study were higher than values reported on various grain legumes (Apath, 1990). Moreover, Lutz and Prytulski (2008) were of the opinion that phytic acid and oxalic acid have the ability to chelate several mineral elements and thereby reducing their availability in the intestinal tract.

Table 2: Anti – nutritional factors in raw jack beans, bambara groundnuts and benne seeds.

Raw Plant seed	CAN(mg/g)	Hg(g/seed)	TI (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, Hg= haemagglutinin, TI= trypsin inhibitor

The results on histopathological changes in the organs are presented in Table 3. The livers from the birds fed control diet showed no visible lesions. However, there were extensive multifocal and lymphocytic infiltration with necrosis of hepatocytes of livers of birds fed 25% and 50% raw jack beans based diets respectively. Moreover, while slight lesions were obtained in birds 25% raw bambara groundnuts and raw benne seeds, those fed 50% raw bambara groundnuts showed severe hepatic congestion and extensive hepatic necrosis respectively.

Table 3. Histopathological lesions of organs of exotic adult cockerels fed raw jack beans, bambara groundnuts and benne seeds

ORGANS	LESIONS	Control	25% RJB	50% RJB	25% RBN	50% RBN	25% RBS	50% RBS
LIVER	Congestion	-	++	++	++	++	++	++
	Necrosis	-	++	++	-	++	-	-
	Degeneration of hepatocytes	-	++	++	½	++	-	-
KIDNEY	Congestion	-	++	++	-	++	-	-
	Hyperemia	-	+	++	M	+	-	-
PANCREAS	Hypertrophy and necrosis	-	+	++	+	++	-	-
TESTIS	Degeneration of seminiferous epithelial cells	-	+	++	+	+	+	+

Key : RJB = Raw jack beans, RBG = Raw Bambara groundnuts, RBS = Raw Benne seeds ; ++ = Present but distinct; + = Present but moderate ; ½ = Present but less marked than usual; M = Minimal or doubtful; - = No visible lesion.

No visible lesions were observed in the pancreas from birds fed control diet and raw benne seed – based diets. However, the pancreas from birds fed raw jack beans and bambara groundnut showed necrosis of glandular cells, lymphoid aggregates, thickening of the supporting stroma and multifocal interstitial lymphocytic infiltration.

The kidneys from birds fed on control diet, 25% raw bambara groundnuts and 25% and 50% raw benne seeds based diets showed no visible lesion respectively. However, the kidneys from birds fed 25% and 50% raw jack beans and 50% raw bambara groundnuts showed widespread lymphocytic infiltrations of the renal interstitium.

In the testes of birds fed 25% and 50% raw jack beans, extensive degeneration of the seminiferous tubular epithelia cells with widespread interstitial lymphocytic infiltrations of the stroma was observed. The testes of birds fed 25% and 50% raw bambara groundnuts and 50% raw benne seeds showed moderate degeneration of seminiferous epithelium respectively. No visible lesions were however obtained in testes of birds fed control diet and 25% raw benne seeds respectively.

The lesions observed in the livers, pancreas and kidney in the birds fed 25% and 50% raw jackbeans and 50% raw bambara groundnuts show similarities with those reported by Aletor and Fetuga(1984b) and Ologhobo *et al.*, (1993). The multifocal aggregations of lymphoid cells of liver, lymphocytic infiltrations of the renal interstitium of kidney, and pancreatic necrosis are clear indications of malfunctioning of the body organs thus with a likely occurrence of poor growth and even death in the birds upon long term consumption of the raw legumes. The presence of histopathological lesions was less marked in birds fed benne seeds and this could be attributed to non – existence of haemagglutinin and trypsin inhibitor activities. The severe histopathological lesions especially in birds fed 50% raw jack beans could be attributed to the combined effects of canavanine, haemagglutinin and trypsin inhibitor activities. According to Russell *et al.*, (2008), haemagglutinin alone can interfere with some metabolic processes in the liver and cause intensive inflammation with destruction of epithelial cells. On the mechanism of canavanine toxicity in monogastrics and even rats, D'Mello (1993), reported a catabolism of the toxic anti –nutritional factor into another toxic compound, canaline, with a negative effect on the liver through a decrease in hepatic ornithine decarboxylase and a subsequent accumulation of ornithine in body fluids. Crine and Lemieux (1982) also reported an incorporation of canavanine into liver enzymes of rats, thereby yielding proteins with reduced activity.

The extensive degeneration of the seminiferous tubular epithelia cells with widespread interstitial lymphocytic infiltrations of the stroma of testes of birds fed raw jack beans in this study are an indication of reduction in semen characteristics and quality. Nutritional toxicity has been reported to affect spermatogenesis, which subsequently reduces sperm concentration (Akanji et al., 2007). In a study carried out by Youssef *et al.*, (1995), rabbits were fed with food containing toxic carbofuran and glyphosate and there were reductions in sperm concentration as a result of direct cytotoxic effects on spermatogenesis and/or indirectly via hypothalamic – pituitary – testis – axis, which controls the reproductive efficiency. Goldman *et al.*, (1990) also found the chlordimeform (toxic substance) caused decreases in the concentrations of serum gonadotropins and testosterone in rats.

1.3 Conclusion

The results presented clearly demonstrate that the potentials of jack bean is limited by the presence of toxic anti-nutritional factors when fed to the birds either at 25% or 50% level. On the other hand, the lesions observed in the organs from birds fed raw bambara groundnuts and benne seeds showed lower level of toxicity when compared to jack bean. These findings portray raw jack bean seed as the most toxic of the three plant seeds used in this study. Adequate processing is therefore necessary to be carried out before their usage as poultry feed ingredient.

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