Effects of Moringa Oleifera seed meal-based diet on selected Biochemical Parameters in Albino Rats

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ABSTRACT

Background: *Malnutrition is a major societal issue. Cheap and readily available protein from plants source (such as Moringa oleifera) can be helpful in solving the problem.*

Objective: *The effect of Moringa oleifera seed meal-based diet on the serum proteins profile, triacylglycerol, cholesterol level, and organ to body weight ratio in rats was investigated in this study.*

Methods: *Fifteen weanling albino rats averagely weighing 45.85 ± 0.13 g were randomized into 3 groups comprising of five animals each. Group 1 (control) was fed with a soybean meal-based diet, Group 2 and 3 were fed with raw and defatted Moringa oleifera seed meal-based diet respectively. Daily feed intake and weekly bodyweight of the animal were monitored.*

Results: *The result revealed no significant difference ($p>0.05$) in serum total protein, total and conjugated bilirubin of experimental rats fed the defatted Moringa oleifera seed meal-based diet when compared with the control but with a significant increase ($p<0.05$) in those fed with the raw Moringa oleifera seed meal-based diet. There was a significant reduction ($p<0.05$) in the globulin concentration of Group 3 and Group 2. There was no significant difference ($p>0.05$) in the triacylglycerol, cholesterol level, and the organ-body weight ratio of rats fed with the raw and defatted Moringa oleifera seed meal-based diet.*

Conclusion: *The result obtained suggests that the defatted Moringa oleifera seed meal-based diet compared favourably with the control and could be used as a good source of protein in animal diet.*

Keywords: *Moringa oleifera, soybeans, serum proteins profile*

Introduction

Plant foods are generally the most important source for meeting the nutritional needs of the majority of the population in Nigeria as well as most developing countries [1]. This is because they are cheap and easily available. Legumes, vegetables, roots, and tubers as well as cereals make up the major sources of nutrients [2].

Moringa oleifera seed is a leguminous seed, which is native to India and available in Nigeria. It is acclaimed to be rich in protein and may be of use as a good protein source particularly when the oil has been extracted [3]. It is the most widely cultivated species of the genus, Moringa, which is the sole genus in the Moringaceae family and it has thirteen (13) species among which M. oleifera is the most widely cultivated. The plant is also known for its high nutrient content and exceptional medicinal values.

Moringa oleifera has become an alternative to some leguminous seeds as a source of high-quality protein, oil, and antioxidant compounds [4]. *Moringa oleifera* seed meal has high essential amino acid content, except for lysine (0.015.3g/g protein), threonine (0.030.8g/g protein), and valine (0.043.5g/g protein)), which are present in lower levels than those recommended for 2 to 5-year-old children [5]. The high methionine and cysteine (0.043.6g/g protein) contents are close to those of human and cow milk and chicken eggs [6]. This abundance of essential amino acids encourages using the seeds as an excellent food substitute for legumes, which are usually poor in sulfur-containing amino acids. In this study, we try to explore the effect of *Moringa oleifera* seed meal-based diet on some biochemical markers of liver function as well as its use as an alternate source of plant protein.

MATERIALS AND METHODS

Source, Collection, and Treatment of Plant Materials

Moringa oleifera seeds used were bought from Gadagaya in Kaduna state, and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin with a voucher authentication number I.U.H.V.No.13.The *Moringa oleifera* seeds were oven-dried at 60 °C until, thereafter the seeds were blended and also oven-dried to obtain a constant weight using the magic blender, SHB-515 model (made by Sorex Company Limited, Seoul, Korea). For the defatted, the milled seeds were defatted using the batch solvent method.

Experimental Animals and Treatments

Fifteen (15) Albino rats (*Rattus norvegicus*) of weanling ages with an average weight of (45.85±0.13) g were used for the experiment. The environment was kept clean and disinfected. The rats were acclimatized for one week giving them the standard rodent diet and water *ad libitum*. The initial weights of the rats were taken before the commencement of the study. The rats were randomly distributed into three treatment groups of five rats each. Group 1 consists of animals fed soybean meal-based diet (control). Group 2 consists of animals fed raw *Moringa oleifera* seed meal-based diet. Group 3 consists of animals fed defatted *Moringa oleifera* seed meal-based diet.

Formulation of Diet

The feed ingredients were weighed out and thoroughly mixed manually until it was homogenous. The feeds were then made into pellets and packed inside polythene bags, labeled, and stored in a plastic bucket with a cover at room temperature. The composition (g/Kg) of the diet is as shown in Table 1. After formulation of the diets, the proximate composition of the diets was determined using the method described by [7].

**Vitamin/Mineral mix: Vitamin A 4,000,000 i.u; Vitamin D3, 800,000 i.u; Tocopherols, 400 i.u; Vitamin K_3 800mg, Folacin, 200mg; Thiamine, 600mg; Riboflavin 1,800mg; Niacin, 6000mg; Calcium pathothenate, 4mg; Biotin, 8mg; Manganese, 30,000mg, Zinc, 20,000mg; Iron, 8,000mg; Choline chloride 80,000mg; Copper, 2,000mg; Iodine, 480mg; Cobalt, 80mg; Selenium, 40mg; BHT, 25,00mg Anticaking agent 6000mg.*

Collection and Treatment of Blood Samples:

The rats were fed for six weeks and weighed every week. In the sixth week, the rats were anesthetized with Diethyl ether and sacrificed by simply incising the jugular vein. Blood samples were collected into plain
sample tubes for serum analysis.

Methods

Proximate composition was carried out by the standard method described by [8]. Protein, albumin, bilirubin, total cholesterol, triacylglycerol, oxalate, tannins and phytate were determined as described by Gornall *et al*. [8], Doumas *et al*. [9], Doumas *et al*. [10], Fredrickson *et al*. [11], Tietz [12], Ukpabi & Ejidoh [13], Josyln [14], and Wheel & Ferrel[15] respectively.

Animal Ethics

The research study adheres strictly and conforms to the Principles of Laboratory Animal Care (NIH Publication No. 85-23).

Statistical Analysis

All data are expressed as the mean of five replicates $\pm$ standard error of the mean (S.E.M)/Standard deviation (SD). Statistical evaluation of data was performed by SPSS version 16.using one-way analysis of variance (ANOVA), followed by Dunnett's posthoc test for multiple comparisons. Values were considered statistically significant at $p<0.05$ (confidence level = 95%).

RESULTS

Proximate Composition of Formulated Feeds

The percentage composition of protein, lipid, ash, crude fibre, moisture, and carbohydrate in the formulated feeds is presented in Table 2.
There was no significant difference ($p>0.05$) in

Table 1: Composition of Formulated Diets (g/Kg)

Ingredients	Control	Raw *M. oleifera* seed Meal-Based Diet	Defatted *M. oleifera* Seed meal-based Diet
Cornstarch	516	516	516
Soybean protein	250	-	-
Raw *M. oleifera* protein	-	250	-
Defatted *M. oleifera* protein	-	-	250
Soybean oil	40	-	40
Moringa oil	-	40	-
Fibre	40	40	40
Sucrose	100	100	100
*Vit/Minerals	50	50	50
DL-methonine/Lysine	4	4	4

Table 2: Proximate Composition of Formulated Feeds

Constituents (%)	Control	Defatted *Moringa oleifera* seed meal	Raw *Moringa oleifera* seed meal
Lipid	5.83 ± 0.22[a]	6.00 ± 0.12[a]	6.05 ± 0.04[a]
Protein	15.53 ± 0.53[a]	15.31± 0.31[a]	15.93 ± 0.70[a]
Moisture	7.20 ± 0.20[a]	6.80 ± 0.20[a]	6.80 ± 0.20[a]
Ash	8.00 ± 1.00[a]	8.00 ± 1.00[a]	7.50 ± 0.50[a]
Crude fibre	3.00 ± 0.50[a]	3.00 ± 0.50[a]	2.83 ± 0.45[a]
Carbohydrate	62.44 ± 0.4[a]	60.89 ± 0.23[a]	60.8 9± 0.21[a]

Values are mean of 3 Determinations ± SD.

Table 3: Antinutrients determined in *Moringa oleifera* seeds

Groups	Oxalate (mg/100g)	Phytate (Mol/Kg)	Tannin
***Moringa oleifera* seed(Raw)**	$(8.37 \pm 0.07)\times10^{-5}$	3.98 ± 0.02	1.5 ± 0.1
***Moringa oleifera* seed(Defatted)**	$(2.89 \pm 0.01)\times10^{-4}$	8.48 ± 0.02	1.8 ± 0.2

Values are mean of 3 Determinations ± SD

Table 4: Feed Intake and Growth Rate of Rats Fed with *Moringa oleifera* Seed Meal-Based Diet

Parameters	Control	*M. oleifera* Seed meal (Defatted)	*M. oleifera* Seed meal (Raw)
Initial weight(g)	45.88 ± 0.26 [a]	45.97 ± 0.35 [a]	45.71 ± 0.61 [a]
Final weight(g)	69.36 ± 1.08 [a]	60.42 ± 0.95[b]	53.35 ± 1.25[c]
Body weight gain(g)	23.48 ± 0.66 [a]	14.45 ± 0.67[b]	7.64 ± 0.59 [c]
Feed Intake(g)	40.00 ± 2.00[a]	42.00 ± 2.00 [a]	47.00 ± 7.00 [a]

Values are mean of 5 Determinations ± SD.
Row values with different superscripts are significantly ($p<0.05$) different.

Table 5: Concentration of Serum Protein Profile, Bilirubin and selected lipids profile of Rats Fed on *Moringa oleifera* Seed Meal-Based Diet

Tissues	Control	Defatted *Moringa oleifera* seed meal	Raw *Moringa oleifera* seed meal
Total protein(mg/ml)	48.38 ± 1.72[a]	54.38 ± 3.96[a]	72.60 ± 2.91[b]
Albumin (g/L)	10.93 ± 1.25[a]	40.86 ± 4.81[b]	53.49 ± 1.43[b]
Globulin (g/L)	37.44 ± 1.09[a]	13.52 ± 1.29[b]	9.12 ± 2.15[c]
Total Bilirubin(μmol/L)	4.44 ± 0.35[a]	3.69 ± 0.02[a]	27.01 ± 1.72[b]
Conjugated Bilirubin(μmol/L)	3.33 ± 0.74 [a]	2.03 ± 0.56[a]	14.08 ± 1.64[b]
Cholesterol (mMol/L)	30.24 ± 2.06 [a]	33.60 ± 2.10 [a]	29.10 ± 2.45 [a]
Triglycerides (mMol/L)	12.38 ± 1.50 [a]	10.61 ± 0.44 [a]	11.72 ± 0.44 [a]

Values are mean of 5 Determinations ± SEM.
Row values with different superscripts are significantly ($p<0.05$) different

the proximate composition of the *Moringa oleifera* seed meal-based diet when compared to the control**.**

Antinutrients Composition of the Formulated Diet
The levels of oxalate, Phytate and tannins in the *Moringa oleifera* defatted seed meal is high relative to the raw *Moringa oleifera* seedmeal; the result is presented in Table 3.

Feed Intake and Growth Rate of Rats Fed with *Moringa oleifera* Seed Meal-Based Diet
There was no significant difference ($p>0.05$) in the feed intake of all experimental animals, but there was a decrease in the body weight gain of the rats fed the defatted *M. oleifera* and the raw *M. oleifera* seed meal-based diet when compared with the control. The result for the feed intake and growth rate of rats fed *M. oleifera* seed meal-based diet is presented in Table 4.

Serum Protein Profile and Bilirubin levels of Rats Fed on *Moringa oleifera* Seed Meal-Based Diet
There was no significant difference ($p>0.05$) in the serum total protein for the rats fed the defatted *M.oleifera* seed meal-based and there was a significant increase ($p<0.05$) in the serum total protein of rats fed the raw *M.oleifera* seed meal-based diet when compared with the control.

There was a significant increase ($p<0.05$) in the serum albumin concentration of the rats fed with the defatted *M.oleifera* seed meal and raw *M. oleifera* seed meal-based diet when compared with the control.
There was a significant decrease ($p<0.05$) in the serum globulin concentration of the rats fed with the raw *M. oleifera* seed meal-based diet which was raised in the defatted *M.oleifera* seed meal-based fed rats compared with the control.
There was no significant difference ($p> 0.05$) in the serum total and conjugated bilirubin concentration of the rats fed with the defatted *M. oleifera* seed meal-based diet when compared with the control, but there was a significant increase ($p<0.05$) in the serum total and conjugated bilirubin concentration of the rats fed with the raw *M. oleifera* seed meal-based diet when compared with the control. This is presented in Table 5.

Serum Lipids of Rats Fed with *Moringa oleifera* Seed Meal-Based Diet

There was no significant difference ($p>0.05$) in the serum cholesterol and Triacylglycerol concentration of the rats fed with defatted *M.oleifera* seed meal and raw *M.oleifera* seed meal based diet when compared with the control. This is presented in Table 5.

DISCUSSION

The percentage composition of the formulated diet indicates that the constituents of both the control and experimental diets were not different quantitatively. This implies that any differences which may eventually emanate from the observation of the rats fed with the feed samples could be attributed to the quality.
The significant reduction observed in the growth rate of rats fed on raw and defatted *M. Oleifera* seed meal-based diet may be due to poor utilization of the nutrient in the feed intake, which might be due to the presence of antinutrients that may reduce the bioavailability of the nutrients in the feed. This could be as a result of the quantity of antinutrient factors such as tannins, phytates and oxalates (the only antinutrient carried out in this study) present in the defatted *M. oleifera* seeds which rendered unavailable proteins and minerals content of the seeds. Tannins have been reported to decrease protein quality by decreasing digestibility and palatability [16]. It is possible that these antinutrient factors inhibit trypsin and pepsin with their protein complexing ability [17].
The concentration of proteins, bilirubin, and albumin in the serum is a biomarker of liver function that is used to predict the extent of liver damage [18]. The significant increase ($p<0.05$) in the serum total proteins of rats fed the raw *M. oleifera* seed meal-based diet might be an indication of increased protein intake which could have come from the diet.
Albumin is the major protein present within the blood and represents a reliable test to assess the degree of liver damage in animals. Albumin is synthesized by the liver and is a major protein that circulates in the bloodstream [19]. Low serum albumin has also been associated with low protein intake. The significantly higher concentration of the serum albumin in the rats fed the raw and the defatted *M.oleifera* seed-meal based diet is an indication that there was high protein intake. This observation signifies that the *M.oleifera* seed-meal-based diet is a rich source of plant proteins.
Albumin and globulin level partially represents the nutrition status and immune system [20]. Unlike albumin, globulins are partly synthesized by lymphocytes (γ-globulins/immunoglobulins) in addition to hepatocytes (α-globulins and β-globulins), although the predominant source of these proteins is the liver [21], [22]. The observed decrease in concentration of serum globulin in the rats fed raw *M.oleifera* seed meal-based diet and defatted *M.oleifera* seed meal-based diet might be a manifestation of hepatic toxicity induced by some toxic components of the formulated diet or impaired ability of lymphocytes to synthesize γ-globulins. A possible consequence of this outcome is impaired humoral immunity resulting from low levels of circulating immunoglobulins.
Bilirubin is an important catabolic product of the blood and its biological and diagnostic values have been established [23], [24]. It is removed from the blood by the liver; hence it is a good biomarker of liver function. Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake (as a result of liver disease).The increased level ($p<0.05$) of bilirubin in the serum of rats fed the raw *Moringa oleifera* seed meal-based diet indicates that the liver is affected. This is supported by [25] who reported that a rise in the concentration of serum bilirubin indicates liver damage since the liver serves as an excretory unit rather than a distributing unit for bilirubin. Low serum cholesterol and triacylglycerols reduce the risk of cardiovascular diseases and are among the health benefit attributed to soyabean [26]. The insignificant difference in cholesterol and triglyceride observed in the study may indicate that defatted and raw *Moringa oleifera* seed meal-based diet pose no risk of cardiovascular diseases.

Conclusively, the data obtained in the study showed that the defatted *M. oleifera* seed meal-based diet compared favourably with soybean seed meal which indicates that defatted *M. oleifera* seed meal can be used as a good source of protein if its antinutrients can be eliminated. However, changes in the biomarkers of liver function observed indicate the possible hepatoxicity of this diet perhaps due to inaccurate dosage. Further studies to determine the safety and efficacy of this formulation are warranted to fully explore its nutritional potential as a rich source of plant proteins.

Acknowledgements
I acknowledged the techincal assistance rendered by Technologists in the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State during the study.

Conflicts of interest:
Authors declared no conflict of interest.

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