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Biochemical and Heamatological Indices of Broiler Chickens fed Differently Processed Legume Seed Meals

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Abstract

This study was designed to investigate the implications of feeding broiler chickens with mucuna beans processed by simple domestic methods on the performance, haematological and biochemical parameters. Differently processed bean meals namely dehulling (DUMM), dehulling and cooked (DCMM), soaked and cooked ($SCMM_I$) prolonged soaking and cooking ($SCMM_{II}$) and toasting (TMM) mucuna meals were fed to two hundred and seventy 1 – day old broiler chicks of Anak strain. The birds were divided into six groups of 45 birds per group. Each group was further sub divided to 3 replicates of 15 birds and allocated to six dietary treatments in a completely randomized design. Maize – soybean meal served as control diet. Processed mucuna meal was added to the diet 200g/kg of feed at the expense of soybean meal in the control diet. All diets formulated were isocaloric and iso-nitrogenous. Feed intake was not influenced by the dietary treatments ($P>0.05$). Average daily gain (ADG) was significantly ($P<0.05$) influenced by the dietary treatments. Growth was depressed in birds fed dehulled mucuna meal compared to other dietary treatments. Compared with DUMM, there was significant improvement in ADG in bird fed heat treated mucuna meal. Feed conversion efficiency was significantly improved ($P<0.05$) in birds fed aqueous heated meals (DCMM, $SCMM_I$ and $SCMM_{II}$) compared with those that received DUMM and TMM diets. Dietary treatments significantly ($P<0.05$) affect blood cellular components. The PCV, Hb, and RBC of broilers chickens fed DUMM were reduced with increased MCV and MCH values compared to other treatments. Serum total protein and globulin were affected by the dietary treatments ($P<0.05$). Values obtained in birds fed $SCMM_I$, $SCMM_{II}$, DCMM and TMM were similar and significantly lower than those that received the control and DUMM diets. The result of this study revealed that aqueous heat treatment (cooking) was more effective in improving the nutritive values of mucuna bean meals compared to dehulling and toasting.

Keywords: Mucuna bean, Processing, Performance, Haematology, Biochemical

Introduction

Mucuna bean (*Mucuna pruriens var utilis*) is a tropical legume plant with a high productivity yield of seeds and foliage (Afolabi *et al*., 1985) . The chemical composition and nutritive value of the mucuna beans were investigated by Siddhurraju *et al*. (1996) and Udedibie and Carlini (2000), and the legume seems to have a great nutritional potential as components of livestock feeds, most especially monogastric animals.

In spite of the high protein (28.5 – 35.75%) content of this promising legume, the nutritive value of mucuna bean is marked by the occurrence of trypsin inhibitors, haemagglutinin, tannin, saponin, L - dopa and hydrogen cyanide (Siddhurraju

et al., 1996; Emiola, 2004). Severe inhibitions in feed intake, growth rate and incidence of high mortality in broiler chicks fed raw mucuna beans have been reported by Afolabi *et al*. (1985). Similar observations have been reported (D' mello *et al*., 1983; Ologhobo *et al.,* 1993) in chicks fed raw winged beans and Jack bean. These negative effects have been attributed to the anti-nutritional factors in the beans.

Attempts at improving the nutritive value of legume seeds have been made in various ways by different researchers with conflicting results, indicating not more than partial detoxification (Udedibie and Carlini, 2000; Emiola *et al*., 2003). Friedman *et al*. (1991) in his report on soybean observed that, despite intensive heat treatment, soybean might still contain 20% of residual trypsin inhibitor (TIA) activity. Similar observations were made in soybean isolate by Van Amovengen *et al*. (1998) and Alonso *et al*. (2000).

Haematological and biochemical parameters are important indicators of health status in animals and have been an indispensable tool in the diagnosis, treatment and prognosis of many diseases. The present study was designed to investigate the implications of feeding broiler chickens with mucuna bean processed by simple domestic methods on performance, haematological and biochemical parameters.

Materials and Methods

Sample preparation: The raw *Mucuna pruriens* beans used in this study were purchased from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Five methods of processing were used. These were:

(i) Dehulling and sun-drying (DUMM): Dry mucuna beans were cracked into 6 – 7mm pieces with a hammer mill. The cuticles were removed by winnowing. The dehulled mucuna beans were sun-dried for 48 hours after which they were oven-dried at 85 ^{0}C for 48 hours.

(ii). Dehulling and cooking (DCMM): Dry mucuna beans were cracked into 6-7mm pieces with a hammer mill, the cuticles were removed by winnowing and the beans were cooked in boiling water (96 ^{0}C) for 1 hour. Water was decanted from the cooked beans. Dehulled - cooked beans were sun-dried for 5 days and thereafter oven-dried at 85^0 C for 48 hours.

(iii) Soaking and cooking ($SCMM_I$): Dry mucuna beans were soaked in cold water at room temperature for 24 hours followed by cooking in boiling water at 96 ^{0}C for 1 hour. The soaked - cooked beans were sun-dried for 5 days followed by oven-drying at 85 ^{0}C for 48 hours.

(iv) Prolonged soaking and cooking ($SCMM_{II}$): Dry mucuna beans were soaked in cold water at room temperature for 48 hours followed by cooking in boiling water for 30 minutes. The soaked - cooked beans were sun-dried for 5 days, after which they were oven- dried at 85 ^{0}C for 48 hours.

(*v) Toasting (TMM)*: The dry seeds were toasted to produce toasted seed meals. This involved spreading the seeds thinly in a pan and placing the pan in the oven (120 ^{0}C). They were stirred from time to time to maintain uniform heating. The heating (toasting) was considered adequate when the seeds colour changed to light brown and

became crispy to the touch. The process lasted between 25 to 30 minutes.

Chicks and experimental diets

Two hundred and seventy 1-day old broiler chicks of Anak strain were allocated at random to 6 dietary treatments. Three replicates of 45 chicks per treatment were raised in pens approximately 2.5 m^2. The broilers received diets containing maize-soybean combination as control or one of the differently processed mucuna bean meals preparations incorporated at 200gkg^{-1}. All diets were formulated to provide 230 gkg^{-1} crude protein and 3000 kcal of metabolizable energy at the starter phase and 200 gkg^{-1} crude protein and 2800 kcal metabolizable energy at the finisher phase. Each diet was supplemented with synthetic methionine and lysine to ensure that methionine and lysine were not limiting for growth. The dietary formulations are presented in Tables 1and 2.

The chicks were weighed individually at the beginning of the trial and weekly thereafter until they were 8 weeks old when the experiment was terminated. Food and water were provided *ad libitum* and light was provided 24 hours daily. The daily feed intake of each replicate was obtained as the difference between the amount of feed served and the amount of left over after 24 hours.

Collection and preparation of samples for haematological and biochemical tests

At 56 days, two birds per replicate whose weights were similar to the mean value were selected for haematological and biochemical tests. Blood samples were collected by cardiac puncture through the use of syringe and needle. For the haematological examination, blood was collected in tubes containing EDTA (Ethylene diamine tetra-acetic dipotassium salt). Samples for biochemical measurement were collected into centrifuge tubes already placed in ice cubes to prevent haemolysis. The blood samples were allowed to stand at 4 ^{0}C for 5-6 hours, during which the sera separated from the clot and the clear sera were withdrawn from the tubes into glass vials and kept frozen (-4 ^{0}C) until required for analysis.

Determination of haematological measurements

The white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PCV) were determined according to the method of Dacie and Lewis (1991). The values obtained for RBC, Hb and PCV were used to calculate mean corpuscular volume (MCV), mean corpuscular and hemoglobin concentration (MCHC). The differential WBC counts were obtained by making a differential smear stained with Wrights stain and the percentage count taken for lymphocytes and neutrophils.

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined by methods of Colowick and Kaplan (1955) while serum protein and serum albumin were determined as described by Doumas and Bigss (1972).

Chemical analysis

The diets were analyzed for dry matter, ether extract, ash and crude fibre according to standard methods AOAC (1995). Nitrogen was determined by the Kjeldahl method AOAC (1995), crude protein (N x 6.25) and nitrogen free extract were calculated.

Data Analysis

Data collected were analyzed in a complete randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). When a significant *F*-value for treatment means ($P < 0.05$) was observed in the ANOVA, treatment means were compared using Duncan's multiple range test (Steel and Torrie, 1980).

Results and Discussion

Feed intakes were higher in the heat treated beans while the lowest value was obtained in birds fed DUMM diet. This result is in agreement with the earlier findings of Ologhobo *et al*. (1993) that the nutritive value of legumes is enhanced by heat treatment. The slight differences in feed intake between birds fed with DCMM and those fed $SCMM_{I,}$ $SCMM_{II}$ and TMM could be due attributed to the presence of residual tannins in these diets.

Significant ($P<0.05$) differences were obtained in average daily gain. Growth rate was depressed in birds fed DUMM while there was significant improvement in average daily gain of birds fed heat treated meals. The improved performance may be due to the increase in nutrient digestibility. The depressed growth rate observed in birds fed DUMM diets could be due to residual anti-nutritional factors in the meal, an indication that the anti-nutritive component of mucuna beans are located in the cotyledons. Much earlier, Ferguson *et al*. (2006) reported that the anti-nutritive properties of lupins are located in the cotyledon. Mucuna bean contain trypsin inhibitors, non starch polysaccharides (NSP), tannins, phytate and oxalate (Afolabi *et al*., 1985; Udedibie and Carlini, 2000). Trypsin inhibitors, non starch polysaccharides and tannins have been reported to inhibit weight gain of broiler chickens (D'mello *et al*., 1985; Yu-long *et al*., 2001; Ologhobo *et al*., 2003). Liener (1976) reported that the growth inhibitory property of trypsin inhibitor in rats is caused by the diversion of dietary sulphur amino acids from the synthesis of body tissues to the synthesis of pancreatic enzymes which are relatively rich in these amino acids. Similarly, Kakade *et al*. (1973) suggested that trypsin inhibitor adversely influences the utilization of proteins in rats by increasing the requirements for methionine and lysine.

The efficiency of feed utilization was significantly improved in birds fed aqueous heat treated mucuna meals (DCMM and $SCMM_{II,}$ $SCMM_{I}$) diets but was considerably reduced in those fed DUMM and TMM diets. The improvement in gain to feed ratio in birds fed aqueous heated mucuna bean diets is consistent with the findings of Pusztai *et al*. (1981) in mucuna bean, Ologhobo *et al*. (1993) in lima bean and Udedibie and Carline (2000) in jack bean. This could be attributed to aqueous heating that destroyed all heat labile anti-nutritional factors and caused a reduction in others, and consequently an improvement in the nutritive value of the diets. The differences in DCMM, $SCMM_{I}$ and $SCMM_{II}$ could be attributed to differences in the levels of residual tannins in the diets. The efficiency of feed

utilization was lower in birds fed TMM diets, an indication that dry heat treatment may not be an effective processing method for detoxifying anti-nutritional factors in legume seed as earlier reported by Udedibie and Carlini (2000). The reductions in the efficiency of feed utilization in birds fed DUMM confirmed an earlier observation of Ferguson *et al*. (2006).. The presence of residual trypsin inhibitors in DUMM would implicate this anti-nutritional factor in adversely exerting specific effects on protein metabolism and utilization and consequently efficiency of feed utilization. Other reasons for this might be the increase in digesta viscosity. This, according to Yu-long *et al*. (2001), might be a reflection of the presence of various compounds which slow down the rate diffusion of substrates and digestive enzymes and hinder their effective interaction at the mucosa surface of the intestine, leading to reduced absorption in the small intestine. In this investigation, digesta viscosity was not measured. However, Siddhurraju *et al*. (1996) reported that *Mucuna pruriens* contained non starch polysaccharides (NSP) which have been implicated in reduced nutrient digestibility and absorption. The authors further stated that heat treatment reduced the level of NSP in the bean.

Haematological and biochemical parameters are important indicators of health status in animals and have been an indispensable tool in the diagnosis, treatment and prognosis of many diseases (REF?). There were variations ($p < 0.05$) in the blood cellular constituents due to different dietary treatments. The PCV, Hb, and RBC of broiler chickens fed DUMM were significantly ($P<0.05$) reduced, which suggest a dysfunction of blood haemopoesis. Values obtained in birds fed control diet and other dietary treatments were comparable. This agreed with earlier report of Ikegwuonu and Basir (1977), who postulated a progressive degradation of the erythrocytes during intoxication of lectins from edible legumes. Values of MCV and MCH were increased in the DUMM. Increased MCV, MCH and MCHC have been shown to cause anaemia (Seivered, 1977). There was a decrease in the white blood cell counts of birds fed DUMM. This is a reflection of the decline in the production of white blood cells for defensive action against infections. It probably explains why the birds were more susceptible to various physiological stresses resulting in disease and reduced growth rate. The lymphocytes were significantly increased in birds fed on DUMM. This according to Ikegwuonu and Basir (1977) was attributed to the stimulation of the reticulo-endothelia system by the potent endogenous toxic substances.

Serum total protein and globulin were significantly influenced by the dietary treatments. The values for birds fed $SCMM_I$, $SCMM_{II}$, DCMM and TMM were similar and significantly lower ($P<0.05$) than for those fed with control diet while the least value was observed in birds fed DUMM. The decreased serum total protein in birds fed DUMM appears to be attributable to inhibition of protein utilization by the birds. Kakade and Evans (1966) reported that reduced serum total protein and globulin levels manifest as an alteration in normal systemic protein utilization. This is further supported by the reduced weight gain in birds fed DUMM. The improvement in serum protein in broilers birds fed heat treated mucuna beans indicates a rise in amino acid absorption and

utilization. The hepatic enzymes, glutamic pyruvic transaminase and glutamic oxalo-acetic transaminase, were increased in birds fed DUMM. These results confirm earlier findings of Sathyamorthy *et al*. (1981) and Aletor and Fetuga (1986). These authors reported that hepatic transaminases in rats were significantly increased when fed raw legume seeds. The increased activities of hepatic transaminase are indicative of increased catabolism of amino acids and liver cell damage.

Table 1: Gross composition of experimental diet-starter phase (%DM)

	Control	$SCMM_1$	$SCMM_{11}$	DUMM	DCMM	TMM
Maize	55.50	53.00	53.00	51.00	53.00	51.50
Soybean	30.00	10.00	10.00	10.00	10.00	10.00
Mucuna	-	20.00	20.00	20.00	20.00	20.00
Wheat offal	4.00	2.50	2.50	4.50	2.50	4.00
Fish meal	3.00	4.50	4.50	4.50	4.50	4.50
Blood meal	3.00	5.50	5.50	5.50	5.50	5.50
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50
#Vit/mineral	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Determined proximate composition (%DM)						
Dry matter	92.50	88.36	90.52	93.24	90.82	94.36
Crude protein	23.25	22.92	23.01	23.26	22.85	23.04
Crude fibre	6.52	7.28	7.42	5.14	4.64	7.65
Ether extract	8.35	6.23	6.15	6.85	6.51	7.05
Ash	8.40	9.43	9.21	8.82	8.28	9.26
Calculated ME (kcal/g)	3050.78	3011.76	3014.56	3019.64	3020.28	3019.78

VIT/MINERAL MIX (mg/kg): lodine 385, cobalt 39; Iron 5.232; zinc 7800; manganese 13500; Retinol 450; cholecalciferol 7.5, riboflavin 2600, pantothenic acid 1.4; vit. K300; cyanocobalamin 2, nicotinic acid 3, choline 56.

Table 2: Gross composition of experimental diet-finisher phase (%DM)

Ingredients	Control	$SCMM_I$	$SCMM_{II}$	DUMM	DCMM	TMM
Maize	45.00	43.50	43.50	44.50	43.50	43.50
Soybean	30.00	10.00	10.00	10.00	10.00	10.00
Mucuna	-	20.00	20.00	20.00	20.00	20.00
Wheat offal	8.00	8.50	8.50	8.50	8.50	8.50
Corn bran	10.50	9.00	9.00	9.00	9.00	9.00
Fish meal	1.00	2.50	2.50	2.00	2.00	2.00
Blood meal	1.00	2.00	2.00	1.50	1.50	1.50
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	2.50	2.50	2.50	2.50	2.50	2.50
#Vit/mineral	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Determined proximate composition of experimental diets on (%DM)						
Dry matter	94.03	93.35	92.50	93.65	93.41	94.62
Crude protein	20.42	20.85	20.68	21.15	19.89	20.84
Crude fibre	8.62	8.72	8.84	6.25	6.14	8.68
Ether extract	7.43	6.32	6.28	6.49	5.98	7.24
Ash	8.12	9.42	10.12	9.24	8.20	9.26
NFE	53.26	52.14	54.21	55.93	57.72	53.00
Cal. ME (kcal/g)	2885.15	2800.43	2815.62	2825.55	2806.47	2812.65

#VIT/MINERAL MIX (mg/kg): Iodine 385, cobalt 39; Iron 5.232; zinc 7800; manganese 13500; Retinol 450; cholecalciferol 7.5, riboflavin 2600, pantothenic acid 1.4; vit. K300; cyanocobalamin 2, nicotinic acid 3, choline 56.

Table 3: Performance characteristics of broilers fed differently processed mucuna bean meals (gbd^{-1})

Measurement	Control	$SCMM_I$	$SCMM_{II}$	DUMM	DCMM	TMM	SEM
Initial weight (g/b)	90.00	90.00	90.00	90.00	90.00	90.00	
Final weight (g/b)	2250	1930	2010	1370	2090	1930	
Weight gain (g/b)	2160	1840	1920	1280	2002	1840	
ADG(gbd^{-1})	38.57^a	32.86^c	34.29^b	22.86^d	35.75^b	32.86^c	0.02
ADFI(gbd^{-1})	78.57	70.00	71.43	75.71	77.14	74.28	0.01
ggain/kgfeed	490^a	469^b	480^a	302^d	463^b	442^c	0.19

$^{a,b.c,d}$ Mean with the different superscripts along the same row are significantly different ($p<0.05$)
SEM – standard error of means

Table 4: Effects of differently processed mucuna bean meals on the haematological parameters of broilers

Parameters	Control	$SCMM_I$	$SCMM_{II}$	DUMM	DCMM	TMM	SEM
PCV (%)	31.30[a]	29.00[a]	30.00[a]	24.00[c]	32.35[a]	27.50[b]	1.11
Hb(g/dl)	9.50[a]	8.50[b]	9.36[a]	7.00[c]	10.25[a]	8.20[b]	0.43
RBC ($x10^{12}$/l)	3.90[a]	3.85[a]	3.88[a]	2.40[b]	3.96[a]	3.70[a]	0.22
WBC ($x10^9$/l)	2.45[a]	2.30[b]	2.35[b]	1.10[c]	2.43[a]	1.25[c]	0.23
MCV (fl)	85.36[b]	82.25[bc]	80.75[c]	97.55[a]	84.24[b]	86.50[b]	2.23
MCH (pg/cell)	26.85	26.80	26.28	30.85	25.32	27.33	0.71
Lymphocytes (%)	42.50[c]	51.00[b]	48.00[bc]	68.70[a]	44.52[c]	53.00[b]	3.49
Neutrophils (%)	56.00[a]	53.50[a]	54.00[a]	42.50[b]	54.50[a]	54.00[a]	1.82

[a,b.c,d] Mean with the different superscript along the same row are significantly different ($p<0.05$)

Table 5: Effect of differently processed mucuna bean on serum metabolites of broiler chickens (mg/100ml**)**

	Control	$SCMM_I$	$SCMM_{II}$	DUMM	DCMM	TMM	SEM
Total protein	2.88[a]	2.45[b]	2.53[b]	1.70[c]	2.55[b]	2.35[b]	0.14
Albumin	1.22	0.98	0.96	0.93	1.14	0.94	0.04
Globulin	1.66[a]	1.47[b]	1.57[b]	0.77[c]	1.60[a]	1.41[b]	0.12
GOT	25.00[b]	26.35[b]	25.85[b]	28.50[a]	24.82[b]	23.00[b]	0.88
GPT	23.50[b]	25.80[b]	26.00[b]	30.00[a]	25.00[b]	26.00[b]	1.01

[a,b.c,d] Mean with the different superscript along the same row are significantly different ($p<0.05$**)**

Conclusion

The results of this study show that aqueous heat treatment (cooking) is more effective in improving the nutritive values of mucuna bean meal compared to dehulling and toasting. Mucuna pruriens bean can be incorporated at 200 gkg^{-1} in broiler diet when subjected to aqueous heat treatment without any negative effect on the heamatological parameters.

References

Afolabi O. A., Oshuntogun, B. A., Adewusi, S. R., Fopowo ,O. O., A yorinde, F. O., Grisson, F. E. and Oke, O. L. (1985). Preliminary nutritional and chemical evaluation of raw seeds from *Mucuna solanei:* an under-utilized food source. *Journal of Agricultural and food chemistry 33*, 122-124.

Aletor V. A. and Fetuga, B. L. (1986). Some pathological aspects of raw Lima bean (*Phaseolus lunatus* Linn) fed to growing rats. *Nig. J. Anim. Prod*. 13: 48-57.

Alonoso, R., .Aguirre, A. and Marzo, F. (2000). Effects of extrusion and traditional processing and method on anti – nutrients and in-vitro digestibility of protein and starch

in faba and kidney beans. *J. Agric. Food Chem. 68: 159-165.*

AOAC (1995) Association of Official Analytical Chemist Official Methods of Analysis Washington. D.C. 16th edition

Colowick, S. P. and Kaplan, N. O. (1955) In Methods in enzymology Vol. 1, 2nd edition, NewYork, Academic press, New York.

D'mello J. P. F., Acamovic, T. and Walker, A. G. (1983). Nutrient content and apparent metabolisable energy values of full fat winged bean (*Psophocarpus tetragonolobus*) for young chicks. *Tropical Agriculture (Trinidad)* 60, 290-293

D' mello, J. P. F., Acamovic, T. and Walker, A. G. (1985). Nutritive value of Jackbeans (*Canavalia ensiformis* (L)) for young chicks. *Trop. Agric. (Trinidad)* 62:145-150

Dacie J.V and Lewis S.M Practical haematology (5th Eds.) Longman Press Limited pp. 22-68. (1991)

Doumas B. T. and Bigss, H. C. (1972). Determination of serum albumin. In: Standard methods of clinical chemistry (Copper, G.R. ed.) Academic Press, New York. Pp 175-188

Emenalom O. O. and Udedibie. (1998). Effect of dietary raw, cooked and toasted *Mucuna* pruriens seeds (Velvet bean) on the performance of finisher broilers. *Nig J. Anim. Prod.* 25: 115-118.

Emiola I. A. (2004) Effects of residual anti-nutritional factors in processed legume on the performance, biochemical and reproductive parameters of exotic broilers and cockerels.

Emiola I. A., Ologhobo, A. D., Akinlade, J., Adedeji, O. S. and Bamigbade, O. M. (2003). Effect of inclusion of differently processed mucuna seed meal on performance characteristics of broilers. *Trop. Anim. Prod Invest. 6:13-21*

Ferguson N. S., Guos, R. M. and Iji, P. A Determining the source of anti-nutritional factor(s) found in two varieties of lupin (*L. albus* and *L. angustifoilus*) fed to growing pigs. University of KwaZulu-Natal, Animal and Poultry Science. Unpublished data.

Friedmann, M., Brandon, D.L., Babes and Hymowitz, T. (1991) Effects of heat on the nutritional quality of soybean cultivars. IN: (Friedmann, M., Ed) Nutritional and Toxicological consequences of food processing Plenum Press, New York, pp 339-361

Ikegwuonu F. I. and Basir, O. (1977). Effects of phytohaemagglutinin from immature legume seeds on the function and enzyme activities of the lower and on the histophatolotical changes of some organs of rats. *Toxicol. Appl. Pharma 40: 217-226.*

Kakade M. L. and Evans, R. J. (1966). Growth inhibitors of rats fed navy bean fraction *Phaseolus vulgaris. J. Nutri. 90: 191-198.*

Kakade, M. L., Hoff, D. E. and Liener, I.E. (1973). Contribution of trypsin inhibitor of the deleterious effects of untreated soybean fed to rats. J. Nutr. 103:1772-1779.

Leon, A. M., Caffin, J. P., Plassart, M. and Picard, M. (1991). Effects of con-canavalin A, from jackbean seeds

on short term food intake regulations in chicks and laying hens. *Animal Feed Science and Technology, 32, 297-311*

Liener, I. E. (1976). Legume toxins in relation to protein digestibility. A review. *J. Food Sci. 41: 1076 – 1081*.

Ologhobo, A. D., Apata, D. F. and Oyejide, A. (1993) Utilization of raw jack bean (*Canavalia ensiformis*) and jack been fractions in diets for broiler chicks. *British Poultry Science 34, 323 – 337*

Ologhobo, A. D., Mosenthin, R. and Alaka, O. O. (2003). Histological alterations in the internal organs of growing chicks from feeding raw jackbean or limabean seeds. *Vet. Human Toxicol*. 45 (1): 10-13

Pusztai, A., Clarke, E. M. W., Grant, G. and King, T. P. (1981). The toxicity of *Phaseolus vulgaris* lectins. Nitrogen balance and Immuno chemical Studies. *J. Sci Food Agric 32: 1037-1046.*

Ravindran V. and Ravindran, G. (1988). Nutritional and anti-nutritional characteristic of *Mucuna utilis* bean seeds. *J. Sci. Food and Agric. 46: 71-79*

Sathyamorthy, V., Kamalakannan, V. and Motlag, D. B. (1981). Influence of dietry raw green grain (Phaseolus Aurens, Roxb). And green gram trypsin inhibitors on the activity of certain protein metabolism enzymes in rats. *Ann. Metab Nutr*. 25: 334-340.

Seivered, C.E. (1972). Haematology for Medical Technologist, Lea and Febger, Philadelphia, 4th edition.

Siddhurahju, P., Vijayakumari, K. and Janardhanan, K. (1996). Chemical composition and protein quality of the little known legume, velvet bean (*Mucuna Pruriens* (L) DC. *J. Agric Food Chem. 44, 2636-2641*

Steel, R. G. D. and Torrie, J. H. (1980). Principles and procedures of statistics. A biometrical approach. 2nd Ed, McGraw – Hill Book co., New York

Udedibie, A. B. I. and Carlini, C. R. (2000). Relative effect of dry and moist heat treatments on haemagglutinin and anti-tryptic activities of selected legume grains. *Nig. Poult. Sci. J.* 1: 81 – 87

Van Amevongen, A., Ostafe, V., Meijewo, M. M. T., Guppen, H., Meeradink, G., Berendsen, L. B. J. M., Koets, M. and Wichers, J. H. (1998). Specific-immuno-(chymo) trypsin-inhibitor assays for determination of (residual) activity of Bowman-Birk or Kunitz soybean inhibitors. In: Jansman, A. J. M., Hill, G. D. and Huisman, J. Recent advances of research in Anti-nutritional factors of Legumes seeds and Rape seed. Wageninger Pers. Wageningen. pp 33-37

Yu-Long Yin, Samuel K Baidoo,. Boychuk, J. L. L and Howard H. Simmins (2001). Performance and carcass characteristics of growing pigs and broilers fed diets containing micronised barley, ground barley, wheat and maize. *J. Sci. Food Agric*. 81: 1487 – 1497