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Haematological and Biochemical Indices of Albino Rats Fed Raw Sickle Pod (*Senna obtusifolia*) Seeds

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Abstract

This study was conducted for 30 days to investigate the effects of feeding raw *Senna obtusifolia* seed meal (RSOSM) on the haematological and biochemical indices of albino rats. Five experimental diets were compounded to contain RSOSM at graded levels of 0, 5, 10, 15 and 20% which constituted five (5) treatments designated T1, T2, T3, T4 and T5, respectively. A total of 60 young albino rats with average weight of 70.52 to 81.05 g were randomly assigned to the five dietary treatments in completely randomized block design with three replicates of four rats each. At the end of the experiment, blood samples were collected and analysed for haematological and biochemical indices using standard procedure. Data obtained were subjected to analysis of variance using a computer statistical package (Statistix 9.0). The haematological parameters were significantly ($P<0.05$) affected by the dietary treatments. A fall in the packed cell volume, haemoglobin, red blood cell and white blood cell counts were observed as the levels of RSOSM increases from 0 to 20%. For instance, packed cell volume, haemoglobin and white blood cell count decreased from 42.23 to 26.27%, 12.08 to 9.17 g/dl and 7.83 to 4.37 x 10^3/ml in T1 to T5, respectively. The serum chemistry values were adversely affected by the dietary treatments with the lowest albumin, globulin and total protein recorded in albino rats fed 15 and 20% RSOSM (2.40g/dl, 0.78g/dl and 3.18g/dl) and (2.67 g/dl, 0.60 g/dl and 3.27 g/dl). The total bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were more elevated in rats fed 15% RSOSM (1.32 mmol/l, 37.47 IU/L and 94.35 IU/L) and 20% RSOSM (1.37 mmol/l, 48.20 iμ/l and 149.67 IU/L). It was concluded that inclusion of RSOSM up to 20% in the diets of albino rats had indicated some toxicological evidences on some haematological and serum biochemical parameters. It is recommended that the seeds of *Senna obtusifolia* be thoroughly processed before inclusion in the diets of albino rats and other similar monogastric animals.

Keywords: Haematological and Biochemical Indices, Rat toxicity, raw *Senna obtusifolia* seeds indices

Introduction

Utilization of lesser-known legume has continued to gain more acceptances in livestock feeding especially in the monogastric livestock industry. Oyewole *et al*. (2011) pointed out that the use of non-convention feedstuffs that are not demanded by man for food is one of the approaches to reducing the high feed cost incurred in livestock feeding. An under-exploited lesser-known legume in Nigeria is *Senna obtusifolia*. *Senna obtusifolia* is a pantropical weed that belongs to the family *leguminosae caesapinioideae*. It is an erect bushy annual shrub that grows up to 90 cm tall and propagates through seed. The leaflets consist of three pairs and each leaflet is obovate, 1.5-5 cm long and 1-3 cm wide, mucronate at the apex and the flowers are yellow in colour (Akobundun and Agyakwa, 1998). The chemical composition of the seeds as revealed by Ingweye *et al*. (2010) and Augustine (2016) indicated that it has good nutritional profile but also contain some toxic components which may limit the utilization of the seed as feed ingredient.

Examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body and it plays a vital role in assessing physiological, nutritional and pathological status of animals (Aderemi, 2004). A readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials may be the use of blood analysis, because ingestion of dietary components has measurable effects on blood composition (Church *et al*. 1984; Maxwell *et al*., 1990). It has become absolutely necessary to generate base-line information on the effects of raw *Senna obtusifolia* seed meal on haematological indices and biochemical profile of albino rats. The choice of albino rats for this study was due to their high sensitivity to diverse diets (Akande *et al*. 2010) and based on their digestive physiology, rats are good representative of other monogastric animals such as rabbits, pig and guinea pigs. At the moment, information on the effects of feeding raw *Senna obtusifolia* seed meal on the haematological and biochemical properties of albino rats seems to be scanty therefore, there is the need to conduct more studies to bridge this information gap. In view of the above, this study was designed to investigate the effect of feeding raw *Senna obtusifolia* seed meal on haematological and biochemical indices of albino rats.

Materials and Methods

Location of the study area

The study was conducted at the Animal House, Department of Biological Sciences, Adamawa State University, Mubi, Nigeria. The area lies between latitudes 9^0 $30'$ and 11^0 North of the equator and longitude 13^0 $45'$ East of the Greenwich meridian. The area has minimum and maximum temperatures of 12.7^0 and 37^0 and a minimum and maximum of 37.3 mm and 238.7 mm of mean monthly rainfall (Adebayo, 2004).

Chemical analysis

The proximate composition and levels of anti-nutritional factors of raw *Senna obtusifolia* seed and the experimental diets were determined according to the standard procedure of AOAC (2004).

Experimental diets and treatments

Five experimental diets were compounded and RSOSM was incorporated at 0, 5, 10, 15 and 20%

levels making five (5) experimental diets designated T1, T2, T3, T4 and T5, respectively (Table 1).

Experimental animals and their management

A total of 60 albino rats of mixed sexes with average weight of 70.52 to 81.05 g were used for the experiment. The rats were managed in constructed metal cages. Measured quantity of the experimental diets and water were given to the rats *ad libitum*.

Experimental Design

The 60 albino rats were randomly allotted to the five (5) dietary treatments in a randomized complete block design with three replicates of four (4) rats each.

Blood collection/analysis

At the end of the experiment, blood samples were randomly collected from three albino rats in each treatment using the standard procedure of blood collection and analysis as described by Ochei and Kolhatkar (2000). Blood samples for haematological assay were collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant treated bottles, while blood samples for biochemical indices were collected in EDTA free bottles. The blood samples were analysed for packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) haemoglobin (Hb), Total protein, albumin, globulin, cholesterol, bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Erythrocyte indices (mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV)) were calculated using the formulae of Jain (1986) as follows:

$$MCHC = \frac{Hb(g/dl)}{PVC(\%)} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$MCH(fl) = \frac{Hb(g/dl)}{RBC(\times 10^{6} UL)} \times 10 \ldots\ldots\ldots (2)$$

$$MCV(pg) = \frac{PVC(\%)}{RBC(million)} \times 10 \ldots\ldots\ldots\ldots (3)$$

Table 1: Ingredient Composition and Calculated Analysis of the Experimental Diets

	Inclusion levels of raw *Senna obtusifolia* seed meal (%)				
Ingredient (%)	**T1 (0)**	**T2 (5)**	**T3 (10)**	**T4 (15)**	**T5 (20)**
Maize	49.00	48.00	48.00	48.00	48.00
Maize offal	13.85	13.85	12.90	11.90	7.65
Soya bean	17.80	13.80	9.75	5.75	5.00
Groundnut cake	16.00	16.00	16.00	16.00	16.00
RSOSM	0.00	5.00	10.00	15.00	20.00
Salt	0.30	0.30	0.30	0.30	0.30
Bone meal	2.50	2.50	2.50	2.50	2.50
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.15	0.15	0.15	0.15	0.15
Premix	0.20	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude protein (%)	19.67	19.39	19.45	18.57	18.95
Crude fibre (%)	4.08	4.35	4.65	4.88	4.95
**Energy(kcal/kg)	2957.73	2936.63	2913.45	2906.67	2904.90

***Metabolizable energy (ME) calculated according to the formular of Pauzenga, (1985)*

ME= 37 × %CP + 81 × % EE + 35.5 × % NFE (Pauzenga, 1985)

Experimental animals and their management

A total of 60 albino rats were used for the experiment. The rats were managed in constructed metal cages. Known quantity of feed and water were given to the rats *ad libitum*.

Experimental Design

The 60 albino rats were randomly allotted to the five (5) dietary treatments in a randomized complete block design with three replicates of four (4) rats each.

Results and Discussion

The haematological parameters of albino rats fed raw *Senna obtusifolia* seed meal is presented in Table 2. The parameters were significantly ($P<0.05$) influenced by the experimental diets. The PCV, Hb, RBC and WBC were more depressed in albino rats fed 15 and 20% RSOSM. The values obtained for these parameters were observed to be lower than the normal ranges of 39.6 -52.5%, 13.7 – 17.6 g/dl, (7.27-9.65 x 10^6/ml) and (1.96-8.25 x 10^3/ml) for PCV, Hb, RBC and WBC, reported by Mary and Charles (2008) respectively. The depressive effect observed on these haematological values was attributed to the adverse effects of anti-nutritional factors such as tannins and saponins which affected the utilization of important nutrients such as protein. This could be an indication that RSOSM probably contain tannins and saponins in sufficient amount. Soetan and Oyewale (2009) also pointed out that anti-nutritional factors such as enzyme inhibitors, hemagglutinin factors, tannins and phytic acid inhibit the proteolytic activity of digestive enzymes such as pepsin and trypsin which consequently affected the digestion and availability of nutrients required for blood formation. This is in agreement with the report of Nwanjo *et al*. (2006) who explained that insufficient intake of protein can result to aneamia which will consequently reflect on the haematological parameters. Uhrmacher *et al*. (2010) explained that blood toxicity is usually accompanied with significant changes in the values of haematological values including falls in values of RBC, Hb, PCV and other RBC indices due to possible suppression of erythropoietic processes or haemolysis of the available RBC. Santosh and Richard (1998) further buttressed that saponins due to its amphiphilic properties can cause hemolysis of red blood cells. Haemolysis of red blood cells seems to result from saponin ability to form complexes with cell membrane cholesterol leading in consequence to pore formation and cell permeability and also to cause alteration in the negatively charged carbohydrate portion on the cell surface (Melzig *et al*., 2001; Gauthier *et al*., 2009). The findings of this study on a general note indicated poor erythropoietic processes and immune suppression in the rats especially in the groups fed 15 and 20% RSOSM.

The serum biochemical parameters of albino rats fed the experimental diets (Table 3) were significantly ($P<0.05$) affected. Serum albumin, globulin and total protein were observed to linearly decrease as the level of RSOSM increases in the experimental diets. The lowest values for albumin, globulin and total protein were recorded in albino rats fed 15% and 20% RSOSM. The values obtained were however; lower than the normal ranges of 3.4 to 4.8 g/dl, 1.5 to 2.5 g/dl and 5.2 to7.1 g/dl reported by Mary and Charles (2008). This is an indication of the adverse effects of anti-

nutritional factors on protein utilization and liver function. A persistently low level of albumin in serum signals reduced capacity of the liver and is a sign of progressive liver failure (Phillips and Best, 2004).

Table 2: Haematological Indices of Albino Rats Fed Raw *Senna obtusifolia* Seed Meal

	Inclusion level of RSOSM					
	T1(0)	**T2(5)**	**T3(10)**	**T4(15)**	**T5(20)**	**SEM**
Packed cell volume (%)	42.23	40.43	35.03	26.37	26.27	4.14^{*}
Haemoglobin (g/dl)	12.08	11.77	11.17	9.20	9.17	1.06^{*}
Red blood cell($x10^6$/ml)	6.80	6.51	5.56	4.50	3.87	0.71^{*}
White blood cells($x10^3$/ml)	7.83	7.30	10.30	4.93	4.37	2.23^{*}
MCV(fl)	62.10	62.11	63.00	58.60	67.88	2.50^{NS}
MCH(pg)	17.76^{b}	18.07^{b}	20.08^{a}	20.44^{a}	23.69^{a}	1.33^{*}
MCHC(%)	28.60^{b}	29.11^{b}	31.89^{a}	34.88^{a}	34.90^{a}	1.67^{NS}

MCV = mean corpuscular volume, MCH = corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RSOSM = raw Senna obtusifolia seed meal

Cholesterol levels of the albino rats fed the experimental diets were significantly ($P<0.05$) affected. The results indicated a decreasing trend as the level of RSOSM increases in the experimental diets. The decrease was attributed to the effects of anti-nutritional factors such as saponins in the seeds. Polyphenol compounds are believed to play a significant role in lowering plasma cholesterol by binding to the bile acids which in turn increase feacal loss (Zunft *et al*., 2003). This might be the reason for the low cholesterol levels in rats fed diets containing 15% 20% levels of RSOSM. Guyton and Hall (2006) further pointed out that secondary metabolic compounds can affect fat absorption and transport as well as break down of fat and cholesterol in the liver into bile salts and fat and cholesterol would subsequently excreted.

The serum urea of the albino rats was significantly ($P<0.05$) elevated in groups of rats in T4 and T5 compared to other treatment groups (T1, T2 and T3). This increase was attributed to the adverse effect of anti-nutritional factors such as tannins on protein quality and utilization which is consistent with the report of Iyayi and Tewe (1998) who pointed out that serum urea depends on both quality and quantity of protein supplied in the diet of an animal. Abiola *et al*. (2001) also added that an increase level of urea is linked to poor protein utilization. Kanetko (1989) further buttressed that high level of serum urea is connected to the presence of anti-nutritional factors which can lower the quality of protein indicating imbalance of amino acid in the diet which caused elevated blood urea concentration. Similar findings was reported by Augustine (2016) who similarly observe high serum uric acid in broiler chickens fed raw *Senna obtusifolia* seed meal.

The serum enzymes (AST and ALT) and total bilirubin were observed to be more elevated beyond the normal ranges of 74-134u/l, 18-45 u/l and 0.05-0.15mg/dl in group of rats fed 15 and 20% RSOSM. This is a clear indication of liver toxicity. Phillips and Best (2004) explained that increase levels of ALT and bilirubin

indicates that something is wrong with the liver which is in line with the findings of this study.

Table 3: Serum Chemistry Values of Albino Rats Fed Raw *Senna obtusifolia* Seed Meal

	Inclusion levels of RSOSM (%)					
	T1(0)	**T2(5)**	**T3(10)**	**T4(15)**	**T5(20)**	**SEM**
Total protein (g/dl)	5.05^{a}	5.03^{a}	4.53^{b}	3.18^{c}	3.27^{c}	0.38*
Albumin (g/dl)	3.46^{a}	3.38^{a}	2.87^{b}	2.40^{b}	2.67^{b}	0.20*
Globulin (g/dl)	1.61^{a}	1.65^{a}	1.66^{a}	0.78^{b}	0.60^{b}	0.09*
Cholesterol (mg/dl)	5.63^{a}	4.67^{ab}	4.23^{b}	3.93^{c}	3.63^{c}	0.51*
Urea (mmol/l)	3.60^{c}	3.15^{c}	4.27^{b}	5.08^{a}	5.76^{a}	0.31*
AST (IU/L)	37.84^{cd}	47.89^{d}	59.14^{c}	94.35^{b}	149.67^{a}	4.81*
ALT (IU/L)	30.89^{c}	30.24^{c}	35.48^{b}	37.47^{b}	48.20^{a}	1.99*
TB (mmol/l)	0.52^{c}	0.66^{bc}	0.99^{b}	1.32^{a}	1.37^{a}	0.15*

ALT = alanine aminotransferase, AST = aspartate aminotransferase, TB = total bilirubin, RSOSM = raw Senna obtusifolia seed meal

Conclusion

At the end of this investigation, it was concluded that inclusion of RSOSM beyond 5% revealed some adverse effects on both haematological and biochemical parameters of albino rats. Furthermore, toxicological evidences and poor protein utilization were more prominent in the groups of albino rats fed 15 and 20% raw *Senna obtusifolia* seed meal. Thoroughly processing of *Senna obtusifolia* seed is therefore recommended before incorporation in the diets of albino rats and other similar monogastric animals.

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