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Nutritional Assessment of Raw and Differently Processed Underutilized Legume Seed in Broiler Diet

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Abstract: The objective of the present study was to investigate the effect of raw and differently processed (cooked, dehulled and toasted) kidney bean meal on the performance of broiler chicken. Histological examinations of internal organs were also conducted in a feeding trial that lasted for 56 days. Two hundred and twenty–five day old broiler chicks (Anak strain) were used for the study. There were five treatment groups of three replicates of 15 birds per treatment. Raw and processed kidney bean meal was used to replace 50% protein supplied by soybean in the control diet. Data collected was used to evaluate feed intake, weight gain and efficiency of feed utilization. The weights of liver, pancreas, kidney, heart and lungs were also recorded and tissue samples of each collected for histological examination. Food intake was unaffected by the dietary treatments. Significant ($p<0.05$) differences were obtained in Average Daily Gain (ADG) and efficiency of feed utilization (FCE). ADG in birds fed cooked kidney bean was significantly ($p<0.05$) higher than those fed toasted. ADG was depressed in birds fed raw and dehulled meals. Feed Conversion Efficiency (FCE) followed the same trend as ADG. The relative weight of the pancreas was significantly ($p<0.05$) increased, as a result of acinar hypertrophy. The kidney had severe congestion of glomeruli and distention of the capillary vessels with numerous thrombi in birds fed soaked and decorticated kidney bean meals. The weight of the liver was significantly ($p<0.05$) reduced in birds fed soaked and decorticated beans and it was characterized by marked coagulative necrosis and degeneration of the hepatocytes. The structural alterations were attributed to high concentration of residual anti-nutritional factors in the processed seeds. In conclusion, cooked kidney bean meal can be used to replace 50% protein supplied by soybean meal in broiler starter and finisher diets without any adverse effect on the performance and the intestinal organs.

Key words: Kidney bean seed, processing, performance, organ weights, histology

INTRODUCTION

Kidney bean (Phaseolus vulgaris) an under-exploited grain legume is a potential source of plant protein and energy that could be used to replace soybean in poultry and pig diets. However, this legume contains various biologically active compounds (usually referred to as anti-nutritional factors ANFs) which when consumed by animals and humans, may result in a reduction in the nutritional value compared with that predicted from its amino acid profile. Liener[1] reported that kidney bean contains trypsin inhibitors, amylase inhibitors, haemagglutinin, tannin, phytic acid and oxalates The ANFs negatively affect the nutritive value of bean through direct and indirect reactions: they inhibit protein and carbohydrate digestibility; induce pathological changes in intestine and liver tissue thus affecting metabolism; inhibit a number of enzymes and binds nutrients making them unavailable [2].Inclusion of raw kidney bean in diets of growing animals as the only source of plant protein almost invariably leads to a significant impairment in growth[3] and other undesirable physiological and biochemical alterations[4]. These effects limit the use of raw kidney bean although various processing technique tends to reduce the anti-nutritional factor ANFs content of the seed. Several studies indicate that heat processing e.g cooking and dry heating (toasting) increases the digestible nutrients available to young non ruminant animals, especially young chicks resulting in improved growth. The hulls of legumes consist of poorly digestible glumes that completely enclose the seed.

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Table 1: Chemical composition and anti-nutritional factors in raw and differently processed kidney bean seeds (%)

	Raw	Cooked	Decorticated	Toasted
Crude protein	26.85	24.70	28.01	25.62
Crude fibre	5.33	4.98	3.15	5.26
Ether extract	1.08	0.98	1.03	1.03
Ash	5.29	4.79	4.96	5.15
NFE	61.45	64.55	62.85	62.94
Trypsin inhibitors activity (Tiu/mg protein)[1]	81.70	-	77.80	10.50
Haemagglutinin activity (Hg/mg protein)[2]	39.00	-	34.65	12.50
Tannins (g/100g)	0.57	0.37	0.11	0.39
Phytate (g/100g)	0.96	0.86	0.89	0.92
Oxalate (g/100g)	0.50	0.23	0.41	0.47

[1]Tiu/mg protein = Trypsin inhibitors units/ mg protein
[2]Hg/mg protein = Haemagglutinin units mg/ protein

Removal of this hull should therefore increase the concentration of digestible nutrient level for broiler comparable to that of soybean. However, many authors reported suboptimal performance when broilers are fed processed legume meals[5-6]. This was attributed to varying concentrations of residual trypsin inhibitor and lectins in the meals. Emphasis has been placed on the various ways of inactivating the anti-nutritional factors in the legume seed and the improvement of the nutritive value. However, little attention has been given to the evaluation of the effects of intake of residual anti-nutritional factors in processed legume seeds on performance characteristics and histology of internal organs of broiler chickens. This study was therefore designed to address this link.

MATERIALS AND METHODS

Samples: Mature dry seeds of raw kidney bean were obtained from a local farm centre in Nigeria. The kidney bean was subjected to one of the following processing methods:

Cooking: Dry legume seeds were poured into boiling water (100°C) in a cooking pot and heated for 1 hour. Cooked beans were air dried for 4 days after which they were oven dried at 85°C for 48 h.

Dehulling: The dry seeds were soaked in cold water for between 18-24 h and the outer seed coats were removed by hand. The seeds were sun dried for 4 days after which they were oven dried at 85°C for 48 h.

Toasting: This involved spreading the seeds thinly in a pan placed in an oven (120°C). It was stirred from time to time to maintain uniform heating. Toasting was considered adequate when the beans changed from whitish to light brown and became crispy to touch. The process lasted between 20-25 min.

The raw and differently processed seeds were ground using 2 mm screen and stored separately in a sealed kilner jar until required for chemical analysis and incorporation into diets.

Chemical analysis: Milled samples of raw and differently processed kidney beans were subjected to proximate analysis of the raw and differently processed beans using method of A.O.A.C[7]. Concentrations of haemagglutinin and trypsin inhibitors were determined using respectively, the haemagglutination assay[8] and a modified Kakade method[9]. Tannin and phytate contents were determined by the methods described by[10] and[11] respectively. Oxalate was assayed by a gravimetric method described by[12]. All analyses were done in duplicate.

Experimental diets: Isocaloric and isonitrogenous diets were formulated by incorporating differently processed kidney bean into broiler starter and finisher diets. A maize-soybean meal diet served as control, raw and treated kidney bean meal was used to replace 50% protein supplied by soybean in the experimental diets. All diets were supplemented with methionine and lysine to ensure that methionine and lysine would not limit growth. The experimental diets and their proximate composition are presented in Table 2 and 3.

Experimental birds: Two hundred and twenty-five unsexed broiler chicks of Anak strain (1-day-old) were used for this study. The chicks were wing-banded and randomly divided into five groups of 45 birds and each group was assigned to one of the five dietary treatments (raw, cooked dehulled and toasted kidney bean) in a completely randomized design. Each group was further subdivided into three replicates of 15 birds and each replicate kept on litter in pens measuring 1.4 x 1.6 m. Feed and water were provided ad libitum and uniform light was provided 24 h daily. Feed intake was recorded daily while body weight was recorded weekly. Feed consumption, weight gain and efficiency of feed utilization were used as measures of chicks' performance. The study lasted for 56 days.

Measurements: On the 56th day, two birds per replicate (6 birds/ treatment) were randomly selected, fasted for about 18 h to empty their gastro intestinal tract,weighed individually, slaughtered and eviscerated. The weight of the liver, pancreas, kidney,

Table 2: Dietary ingredients and chemical composition (%) of broiler starter diets

Ingredients	Control	Raw	Cooked	Decorticated	Toasted
Maize	54.50	46.00	45.00	48.00	46.40
Kidney bean	-	24.50	25.50	22.50	24.60
Soybean meal	30.00	15.00	15.00	15.00	15.00
Wheat offal	4.50	3.00	2.50	2.50	2.00
Fish meal	6.50	7.00	7.50	7.50	7.50
Fixed ingredients	4.50	4.50	4.50	4.50	4.50
Proximate composition (%)					
Dry matter	94.68	92.68	91.47	93.12	93.78
Crude protein	23.23	22.98	3.04	23.14	23.11
Crude fibre	5.83	5.94	5.88	4.92	5.11
Ether extract	8.24	5.14	4.92	4.63	5.24
Ash	9.54	10.24	10.56	8.68	9.22
NFE	53.16	55.70	55.60	58.63	57.32
ME (MJ/KG)	13.02	13.14	12.96	13.22	13.06

[1]Fixed ingredients include: bone meal, 2.50; oyster shell, 0.50; vit. Premix, 0.50; methionine, 0.30; lysine, 0.20; salt, 0.50
Premix supplied, per kg diet: Vitamin A, 12,000 IU; Vitamin D_3, 2000 IU; Vitamin E, 50 IU; Vitamin B_1, 1 mg; Vitamin B_2, 3 mg; Vitamin B_6, 1 mg; Vitamin B_{12}, 10 ìg; Vitamin K, 2 mg; copper (cupric sulphate), 175 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40 mg; zinc, 90 mg, iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; salt, 25 g; sodium, 10g

Table 3: Dietry ingrdients and chemical composition of (%) of broiler finishing diets

Ingredient	Control	Raw	Cooked	Decorticated	Toasted
Maize	54.00	49.60	49.75	52.25	50.50
Kidney bean	-	20.40	21.25	18.75	20.50
Soybean meal	25.00	12.50	12.50	12.50	12.50
Wheat offal	15.00	11.50	10.50	10.50	10.50
Fish meal	2.00	2.00	2.00	2.00	2.00
Fixed ingredients[1]	4.00	4.00	4.00	4.00	4.00
Proximate composition (%)					
Dry matter	91.52	92.14	90.60	90.15	91.38
Crude protein	20.56	20.14	20.60	20.55	20.38
Crude fibre	8.25	6.45	6.25	5.82	6.12
Ether extract	6.34	4.63	4.21	4.98	5.06
Ash	7.28	8.65	8.84	8.56	9.02
NFE	57.57	60.13	60.10	61.00	59.42
ME ($KJ^{-1}KG$)	12.80	12.60	12.62	12.78	12.40

[1]Fixed ingredients include: bone meal, 2.50; oyster shell, 0.50; vit. Premix, 0.50; methionine, 0.30; lysine, 0.20; salt, 0.50
Premix supplied, per kg diet: Vitamin A, 12,000 IU; Vitamin D_3, 2000 IU; Vitamin E, 50 IU; Vitamin B_1, 1 mg; Vitamin B_2, 3 mg; Vitamin B_6 1 mg; Vitamin B_{12}, 10 ìg; Vitamin K, 2 mg; copper (cupric sulphate), 175 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40 mg; zinc, 90 mg, iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; salt, 25 g; sodium, 10g

heart and lungs was recorded. For histological analysis, tissue samples of each organ were taken, immersed in formalin (1%), fixed in Bouin's solution for 24 h and embedded in paraffin wax. Sections from each organ were made at a thickness of 5µm with a microtome, stained with hematoxylin-eosin and examined by light microscope.

Data analysis: Data collected were subjected to analysis of variance[13]. Where significant differences were observed between treatments, the means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

The result of proximate composition of raw and differently processed kidney bean revealed that it is a rich source of protein. The raw kidney beans contained 26.65% Crude Protein (CP). This is in agreement with the value reported byLiener[1] while Udedibie and Carlini[12] reported a value of 28.70% for kidney bean in Brazil.

Cooking and toasting tended to reduce the crude protein content possibly due to leaching and vaporization of some nitrogenous compound during processing. The CP was enhanced by dehulling. This is consistent with the report of Buckle and Samdudi[14]. The crude fibre content of the bean is low, which makes it ideal for poultry. Raw kidney bean contained 81.70 Tiu and 39.00 Hg of Trypsin Inhibitors (TI) and Haemagglutinins (Hg) (Table 1). Dehulling marginally reduced the contents of TI and Hg in kidney bean and caused substantial reduction in tannin content. This is in agreement with the findings of[15]. Cooking inactivates TI and Hg while toasting left residual amounts of these anti-nutritional factors. Heat treatments were less effective in the detoxification of tannin, phytate and oxalate. Toasting appeared to be partially effective in inactivating the lectins and trypsin inhibitors in the seeds. This result reaffirms the earlier report by Marqaurdt[16].

Table 4: Performance of broiler chickens fed processed mucuna and kidney bean meals

	AFI g/b/d	ADG g/b/d	FCE
Contro	l69.84	38.02[a]	1.84[c]
Raw	69.56	22.15[c]	3.14[a]
Cooked	72.28	36.86[ab]	1.96[b]
Dehulled	68.75	22.28[c]	3.08[a]
Toasted	71.74	36.14[b]	1.98[b]
Mean	70.44	31.09	2.40
SEM	1.76	5.14	0.85

[a, b, c, d]Means with different superscript, on the same column are significantly different ($p<0.05$)

Average feed intake was not significantly ($p>0.05$) affected by any of the processes. Kidney bean contain amylase inhibitors[1] thus, bird taking improperly processed bean must have energy deficiency problem. This could explain the similar feed intake of the groups fed raw and dehulled kidney bean diets since birds eat to meet their energy requirement.

The results, in respect to the growth, underscored the poor nutritional value of improperly processed legumes as animal feed and buttress the contention[17] that the nutritional value of raw or improperly processed legumes is lower than that predicted from their protein content or amino acid composition. Significant ($p<0.05$) differences in Average Daily Gain (ADG) and efficiency of feed utilization were observed. The depressed ADG in birds fed raw and dehulled kidney bean diets could be attributed to higher intake of TI and Hg by these groups. These ANFs have been implicated in growth depression in broiler chicks[18]. Depressed growth could be as a result of combination of endogenous losses of essential amino acids especially sulphur containing amino acids which are important components of trypsin and decreased proteolysis of dietary proteins. Similarly, lectins are known to exert deleterious effects via structural and functional disruptions of the intestinal microvilli resulting in reduced nutrient absorption. The improvement in growth recorded in the groups fed cooked and toasted kidney bean meal could be attributed to the inactivation or reductions in TI and Hg in the meals.

Efficiency of feed utilization was significantly ($p<0.05$) different among the treatment groups. This is consistent with the observation of[19] who reported a reduction in the efficiency of feed utilization when diets containing TI was fed to monogastric animals. The improved value obtained in the group fed cooked kidney bean meal diets is consistent with the finding of[20]. The presence of residual TI and Hg in the toasted meal could account for the observed differences in the efficiency of feed utilization of birds fed toasted and cooked meals.

Table 5: Relative weights of organ of broiler chicken fed processed legume meals (% live weight)

	Liver	Pancreas	Kidney	Lung	Heart
Control	3.24[a]	0.36[d]	0.63	0.05	0.43
Raw	2.55[d]	0.46[a]	0.70	0.07	0.42
Cooked	3.14[b]	0.36[d]	0.63	0.05	0.41
Dehulled	2.70[d]	0.44[b]	0.67	0.08	0.42
Toasted	2.95[c]	0.40[c]	0.65	0.06	0.40
Mean	2.92	0.40	0.65	0.06	0.42
SEM	0.04	0.01	0.006	0.004	0.002

[a, b, c, d]Means with different superscript, on the same column are significantly different ($p<0.05$)

The toxic effects of anti-nutritional factors in raw and processed kidney bean diets caused a significant ($p<0.05$) reduction in the relative weights of the liver and an increase in the weights of the pancreas and the kidney. Weights of the heart and lungs were not affected by the treatments. These observations corroborate previous findings of[21]. The fact that raw and dehulled kidney bean diets resulted in the largest pancreatic, implicates trypsin inhibitors as the key factor responsible for this physiological anomaly. Lyman and Lepkovsky[22] and[1] reported that inactivation of trypsin elicits release of cholecystokinin which stimulates pancreatic production of digestive enzymes including trypsin and chymotrypsin which leads to enlarged pancreas as a result of hypertrophy and hyperplasia. The decrease in weight of the liver in birds fed with raw and dehulled meal could be attributed to an attempt to detoxify the toxic components of the diets.

The changes in the internal structure of organs and the associated intracellular components due to feeding raw and dehulled kidney bean indicates alterations in the metabolic and secretory functions of those organs (Table 5). The changes in the pancreatic acinar of cells of birds fed raw and dehulled bean meals are in keeping with previous reports[23], who observed decreased pancreatic enzyme activities in pigs fed diets containing kidney beans. The authors suggested that such effects are the consequences of poor protein digestibility and interference with systemic protein utilization, resulting in insufficient amino acids for protein synthesis. Similar alterations in the duodenum and pancreas have been reported in other studies with broilers fed different levels of faba bean and peas[24, 25]. The degeneration of the hepatocytes and coagulative necrosis in the liver and congestion of the glomerulus in the kidney observed in birds fed raw and dehulled kidney bean meals were less marked in birds fed toasted meal while no changes were noticed in birds fed the cooked meal. The absence of histological

Table 6: Histological response of organs of broiler chickens fed raw and processed kidney bean meals

Organs	Control	Raw	Cooked	Decorticated	Toasted
Liver					
Congestion of sinusoids	-	**	-	**	*
Coagulative necrosis	-	**	-	**	*
Degeneration of hepatocyytes	-	**	-	**	D
Kidney					
Congestion of glomeruli	-	**	-	**	*
Distention of capillary vessels with numerous thrombi	D	**	-	*	*
Hyperemia	-	-	-	-	-
Pancreas					
Acinar hypertrophy	-	**	-	**	*
Lungs					
Congestion of alveolar walls	-	*	-	*	-
Oedema	-	*	-	*	-
Thickening of alveolar septa	-	D	-	-	-
Heart					
Congestion	-	-	-	-	-

** = present and distinct * = present but less marked than usual D = doubtful/ minimal - = no lesion

lesions in these birds could be attributed to the lower concentrations of residual anti-nutritional factors in the toasted and cooked kidney bean.

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