DOI: 10.2478/arl-2020-0011
Research Article

Ziziphus mauritiana* (Jujube) Seed as a Protein Source in the Diet Promote Growth Performance and Stabilized Hematology, Lipid Profile and Serum Chemistry Profile of *Rattus norvegicus

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Received March, 2020; Revised April, 2020; Accepted May, 2020

Abstract

The increase search for easily available and cheaper alternative sources of proteins to meet up the increase protein demand necessitated the present study which was design to assess the effects of graded levels of jujube (*Ziziphus mauritiana*) seed inclusion in the diets of weaners rats. Twenty-five weaner rats were allocated into five groups of formulated diet consisting of treatment 1 - 5 in which soya bean was replaced with *Ziziphus mauritiana* seed at 0% (control), 25%, 50%, 75%, and 100% respectively for 3 weeks. Effects of the diets on lipid profile, haematology and serum chemistry were evaluated. Rats fed 25%, 50% and 100% *Z. mauritiana* had significantly higher feed intake and weight gain when compared with the normal control. All levels of inclusion tested increases the concentrations of serum total proteins and bilirubin but decreases the cholesterol and platelet counts when compared with the control. No diet related alterations were recorded for the relative organ's ratio, aspartate transaminase, alanine transaminase, albumins, urea, creatinine, sodium, chloride, high- and low-density lipoprotein, triglyceride and hematological indices analyzed. However, seed inclusion only at 100% significantly ($p < 0.05$) increases the total white blood cell count and relative liver weight ratio. *Ziziphus mauritiana* seed possess good dietary protein quality for optimal growth of rats and does not elicit any adverse effect on haematological and serum biochemical indices of organs integrity.

Key words: *Ziziphus mauritiana*, hematology, lipid profile, serum chemistry, diet.

Introduction

Malnutrition is one of the main causes of high infant mortality in developing countries especially in sub-saharan Africa [1]. According to WHO [2], about 30% of the global population are malnourished resulting in about daily mortality of 40,000 children [2]. Self-sufficiency in food production in order to equilibrate food demand with supply and reduced malnutrition is one of the main goals of both state and federal government of Nigeria [3].

The consumption of animal protein sources in developing countries is ever increasing, however, the demand for animal protein sources in Nigeria is higher than the supply [4]. This has strongly obliged the necessity to explore easily available and cheaper alternative sources of proteins as a means of alleviating the overwhelming shortage of animal protein [5].

Ziziphus mauritiana Lam (family; Rhamnaceae) also known as Jujube is a tropical fruit tree that grows in arid and semiarid regions of the tropics [6], the plant is also called magarya by traditional herbalist of northern Nigeria [7] where it is commonly used for the treatment of convulsion, epilepsy, diarrhea, sexual disability, ulcers, digestive and urinary discomfort, cough, fever, sleep disorders, obesity, burning sensations,

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fever, skin disease, wound, ulcers and stomatitis [8]. Various parts of *Ziziphus mauritiana* have also been reported for immunomodulatory [9], antioxidant [10], anticancer [11], anti-diarrhoeal [12] hypoglycemic, hypolipidemic [13], antiulcer [14], antimicrobial [15], and antiplasmodial [16] activities.

However, despite the medicinal properties of *Ziziphus mauritiana*, its seed is less explored, underutilized and are often discarded as a waste. However, recent studies have indicated that the less underutilized seed could serve as a rich source of nutrient thus contribute to solving the problems of malnutrition. To this end the present study investigated the effect of replacements of dietary proteins with *Ziziphus mauritiana* seed on growth performance, hematology, lipid profile and serum chemistry of albino rats.

Material and Methods

Sample collections

Matured fruits of *Ziziphus mauritiana* fruits were picked directly from the trees in March, 2017 from Barnawa area of Kaduna, Kaduna State. The sample was identified and authenticated at the herbarium unit of Department of Biological Sciences Ahmadu Bello University where existing voucher number of the specimen (No. 7072) was given.

Chemicals and Reagent

Biochemical assay kits including, AST, ALT, ALP, Total proteins, albumin, urea and creatinine were

product of Randox Liquizyme assay kits. Total cholesterol, triglycerides and high-density lipoprotein (HDL)-cholesterol kits were product of agape diagnostic ltd.

Experimental animals

Twenty-five (25) weaners rats weighing 60 ± 5.45 g (4-5 weeks old) were obtained from Animal Holding Unit, School of life science, Federal University of Technology, Minna, Nigeria. Animals were kept and used in compliance with internationally accepted principles for human handling and use of laboratory animals as contained in the Canadian council on Animal Care Guidelines and Protocol Review.

Sample preparation

The fruits were macerated in water to remove the pulp and the seeds were rinsed in clean water. Thereafter the seeds were spread out and sun dried. The dried seeds were grounded and sieved through a mesh to obtain a fine powder which was stored in air tight container.

Experimental design and treatments

Five diets were formulated consisting of 5 treatments (A-E) in which soya bean was replaced with *Ziziphus mauritiana* seed at 0%, 25%, 50%, 75%, and 100% respectively as shown in Table 1. The animals were fed once (9:00 am) daily and water was given ad libidum. The amount of feed taken by the individual rats was estimated daily by deducting the left-over feed from the daily feed given.

Table 1.

Nutrient formulation

Ingredients (%)	A	B	C	D	E
Maize	450	450	450	450	450
Maize offal	350	350	350	350	350
Soya beans	100	75	50	25	00
<i>Z. mauritiana</i>	0	25	50	75	100
Palm kernel cake	40	40	40	40	40
Bone meal	20	20	20	20	20
Minerals	7.5	7.5	7.5	7.5	7.5
Methionine	2.5	2.5	2.5	2.5	2.5
Lysine	2.5	2.5	2.5	2.5	2.5
Vitamins	25	25	25	25	25
Salt	2.5	2.5	2.5	2.5	2.5

Blood collection and Serum Preparation

After 21 days of feeding trial, the animals were sacrificed under ether anaesthesia and blood was collected in EDTA bottle for haematological analysis. Another set of blood was collected in

EDTA free sample bottle, the blood was allowed to clot and centrifuge at 3000rpm for 10 minutes [17-18]. The plasma samples were collected and kept in a freezer (-20°C) until needed for biochemical analyses.

Analysis of Biochemical and Haematology Parameters

The estimation of biochemical parameters including transaminases [19] total protein [20], albumin and bilirubin [21], urea, creatinine and electrolyte concentrations from serum were assayed using standard protocols [22]. Serum concentrations of lipid profile including total cholesterol, triglycerides and high density lipoprotein (HDL)-cholesterol were assayed by enzymatic colorimetric methods using commercial kits according to the manufacturer's instructions. VLDL-cholesterol was estimated as TG/5, and LDL-cholesterol was calculated using Friedewald formula [23] as follows:

$$\text{LDL (mg/dl)} = \text{TC} - (\text{HDL} + \text{VLDL})$$

The hematological components including hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) and platelet count (PLT) were determined using the automated hematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis [24].

Statistical Analysis

Statistical analysis system was used for data analysis. Mean $\pm$ SD were presented for each analysis while one-way analysis of variance and Duncan's Multiple Range Test were used for group comparison at $p < 0.05$ significance

Results

Feed intake and Weight gain

After three (3) weeks of feeding trials rats fed 75% *Z. mauritiana* proteins had the least feed intake (127.15 $\pm$ 0.78g) comparable with the normal control (121.70 $\pm$ 0.71g), rats fed 25%, 50% and 100% *Z. mauritiana* had significantly higher feed intake of 157.70 $\pm$ 5.09 g, 150.35 $\pm$ 0.71g and 145.45 $\pm$ 0.92g respectively (Table 2). Rats fed 50% and 100 % *Z. mauritiana* proteins had the

highest weight gain of 27.50 $\pm$ 0.00 g and 28.50 $\pm$ 4.92 g respectively when compared with the normal control (Table 3).

Table 2.
Weekly feed Intake of rats fed graded level of *Z. mauritiana* as protein source

Feed	Week1	Week2	Week3
0%	148.75 $\pm$ 0.35 ^d	132.85 $\pm$ 0.35 ^c	121.70 $\pm$ 0.71 ^a
25%	121.85 $\pm$ 0.71 ^b	182.45 $\pm$ 1.06 ^d	157.70 $\pm$ 5.09 ^c
50%	92.65 $\pm$ 0.71 ^a	152.05 $\pm$ 0.71 ^b	150.35 $\pm$ 0.71 ^c
75%	139.90 $\pm$ 5.52 ^c	122.55 $\pm$ 2.90 ^a	127.15 $\pm$ 0.78 ^a
100%	184.90 $\pm$ 1.56 ^e	147.00 $\pm$ 1.13 ^b	145.45 $\pm$ 0.92 ^b

Data are Mean $\pm$ SD of triplicate determination. Data followed by different superscript alphabet in each column are significantly different ($p < 0.05$)

Table 3.
Weight changes of rats fed graded level of *Z. mauritiana* as protein source

Feed %	Initial	Final	Weight gain
0	64.50 $\pm$ 17.68	82.50 $\pm$ 30.41	18.00 $\pm$ 24.40 ^a
25	80.00 $\pm$ 1.41	96.50 $\pm$ 3.54	16.50 $\pm$ 3.18 ^a
50	88.00 $\pm$ 0.00	115.50 $\pm$ 0.00	27.50 $\pm$ 0.00 ^b
75	94.50 $\pm$ 34.65	115.00 $\pm$ 12.75	20.50 $\pm$ 1.92 ^a
100	88.50 $\pm$ 12.02	97.00 $\pm$ 9.89	28.50 $\pm$ 4.92 ^b

Data are Mean $\pm$ SD of triplicate determination. Data followed by different superscript alphabet in each column are significantly different ($p < 0.05$)

Relative organs body weight

The relative organs body weight of the animal's kidney, lung, heart and spleen were not significantly ($p > 0.05$) different from the control values. However, the relative liver body weight ratio was significantly ($p < 0.05$) higher in rats fed 100% *Z. mauritiana* protein when compared with the control and other experimental groups (Table 4).

Table 4.
Relative organ weight (g) ratio of rat fed graded level of *Z. mauritiana* as protein source

Feed	Liver	Kidney	Lung	Heart	Spleen
0%	0.025 $\pm$ 0.19 ^a	0.005 $\pm$ 0.00 ^a	0.014 $\pm$ 0.00 ^a	0.003 $\pm$ 0.00 ^a	0.003 $\pm$ 0.00 ^a
25%	0.028 $\pm$ 0.00 ^a	0.005 $\pm$ 0.00 ^a	0.010 $\pm$ 0.00 ^a	0.004 $\pm$ 0.00 ^a	0.004 $\pm$ 0.00 ^a
50%	0.021 $\pm$ 0.01 ^a	0.006 $\pm$ 0.00 ^a	0.014 $\pm$ 0.00 ^a	0.004 $\pm$ 0.00 ^a	0.003 $\pm$ 0.00 ^a
75%	0.027 $\pm$ 0.28 ^a	0.005 $\pm$ 0.00 ^a	0.012 $\pm$ 0.00 ^a	0.004 $\pm$ 0.00 ^a	0.003 $\pm$ 0.00 ^a
100%	0.045 $\pm$ 0.00 ^b	0.005 $\pm$ 0.00 ^a	0.012 $\pm$ 0.00 ^a	0.004 $\pm$ 0.00 ^a	0.003 $\pm$ 0.00 ^a

Data are Mean $\pm$ SD of triplicate determination. Data followed by different superscript alphabet are significantly different ($p < 0.05$).

Biochemical

The serum activities of aspartate transaminase, alanine transaminase (figure 1) and the concentrations of albumins (figure 2), urea (figure

3), creatinine (figure 4), sodium and chloride (figure 5) in rats fed 25%, 50%, 75% and 100% *Z. mauritiana* proteins were not significantly ($p>0.05$) different from the control values.

Figure 1. Effect of graded level of *Z. mauritiana* inclusion as protein source on transaminase activities in rats

Figure 2. Effect of graded level of *Z. mauritiana* inclusion as protein source on albumin and total proteins concentration in rats

Figure 3. Effect of graded level of *Z. mauritiana* inclusion as protein source on urea concentration in rats

Figure 4. Effect of graded level of *Z. mauritiana* inclusion as protein source on creatinine and bilirubin concentrations in rats

Figure 5. Effect of graded level of *Z. mauritiana* inclusion as protein source on sodium concentration in rats

Figure 6. Effect of graded level of *Z. mauritiana* inclusion as protein source on lipid profile in rats

The serum concentrations of high-density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride in rats fed 25%, 50%, 75% and 100% *Z. mauritiana* proteins were not significantly ($p>0.05$) different from the control values. However, the cholesterol concentrations were significantly ($p<0.05$) lower in rats fed 25%, 50%, 75% and 100% *Z. mauritiana* protein when compared with the control value (figure 6). When compared with the normal control, the concentrations of total proteins were higher in rats fed 25%, 50%, and 100% *Z. mauritiana* proteins but lower in rats fed 75% *Z. mauritiana* proteins. Similarly, bilirubin was higher in rats fed 25%, 75%, and 100% *Z. mauritiana* proteins when compared with the control.

Hematology

The hematological parameters including hemoglobin, packed cell volume, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and red blood cell in rats fed 25%, 50%, 75% and 100% *Z. mauritiana* feed were not significantly different ($p<0.05$) when compared with the normal control. The platelet counts were significantly lowered in rats fed 25%, 50%, 75% and 100% *Z. mauritiana* feed when compared with the control, while total white blood cell count was higher only in rats fed 100% *Z. mauritiana* feed proteins when compared with the control (Table 5).

Table 5.
Hematological parameters of rat fed graded level of *Z. mauritiana* as protein source

%	HB	PCV	MCH	MCH	MCHC	RBC	PLT	TWBC
0	14.55±0.64 ^a	43.50±2.12 ^a	53.00±0.00 ^a	17.00±1.42 ^a	31.00±2.82 ^a	8.80±0.42 ^a	467.43±9.56 ^b	7.32±0.43 ^a
25	13.30±0.42 ^a	40.00±0.00 ^a	55.00±1.41 ^a	17.50±2.12 ^a	30.00±2.83 ^a	7.75±0.92 ^a	215.50±12.53 ^a	8.10±1.41 ^a
50	13.75±1.06 ^a	41.50±3.54 ^a	57.50±2.12 ^a	17.00±1.42 ^a	28.50±2.12 ^a	8.10±0.00 ^a	215.00±73.54 ^a	7.60±0.54 ^a
75	14.30±0.42 ^a	43.50±2.12 ^a	58.00±5.66 ^a	18.50±0.71 ^a	31.50±0.71 ^a	7.70±0.71 ^a	286.50±54.45 ^a	7.20±0.69 ^a
100	14.20±1.27 ^a	44.50±3.54 ^a	59.50±4.95 ^a	18.00±1.41 ^a	33.00±4.24 ^a	7.95±0.21 ^a	287.00±15.56 ^a	13.10±1.42 ^b

Discussions

Analysis of hematological parameters can be used to assess the deleterious effects of a test substances on the blood cells and also served as a useful predictor of nutritional and malnutrition status of organism exposed to the test food substances [25], to this end, it was necessary to determine the effect of the *Z. mauritiana* as a protein source on hematological parameters. Consequently, the present study showed that *Z. mauritiana* feed at 25%, 50%, 75% and 100% proteins did not induce obvious toxic changes in the red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) of rats. The absence of significant changes on these indices may suggest that there were no destruction of matured RBC's and no change in the rate of production of erythrocytes (erythropoiesis[26-27]). It also indicates that the test protein source did not possess toxic substances that can cause an anemic condition in rats. The significant increase in the total WBCs observed in rats fed with *Z. mauritiana* as 100% proteins could be attributed to the immunological responses of the animals in response to the stress

induced by the components of the *Z. mauritiana* which stimulate leucopoiesis in the bone marrow. However, this property could be useful in enhancing defense system of animals and will thus increase the animal's capability to fight infection [28].

Alteration in biochemical and hematological parameters has been widely accepted and easily assessable indicator of organs impairment and other detrimental effect of drug or food substance consumed to the body systems [29]. Alanine aminotransferase and aspartate aminotransferase are liver based biochemical enzyme that plays important role in protein metabolism. These enzymes are release to serum at certain concentrations, however, when liver is impaired, high amount of these enzymes find their way into the serum, through leakage [30]. These enzymes therefore, become a valuable tool in clinical diagnosis of pathological damage to liver [31]. Consequently, the serum AST and ALT levels were not increased by feeding rats with *Z. mauritiana* as 25%, 50%, 75% and 100% protein source and this indicated that the activity of the functional integrity of the liver was preserved. These also indicated that the amino acid

metabolism and energy generation of the animals has not been compromised.

The observed increase in the *total proteins*, contents in rats fed 100% *Z. mauritiana* suggests a compromise of the synthetic ability of the liver. This 100% *Z. mauritiana* protein source might have increased the functional activity of the liver by interfering with the equilibrium in the rate of synthesis and destruction, removal or clearance of total proteins from the system of the animals [32]. Such increase in total protein could, however, lead to dehydration which is detrimental to cellular homeostasis [33]. This will negatively affect the metabolic activities of the liver and consequently the health of the animals. However, no significant differences in the *serum total proteins, concentrations* in rats fed 25%, 50% and 75% *Z. mauritiana* protein source when compared with the control. This indicates that the activity of the liver was preserved. Albumin is a protein synthesized by the liver and plays a varieties of roles such as translocations of various hormones, minerals, fatty acids and enzymes [34]. Thus commonly used to assess nutritional status and liver integrity [35-36]. In the present study, no feeding related change in albumin levels an indication of adequate nutritional status and preserved liver integrity

The kidneys control the excretion of urea, creatinine and reabsorption of electrolytes into the blood. Defeat in activities of kidney will result in accumulation of electrolytes, urea and creatinine in the biological fluid [37]. The non-significant ($p>0.05$) changes in serum concentrations of sodium and chloride suggests that the normal excretion of these electrolytes by the kidney has not been compromised [38]. Urea and creatinine concentrations were also found not to be significantly altered thus suggesting that the functional integrity of kidney has been preserved and thus no renal dysfunction could be attributed to the feeding of rats with *Z. mauritiana* at 25%, 50%, 75% and 100% protein source

Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) are known as lipid profile and increase concentrations of these parameters suggest an abnormal lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases [39]. Thus, assessment of these lipid profile is crucial in evaluating nutritional adequacy and effect of food substance. Consequently, the lipid profile analysis showed that the mean cholesterol concentrations were significantly ($p < 0.05$) lower in rats fed *Z. mauritiana* at 25%, 50%, 75% and 100% protein source when compared with the control. This is an indication of hypocholesterolemic effect of *Z.*

mauritiana. In this study, no such alterations in the HDL-C, LDL-C and triglyceride concentrations were recorded after feeding rats with *Z. mauritiana* at 25%, 50%, 75% and 100% protein source and this supports the non-toxic nature of *Z. mauritiana*.

Body weight gain and internal organ weights are simple but strong and sensitive indices of toxicity after exposure to toxic substances [40]. Interestingly, rats fed 50% and 100 % *Z. mauritiana* proteins had the highest weight gain of 27.50 ± 0.00 g and 28.50 ± 4.92 g respectively when compared with the normal control indicating the inherent weight gaining potential of the feed. The observed significant weight gain could have been mediated via appetite stimulatory or other mechanisms. This correlate with the higher feed intake of 157.70 ± 5.09 g, 150.35 ± 0.71 g and 145.45 ± 0.92 g at 25%, 50% and 100% *Z. mauritiana* protein source. Changes in the relative organ weight are strong indications of organ enlargement (organomegally, hypertrophy or hyperplasia) or organs constriction (hypotrophy) as a secondary effect that follows biochemical alterations [27, 29]. Fortunately, the organ body weight ratio of rat kidney, lung, and heart compared favorably ($p>0.05$) with those of the controls and no significant change was observed at 25%, 50% and 75% *Z. mauritiana* protein source. However, the liver hypertrophy observed in rats fed 100% *Z. mauritiana* call for concern Since no major alterations were found in the organ weight, hematological and biochemical parameters. The results of this study have established that feeding of rats with *Z. mauritiana* at 25%, 50%, and 75% has no adverse effect on the functions of the liver, kidney and bone marrow in albino rats.

Conclusions

Ziziphus mauritiana seed possess good dietary protein quality for optimal growth of rats and does not elicit any adverse effect on haematological and serum biochemical indices of organs integrity thus could be considered a potential and safe source of proteins for proper growth.

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