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Evaluation of the Haematological and Serum Biochemical Parameters of Mice Fed with Three Varieties of Unprocessed Lima Beans (Phaeolus lunatus)

SOETAN, K.O.* ADEBIYI, E.O. ATANDA, T.N.
Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria

Abstract
Lima Bean (LB) (*Phaseolus lunatus*) is an underutilized legume. This study evaluated effects of feeding three varieties of unprocessed LB diets (LB011, LB001 and LB015) from IITA, Ibadan on the haematology and serum chemistry of mice. 40 mice weighing 17g-32g, divided into four groups (A-D), of 10 mice per group were used. Group A (control) received commercial mice feed while groups B, C and D received 50% LB diet inclusions of LB011, LB001 and LB015 respectively. All analyses were done using standard protocols and statistical analysis was done using students' t-test. Group D mice recorded the highest packed cell volume (40.00±1.00%), Haemoglobin (13.43±0.12 g/dl) and red blood cells counts (6.52±0.06 10^6/ul). Group C mice gave the highest white blood cells counts (5688±370.50 10^9/ul). For serum chemistry, Group D mice recorded the highest ALP (224.7±23.71 U/L), Group C mice gave the highest ALT (137±8.87 U/L) while Group A mice had highest AST (256.8±33.34 U/L). The study concluded that consumption of the different varieties of unprocessed lima beans did not produce significant adverse effects on the haematology and serum biochemistry of the mice.
Keywords: Lima beans, mice, biochemical, IITA.

Introduction

Lima bean (LB) (*Phaseolus lunatus*) is a legume belonging to the Family: *Fabacea* and Genus: *Phaseolus*. LB is rich in plant protein (Bello-Perez *et al.* 2007; Falaye *et al*. 2014) and it is one of the under-utilized legumes in Nigeria (Aletor and Aladetimi, 1989) and West Africa (Asante *et al*. 2008).

Lima beans was introduced to West and Central Africa by the Portuguese explorers in the 16th century of which the bean now forms the major food legume in eastern and southern Africa (Aghkhani *et al.,* 2012).

In Nigeria lima bean is second in importance after cowpea (Asante *et al*., 2008). In West Africa, lima bean is an underutilized legume. Lima bean is widely cultivated in the south-western, south-eastern and the middle belt regions of Nigeria (Aletor and Aladetimi, 1989). Lima bean has a rich source of protein (21-26%) and high carbohydrate (55-64%), low fat (1.0-2.3%) and fiber levels (3.2-6.8%), high levels of minerals such as potassium, zinc, calcium and iron, and low levels of sodium and phosphorus (Oshodi and Aletor, 1993; Kizito, 2010). However, lima bean contains some anti-nutritional factors such as lectins, trypsin inhibitors, phytates and oxalates (Ologhobo and Fetuga, 1982; Fasoyiro *et al.,* 2006**)** which can be reduced by modern and traditional food processing methodologies including soaking, germination, dehulling, cooking and fermentation (Honke *et al.,* 1998; Fasoyiro *et al.,* 2006).

The global demand for protein is continually increasing and so protein sources are required from both conventional and lesser known sources of protein (Eltayeb *et al*. 2010) and the plants proteins play a major and vital role in the global demand for food supply.

The continually increasing world population most especially in third world countries and developing nations as in Nigeria has called for urgent intervention and improvement in the livestock sub-sector. The deficiency of animal proteins available for human consumption is a major problem that needs to be critically assessed and addressed in Nigeria and other African Countries (Okah and Ibeawuchi, 2011). The level of animal proteins in diets of most Nigerians is very low and consequently reached a critical level (Okah and Ibeawuchi 2011).

Many Protein supplements are very expensive and their use in ruminant animal production competes with monogastric animal production as well as human nutrition. There is the need to then address the issue of insufficiency of dietary supplements in animal nutrition especially the protein supplements which is becoming a ravaging menace to the Livestock sub-sector and human consumption (Okah and Ibeawuchi, 2011). This can then be achieved by sourcing for alternative protein feedstuff that will attract reduced competition amongst Ruminant animals, monogastric animals and humans.

The effects of processed and unprocessed lima bean seed meal have been reported in *Clarias gariepinus* (Falaye *et al*., 2014), on Nile Tilapia Fish (*Oreochromis niloticus*) (Adeparusi and Ajayi 2004), on Rats (Oboh and Omofoma 2008), on Growing Chicks (Ologhobo *et al*., 1993) but there is a dearth of information on its effect on the performance of mice with regards to haematological, biochemical and hepatorenal parameters.

Lesser-known and underutilized legumes are now been utilized as sources of animal feeds because of the competitive demand for conventional plant proteins (Ari and Ayanwale 2012; Adewale *et al*., 2013). This calls

for the need to evaluate the haematological and biochemical effects of these lesser-known legumes on animals.

There is very little of information from literature on the effect(s) of *Phaseolus lunatus* seeds on haematology and serum biochemical parameters of male mice and this study aims at investigating the effect of oral consumption of unprocessed *Phaseolus lunatus* seeds on the haematology, serum biochemical parameters and organ histopathology of male mice.

Materials and Methods

Experimental Animals

Forty male mice weighing between 20 g and 22 g were selected for this study. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water. The mice were acclimatized to laboratory conditions for a period of two weeks before the commencement of the experiments. All the procedures were carried out in compliance with the recommendations on the standard use of laboratory animals (World Medical Association and American Physiological Society, 2002).

Source of the Lima Beans (*Phaseolus lunatus*)

The three varieties of the Lima beans (LB) used for this study, LB011, LB001 and LB015 were obtained from the Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

Figure 1: The three varieties of Lima beans (*Phaeolus lunatus*). Based on seed coat colour (Accession numbers; LB-011, LB-001 and LB-015); white, black and light brown seeds respectively. **Source: (Soetan *et al.*, 2018).**

The three varieties of Lima beans (LB-011, LB 001 and LB 015) seeds were separately ground into powdery form with an electric blender (Blender/Miller III, model MS-223, Taiwan, China). The ground seeds were then incorporated into commercial mice feed (Vital® Feed), at a ratio of 1:1. The nutritional constituents of the commercial mice feed were: crude protein (13%), crude fibre (15%), fat (8%), calcium (0.9%), phosphorus (0.35%), metabolizable energy (2,600 Kcal/kg).

The forty male mice were randomly divided into four groups with each group consisting of ten mice. The four groups of rats were given the following feeds for 21 days:

Group A mice served as the control and were fed with commercial mice feed only
Group B mice were fed with the commercial mice feed + LB011
Group C mice were fed with the commercial mice feed + LB001
Group D mice were fed with the commercial mice feed + LB015.

Haematology and Serum biochemistry

Collection of Blood Samples

At the end of the 21 days feeding, bleeding of each mice was done through the orbital sinus into clean heparinized bottles and used for haematological studies and some blood samples collected into clean non-heparinized bottles were allowed to clot to obtain the serum. The serum separated from the clot and was decanted into clean sample bottles for serum chemical analysis. The packed cell volume (PCV) and haemoglobin

concentration were determined with the microhematocrit and cyanmethaemoglobin procedures, respectively (Jain, 1986). The erythrocyte count was determined using the haematocytometry method (Jain, 1986). Total white blood cells (WBC) counts were made in a haemocytometer with the WBC diluting fluid and differential leucocytes counts were done by counting the different types of WBC from Giemsa stained slides viewed from each of the 30 fields of an oil immersion objective of a microscope (Coles, 1989). The erythrocyte indices; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from the values obtained for RBC count, Hb and PCV values (Duncan *et al*., 1994).

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using a photoelectric colorimeter (Duncan *et al*., 1994). The alkaline phosphatase (ALP) activity was assayed using the method of (Talwar and Srivastava, 2004). Serum urea and creatinine values were determined using method described by (Coles, 1989). The total protein was measured using biuret reaction, while albumin was measured by the colorimetric estimation using sigma diagnostic reagent (Sigma Diagnostic, UK.), which contained bromcresol green (BCG). The globulin was calculated from the difference between the values for total protein and albumin.

Statistical analysis

The data generated from the study were analyzed using the Graphpad prism version 7.03.

Results

Table 1: Results of red blood cells counts of mice fed with different varieties of Lima beans (*Phaeolus lunatus*).

Groups	(PCV) (%)	(Hb) (g/dl)	(RBC) (10^6/ul)	(MCV) (fL)	(MCH) (pg)	(MCHC) g/dL	PLATELETS (/ul)
A	40.25± 1.50	13.43± 0.56	6.50± 0.05	61.94± 1.98	20.66± 0.76	33.36± 0.25	118750± 3594
B	35± 5.57	11.47± 1.86	5.43± 1.21	65.09± 4.02	21.31± 1.26	32.75± 0.46	102667± 2100
C	33± 8.41	10.9± 2.75	5.45± 1.49	60.87± 3.45	20.12± 1.07	33.05± 0.23	104250± 19259
D	40± 1.00	13.43± 0.12	6.52± 0.06	61.35± 1.51	20.62± 0.16	33.59± 0.60	98667± 10214

n=10

The packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell cells (RBC) and platelets values of all the mice fed with 50% LB011, LB001 and LB015 inclusion diets (groups B, C and D respectively), were not significantly different from those of the control mice (group A) fed on commercial mice feed.

Table 2: Results of white blood cells counts of mice fed with different varieties of Lima beans (*Phaeolus lunatus*).

Groups	(WBC) (10^9/ul)	LYMPHOCYTES (10^3/ul)	NEUTROPHILS (10^3/ul)	BASOPHILS (10^3/ul)	MONOCYTES (10^3/ul)
A	5595± 378.30	63.75± 1.50	32.5± 2.38	2.00± 0.82	1.75± 0.96
B	5567± 907.40	58.00± 3.61	38± 3.46	2.33± 1.16	1.67± 0.58
C	5688± 370.50	61.00± 2.16	34.25± 2.22	2.5± 0.58	2.25± 0.50
D	5483± 475.20	61.33± 5.86	34.33± 4.73	2.33± 0.58	2.00± 1.00

n=10

The white blood cells counts of all the mice fed with 50% LB011, LB001 and LB015 inclusion diets (groups B, C and D respectively), were not significantly different from those of the control mice (group A) fed on commercial mice feed.

Table 3: Results of serum biochemistry of mice fed with different varieties of Lima beans (*Phaeolus lunatus*).

Groups	Protein g/dl	Albumin g/dl	Globulin g/dl	A:G ratio	ALT U/L	AST U/L	ALP U/L	Urea mg/dl	Creatinine mg/dl
A	6.05± 0.27	2.93± 0.15	3.1± 0.20	0.9± 0.08	87.0± 21.46	256.8± 33.34	176.5± 38.10	15.25± 1.71	0.63± 0.05
B	6.57± 0.21	3.33± 0.06	3.23± 0.15	1.0± 0.00	80.67± 45.17	141± 89.27	185± 51.22	14.33± 2.52	0.60± 0.10
C	5.93± 0.22*	2.93± 0.17	3.00± 0.18	0.925± 0.10	137.0± 8.87	200.5± 49.28	165.8± 56.60	14.5± 2.38	0.55± 0.06
D	6.6± 0.20	3.30± 0.17	3.30± 0.10	1.0± 0.10	95.33± 31.53	223.7± 24.01	224.7± 23.71	18± 1.00	0.57± 0.12

n=10 * significant at $p<0.05$

There were no statistically significant differences in the values for ALT, AST and ALP, urea and creatinine of the mice fed with 50% LB011, LB001 and LB015 inclusion diets (groups B, C and D respectively), as compared to the control mice. However, total protein values for the mice in group C were significantly reduced as compared with the values for the control mice (group A).

Discussions

The statistically non-significant values recorded for packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell cells (RBC) and platelets of all the mice fed with 50% LB011, LB001 and LB015 inclusion diets (groups B, C and D respectively), as compared with those of the control mice (group A) fed on commercial mice feed showed that the unprocessed lima beans did not have adverse effects on the red blood cells of the mice. Red blood cells function mainly in the transportation of oxygen to the tissues of the body. Thus, any pathological condition affecting the red blood cells adversely affects its function and this may be detrimental to the body (Agbor *et al*., 2005).

The statistically non-significant values recorded for the white blood cells counts of all the mice fed with 50% LB011, LB001 and LB015 inclusion diets (groups B, C and D respectively) as compared with the control mice (group A) fed on commercial mice feed, also showed that the unprocessed lima beans did not have adverse effects on the white blood cells of the mice. White blood cells play active functions in the fighting of infections in the body. A decrease in white blood cells, neutrophil and lymphocyte counts is positively correlated with susceptibility to infection, leukaemia and possible compromise of cellular and humoral mediated immunity (Bochner *et al*., 1991).

The ALT, AST and ALP, urea and creatinine values recorded of all the mice fed with 50% LB011, LB001 and LB015 inclusion diets (groups B, C and D respectively) were not statistically different from those of the control mice (group A) fed on commercial mice feed. This showed that the unprocessed lima beans did not have adverse effects on the white blood cells of the mice.

However, the total protein values for the mice in group C were significantly reduced as compared with the values for the control mice (group A). ALT and AST are important marker enzymes of damage to the plasma membrane of the liver cells (Shahjahan *et al*., 2004). Their presence in high concentrations above the normal reference values in the serum may provide information on organ dysfunction (Toro and Ackermann, 1975). The lack of significant effect of the unprocessed lima beans on the ALT values of all the mice fed with the different varieties of unprocessed lima beans implies that LB will not cause serious impairment to liver function. ALP is often used to assess the integrity of plasma membrane (Akanji *et al*., 1993) and is localized

predominantly in the plasma membrane of the microvilli of the bile canaliculi (Talwar and Srivastava, 2004).

The significant reductions observed in the values of total protein for the mice in group C may be associated with the protein constituent of the LB 001, rather than hepatic toxicity. LB 001 recorded the least value for crude protein (22.40+0.95%) (Soetan and Atanda, 2018). Under normal conditions, most plasma proteins are synthesized in the liver. Urea and creatinine are measured as indicators of renal functions (Duncan and Prasse, 2011).

The kidney is the most important route of urea excretion. Urea concentration in the glomerular filtrate is the same as that in the blood.

Conclusions

The study concluded that consumption of the different varieties of unprocessed lima beans did not produce significant adverse effects on the haematology and serum biochemistry of the mice. Lima bean could therefore serve as a source of protein for animal feeds. There is however a need for further studies to assess the effects of feeding the unprocessed lima beans on some vital body organs like the liver, kidneys, intestines, and the testes of the mice.

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