Nutritional evaluation of *Moringa oleifera* leaf and the effect of its bio-fortification with animal feed on physical changes and organ weights in male albino rats

Abstract

Current research investigated the nutritional value of *Moringa oleifera* leaf. Four diets, different in their composition were used on sixteen male albino rats (n=4). Commercial vitamins and mineral premix (75.0 g) were used solely in diet 1; diet 2 contained 37.5 g of the vitamin-mineral premix and 37.5 g MOL. Diet 3 contained 19.0 g of vitamin-mineral premix and 56.0 g of MOL. Diet 4 contained only MOL (75.0 g) as the sole source of vitamins and mineral. Diets 1, 2, 3 and 4 were provided for groups A (control), B, C and D respectively. Nutritional evaluation of the *Moringa oleifera* leaf contained protein (28.23±0.02%), dry matter (25.56±05%), Calcium (723.01 ±0.11 mg), Magnesium (677.28±0.00 mg) and Zinc (214.51±0.02 mg). In relation to the respective diets on feed consumption, body weight gain and growth performance, results showed a significant decrease ($p < 0.05$) in dose dependent manners compared with control (A). Groups C and D showed significant decrease ($p > 0.05$) in efficiency of feed conversion when compared to control. The organs of all the test groups showed no significant difference ($p > 0.05$) in weight compared to control. Conclusively, the study suggests the use of MOL may be needful only as a supplement, condiment or ingredient to enrich diets with essential vitamins and minerals but not for growth or body weight gain.

Keywords: *Moringa oleifera* leave-meal, premix, vitamin-minerals and weight gain.

1.0 Introduction

In different parts of the world, *Moringa oleifera* (MO) is widely cultivated specie of *Moringaceae* (Serem *et al*., 2017). It is known by different names. Among the Yorubas, it is known as ewe igbal, the Igbos call it okwe oyibo, the Hausas call it zogale, the Fulani call it “gawara. In English language it is called many names (miracle tree, mother’s best friend, never die and Benzolive tree) (Ukachi *et al*., 2019). MO has been used as a regular ingredient in conventional foods in Nigeria and many other countries (Anwar and Bhanger, 2003), and according to Fuglie (2005), MO plant forms the basis for several nutritional programs in many poor countries by charitable organizations, given that the leaves of the trees contain essential nutrients. The leaves are considered to provide immense possibilities for those who are nutritionally challenged as it may be regarded as a protein and minerals supplement (Rajangam, 2011). The plant possesses the potential to improve blood supply, improve nutrition, and boost food security, and support sustainable land practice use (Price, 2007). Researchers at the Asian Vegetable Research and Development Center (AVRDC, 2006) reported that leaves from four (4) different moringa species (*Moringa oleifera*, *Moringa peregrina, Moringa stenopetala* and *Moringa drouhardii*) contained high levels of nutrients, vitamins and antioxidants (Price, 2007). Bureau of plant industry reported MO as an outstanding source of nutrients among the *Moringa* species. It's leaves (weight per weight) have the calcium equivalent of four times that of milk, the vitamin C content is seven times that of oranges, while its potassium is three times that of bananas, three

times the iron of spinach, four times the amount of vitamin A in carrots, and two times the protein in milk (Kamal, 2008). The nutritional and medicinal property of MO leaves suggest it as a good option for the replacement; hence this study investigated the nutritional composition of *Moringa oleifera* leave and the effect of its bio-fortification with animal feed on physical changes and organ weight.

Fig.1a. Fresh *Moringa oleifera leaf*

1b. *Moringa oleifera leave meal.*

2.0 Materials and Methods

2.1 Chemicals and reagents

Vitamins, mineral-premixes and other chemicals used for the study were of standard food grades.

2.2 Plant collection and preparation

Moringa oleifera fresh leaves were obtained from Oke-ijetu, Osogbo, Osun state, Nigeria. The harvested moringa leaves were air-dried until they were crispy. The crispy *Moringa oleifera* leaves were pulverized with a clean and dry electric blender (Model: DMN/DIC/PMT/02-03/206) to obtain *Moringa oleifera* leaf powder (MOLP).

2.3 Extraction for MO leaves for proximate, mineral and vitamins analyses

Moringa oleifera leave powder (5 g) was weighed and transferred to a 150 ml round flask, 10 ml of 33% potassium hydroxide, $KOH_{(aq)}$ and 40 ml absolute ethanol were added, and boiled at reflux for 30 mins. It was then cooled very rapidly, followed with the addition of 17 ml of 25% hydrochloric acid, HCl and cooled again. Petroleum ether (50 ml) was added, shaked vigorously for 3 mins and waited to complete separation of the two phases. The organic phase was

removed and filtered through anhydrous sodium phases, evaporated to dryness in vacuum at a temperature of 35 ^{o}C and concentrated until 1 ml was obtained.

2.4 Proximate analysis

Proximate chemical composition of the *Moringa oleifera* leaf sample was determined by adopting procedures of AOAC (2005).

2.5 Mineral analysis

Sample (1.0 g) was weighed and subjected to dry ashing in a well cleaned crucible at 550 ^{o}C in a muffle furnace. The resultant ash was dissolved in 5.0 ml of HNO_3/HCl/H_2O (1:2:3) and heated gently on a heating mantle until brown fumes disappeared. Distilled water (5.0 ml) was added to each of the sample in crucible and heated until colorless solution was obtained. The mineral solution was filtered into a 100.0ml volumetric flask through filter paper, and the volume was made to the mark with distilled water. The solution was analyzed twice for its elemental composition using parking Elmer 403 model of atomic absorption spectrophotometer.

2.6 Vitamins analysis

The general technique for determining vitamins can be used for biological liquids and plants or animal tissue. The general principle is the same but the extraction methods differ depending on the matter being analyzed. This analysis was conducted following the method of AOAC (2005).

2.6.1 Vitamin A

The aqueous phase obtained in 2.10.1 was concentrated in a vacuum, the extract was re-dissolved in a mixture containing chloroform and trifluoroacetic acid (4:1v/v). Absorbance was taken at 620 nm following the method of AOAC (2005).

2.6.2 Vitamins B

After the action of potassium ferricyanide in the presence of potash, on little quantity of the sample extract, fluorescence was determined using a 360 nm primary filter and a 460 nm secondary filter to determine the vitamins B1, 2 and 3 following the method of AOAC (2005).

2.6.3 Vitamin C

A solution of the sample was made and 20 ml of the solution was pipetted into a 100 ml volumetric flask and diluted to volume with the extracting solution (ethanol). It was then filtered through a fluted filter paper and suitable aliquot was titrated with the dyestuff solution following the method of AOAC (2005).

Blank correction was made for the volume of extracting solution involved. The ascorbic acid content was calculated as stated below.

Ascorbic acid (mg/100 g) = (x B) × (F/E) × (V/Y)

x = average ml for sample titration

B= average ml for blank titration

F = ml of ascorbic acid equivalent to 1.0 ml indophenol standard solution

E = volume of ascorbic acid/ml

V = volume of initial assay solution

Y = volume of sample aliquot titrated by analytical chemist (AOAC 2005).

2.7 *Moringa oleifera* meal preparation

The Blanch diet and premix were purchased from Blessing feed mill at Niyi Ibikunle store, Osogbo, Osun State, Nigeria. The *Moringa oleifera* powder was then mixed in different proportions of the rats' diet per test group before administration to the animals.

2.8 Experimental diets

The basic ingredients used for the formulated diets (per 25 kg of feed) used are shown in **Table 1**. Commercial vitamins and mineral premix were used solely in diet 1, diet 2 consists 37.5 g of the vitamin-mineral premix and the other half (37.5 g) replaced by *Moringa oleifera* leaf meal (MOL). Diet 3 consists 19.0 g of vitamin-mineral premix in diet and 56.0 g of MOL. Diet 4 contains only MOL (75.0 g) as the sole source of vitamins and mineral premix.

2.9 Experimental animals and design

Sixteen (16) male albino rats weighing 155.50 - 165.50 g were randomly distributed into four groups per treatment. Allocation to groups was based on initial weight of the rats and group mean weight. Rats were acclimatized for two weeks, after which group A (Control) was given diet containing 75.0 g of vitamin and mineral premix only, B, C and D were provided with diets containing 37.5 g vitamin-mineral premix + 37.5 g MOL, 19.0 g vitamin-mineral premix + 56.0 g MOL and 75.0 g MOL respectively, fed *ad libitum* for 21 days.

2.10 Housing and feeding

The rats were housed in metabolic cages and provided diets. Feeds were weighed each morning and fed in two portions (morning and evening) to minimize wastage. At the end of the day, feed left in the troughs were weighed and subtracted from the total weight of feed (a total of 160 g) provided for the day to get the daily feed intake. Average weekly feed intake for each of the rat groups was calculated for the entire experimental period. Water was provided *ad libitum* daily. Weights of all the animals were taken before and during the experiment, and records kept weekly, following Serem *et al*. (2017).

2.11 Determination of feed consumption

Feed consumption (FC) is the difference between quantity of the left over feed and quantity of the feed provided.

Feed consumption, FC (g) = quantity of feed provided (QFP) - quantity of left over feed (QLF)

2.12 Determination of body weight gain

The body weight gain (BWG) was determined weekly. It represents the difference between the weight of the current week (Wc) and that of the previous week (Wp). It is determined as follows:

Body weight gain (g) = weight of the previous week - weight of the current week.

2.13 Determination of efficiency of feed conversion: This is the quantity of feed required to effect one unit of body weight gain. It is calculated as follow:

Efficiency of feed conversion (EFC) = BWG/FC

2.14 Growth study

All animals were weighed before and during the experimental period. Their weights were taken daily and body weight gains were determined weekly.

4.0 Results

Table 1: Proximate, minerals and vitamins analyses of *Moringa oleifera* leaves per 100 g

Composition	Quantitative content
Proximate	
% Protein	28.23±0.02
% Moisture content	7.49±0.11
% Fat	5.62±0.01
% Ash content	4.29±0.15
% Crude fiber	0.95±1.25

% Carbohydrate	46.48±0.03
% Dry matter	25.56±0.05
Energy	250.20±0.05Kcal
Minerals	
Ca	723.01 ±0.11 mg
Mg	677.28±0.00 mg
Zn	548.51±0.01 mg
Na	214.51±0.02 mg
Vitamins	
Vitamin A	16.88±0.06 mg
Vitamin B1	2.65±0.01 mg
Vitamin B2	20.60±0.03 mg
Vitamin B3	9.20±0.03 mg
Vitamin C	30.10±0.08 mg

Values were expressed as mean of three determinations ± SEM.

Table 2: Effect of MOL on feed consumption (g) a: raw data; b: statistical data

a

Group	Week 1	Week 2	Week 3
A (control)	924	990	1044
B	920	966	982
C	894	956	934
D	513	537	616

b

Group	Week 1	Week 2	Week 3
A (control)	6.16± 0.17^{c}	8.30± 0.19^{e}	11.02± 1.11^{f}
B	6.14± 0.09^{c}	8.00± 0.11^{d}	8.00± 0.15^{d}
C	4.99± 0.01^{b}	7.97± 0.03^{d}	7.90± 0.09^{d}
D	4.00± 0.25^{a}	4.60± 0.02^{b}	5.10± 0.06^{b}

[b] Values were expressed as mean of three determinations ±

SEM. Differences were considered significant at $p < 0.05$. Different alphabets across the rows and down the columns represent significant difference.

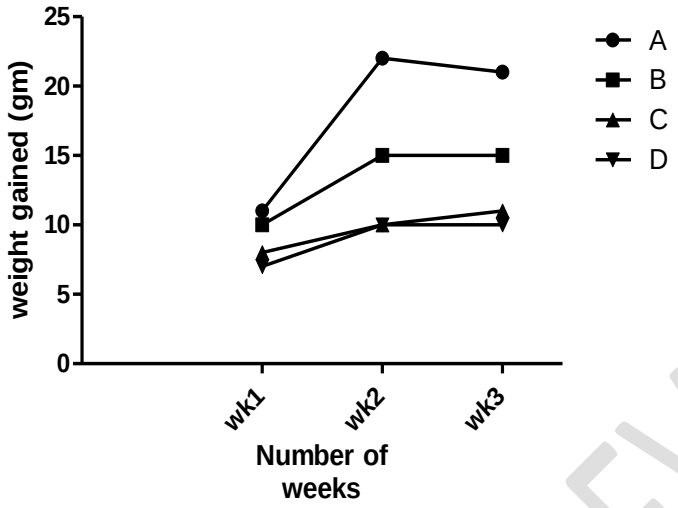

Fig 2: Effect of MOL on weekly body weight gain

Table 3: Effect of MOL on efficiency of feed conversion

Groups	Week 1	Week 2	Week 3
A (Control)	0.012 ± 0.05^{c}	0.012 ± 0.03^{c}	0.012 ± 0.05^{c}
B	0.012 ± 0.03^{c}	0.012 ± 0.00^{c}	0.012 ± 0.01^{c}
C	0.011 ± 0.05^{b}	0.011 ± 0.04^{b}	0.011 ± 0.06^{b}
D	0.010 ± 0.03^{a}	0.010 ± 0.01^{a}	0.010 ± 0.00^{a}

Values were expressed as mean of three determinations ± SEM. Differences were considered significant at $p < 0.05$. Different alphabets across the rows and down the columns represent significant difference.

Table 4: Effect of MOL on organ weight (g)

Rat organs	A	B	C	D
Liver, L	4.83±0.01	4.82±0.01	4.80±0.02	4.81±0.01
Heart, H	4.59±0.00	4.56±0.03	4.55±0.03	4.52±0.04
Kidney, K	4.81±0.03	4.86±0.01	4.77±0.05	4.81±0.01

Values were expressed as mean of three determinations ± SEM. Differences were considered significant at $p < 0.05$. Different alphabets across the rows and down the columns represent significant difference.

Table 1 showed the proximate, minerals and vitamins of MOL, with 25.56±0.05%, 46.48±0.03%, 28.23±0.02% and 5.62±0.01% dry matter, carbohydrate, protein and fat respectively. The mineral content of the leaves indicated that Ca (723.01±0.11 mg), Mg (677.28±0.00 mg), Zn (548.51±0.01 mg) and Na (214.51±0.11 mg) are the most abundant amongst other. Among the vitamins, Vitamins C and B2 are more abundant. Differences in contents could be attributed to differences in ecological zones, climatic conditions and the physiological stage of harvesting the *Moringa oleifera* plants.

Table 2 showed weekly feed consumption of all the rats. All groups showed remarkable weekly increase ($p < 0.05$) in feed consumption. Groups fed with diets containing MOLM showed a significant reduction ($p < 0.05$) in feed consumption, in dose manners compared with control (A).

In figure 2, no significant difference ($p > 0.05$) was observed in body weight gain in all the groups at week 1. A significant reduction ($p < 0.05$) was observed in body weight of MOLM fed groups at week 2 and 3 compared with control (A).

Table 3 showed no significant difference ($p > 0.05$) in the weekly efficiency of feed conversion of each group. Group B showed no significant difference ($p > 0.05$) at week 1, 2 and 3 when compared with control (A). Groups C and D showed significant decrease ($p < 0.05$) in efficiency of feed conversion at week 1, 2 and 3 when compared with control (A).

In Table 4, MOL showed no significant changes ($p > 0.05$) in organ weights of the rats treated with MOL in graded levels compared with control (A).

5.0 Discussion and Conclusion

Moringa oleifera leaves have been reported to be a rich source of minerals, vitamins and natural anti-oxidants (Jayanti *et al*., 2017). The use of inexpensive alternative substances from plant sources that possess pharmaceutical potentials which can be used as substitute to the costly modern vitamins and mineral supplements is thus essential (Yang *et al.,* 2006).

The proximate result, percentage (dry matter, proteins and ash contents) of MOL is collaborating with the findings of Patel *et al.* (2020) as 93.16, 24.44 and 7.44 respectively. The crude protein content of MOL in this study was close to 27.51% reported by Oduro *et al*. (2008), and 29.55% recorded by Nuhu (2010) in Ghana but higher than 23.30% reported by Gakuya *et*

al. (2014). Some of the minerals and vitamins present in MOL are collaborating with the findings of Zanum *et al*. (2012); Madukwe *et al*. (2013); Gakuya *et al*. (2014); Patel *et al.* (2020).

This study revealed a remarkable decrease ($p < 0.05$) in the feed consumption of rats fed with MOL, particularly at graded levels of 56.0 and 75.0 g, at week 2 and week 3 as compared with control (A). The study was in agreement with Kout *et al.* (2015) who observed lowered cumulative feed consumption at graded levels of MOL (0.0, 0.2, 0.4 and 0.6%) in broilers. This may simply imply that MOL did not enhance the feed intake of the rats, which may be due to the taste of MO leaves in the diets in which the rats could possibly deter from the feed.

Our findings showed that MOL has a body weight reducing potency. This is in contrast with the findings of Okafor *et al.* (2014); Banjo (2012); Gadzirayi *et al.* (2012); Kout *et al.* (2015) who reported that *M. oleifera* supplemented groups recorded a higher daily weight gain and showed that birds fed on moringa leaf powder gained significantly higher body weights than birds fed with control diet respectively. Our study thus suggests the use of MOL may be needful in the management of excessive body weight gain or obesity.

The efficiency of feed conversion of MOL in the experimental rats as shown in the group provided diet containing 37.5 g vitamin-mineral premix + 37.5 g MOL (Table 4) showed no significant changes when compared with the control rats. However, the reduction in the efficiency of feed conversion observed in other MOL diets treated groups might be due to the low feed intake or low feed consumption in dose dependent manners observed in Table 2.

Changes not observed in the MOL treated groups (Table 5) might simply suggest MOL has no effect on organ weight.

Conclusively, the study suggests the use of MOL may be needful only as a supplement, condiment or ingredient to enrich diets with essential vitamins and minerals but not for growth or body weight gain.

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