Protein Uptake and Utilization of *Moringa olifera* Leaves and Seeds Fed to Rats as a Dietary Supplement

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Authors' contributions

This work was carried out in collaboration among all authors. Author NNU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JIO and AIA managed the analyses of the study. Author NNU managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background/Objective: Protein malnutrition is detrimental at any point in life. Protein malnutrition prenatally has also been shown to have significant lifelong effects. The objective of this study was to evaluate the protein uptake and utilization of *Moringa olifera* leaves and seeds fed to rats as a dietary supplement.
Materials and Methods: The leaves and seeds were harvested from the forest. The leaves were washed, drained and divided into three portions. The first portion was processed raw, the sun and shade dried samples were the second and third portion. The seeds were cracked and divided into six portions. The other five portions were fermented for 24, 48, 72, 96 and 120 h respectively. The blends of the shade dried leaves (SHDL) and 69 h fermented seed (FMS) provided 10% protein for two groups of rats. The diets were fed to twenty (20) adult albino rats in a 12-day N and mineral balance study. Casein was used as control. The result generated were statistically analyzed using SPSS version 22.

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Results: The result showed that over 70% of the nitrogen consumed by all the two groups of rats fed the test diets were absorbed and retained. Among the test diet group, the FMS diet had the highest absorbed nitrogen (0.94 g), retained nitrogen (0.55 g), biological value (76%) and net protein utilization (52%) which was significantly different from the other groups. The SHDL had the most digested nitrogen (94%), highest maintenance food intake (74.09%) and weight gain (34.28%) that were comparable with the reference protein casein at ($p>0.05$). The FMS group had the highest liver, small intestine and lung weight. The mineral metabolism of all the rats fed the test diets were absorbed and retained.

Conclusion: Generally, both the leaves and the seeds of *Moringa olifera* have good nutrient profile which could promote growth, they should be consumed to diversify diet and reduced micronutrient deficiency.

1. INTRODUCTION

Dietary protein is indispensable to deliver the nitrogen and amino acids required to meet metabolic demands, and particularly the renewal of body proteins. [1,2]. Human diet should be nutritionally balanced, especially protein to meet the growth and maintenance needs of both children and adult [3]. A good quality protein is one with high digestibility. This is the protein that will supply sufficient amino acid (AA) to cover requirements when consumed at a level that complies with the global protein requirement.

Plant protein sources tend to contain less overall protein and have lower lysine content as a percentage of total protein [4] and slightly poorer digestibility [5] than animal protein [6]. A predominant intake of plant protein might lead to inadequate intake of protein and (AA) amino acid when compared to requirements. The utilization of these plant foods is sometimes limited by the presence of some anti-nutrients, low palatability, digestibility and processing techniques [7]. To optimize their utilitilization, several detoxification/processing techniques have to be used [8]. Many processing methods have been shown to improve the nutritive value of plant foods for human nutrition [9,10,11,12]. Food processing includes any treatment or action that changes or converts raw plant or animal materials into safe, edible and more palatable food [13]. The food flavouring condiments are prepared by traditional methods of solid substrate fermentation resulting in extensive hydrolysis of protein and carbohydrate components [14,15,8]. In addition to increasing the shelflife and reduction in anti-nutritional factors, fermentation markedly improve the digestibility, nutritive value and flavour of food.

Protein quality is the ability of a food protein to support body growth and maintenance. Biological evaluation has the ability to support growth and maintenance and it is the only reliable method for the determination of protein quality. Quality of a protein is determined by assessing its essential amino acid composition, digestibility and bioavailability of amino acids [16]. The composition of various proteins may be so unique thereby influencing the physiological function in the human body. The quality of protein is vital when considering the nutritional benefits of a food product [16]. Biological evaluation indices of protein quality include but not limited to protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU), and true digestibility (TD) [17].

There has been some reports on the nutrient composition of *Moringa oleifera* leaves and seeds. This research made an attempt to explore their food potentials as a source of protein and minerals. The protein uptake and utilization of *Moringa olifera* leaves and seeds fed to rats as a dietary supplement was examined.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The fresh *Moringa oleifera* leaves and seeds were collected from the forest in Nri Community of Anambra State of Nigeria. Casein, mineral mix, vitamin mix, soy bean oil and corn starch were all purchased in a store in Lagos, Nigeria.

The edible leaves were divided into three portions. One portion was processed raw, the second portion shade dried and the third portion was sun dried. The seeds were scraped from the pod to obtain clean seeds. The seeds were cracked, boiled, drained and fermented by inherent micro-flora of the seeds for 24, 48, 72, 96 and 120 h at 28.0±2°C using ash as catalyst.

The fermented seeds were dried until bristle in an air oven at 55°C and ground using hammer mill into fine flour (70 mm-mesh screen). The flour from each samples were packaged, labeled and stored in an air tight container until needed for further use.

2.2 Diet Formulation

Four diets were formulated. These included (a) Casein diet. (b) Shade dried leaves of *Moringa oleifera* (c) 96 h fermented *Moringa oleifera*. (d) Nitrogen free diet. The diets were meant to provide 10% protein to the rats [9]. In order to provide this level of protein, 96 h fermented seed and the shade dried leaves that had the highest protein content was used. The casein diet was the reference or control. The fourth diet was nitrogen free (Table 1).

All the experimental diets were prepared by incorporating the flours and the protein free diet at the expense of the cornstarch-sucrose mixture of 1:1 ratio to obtain the required 1000 g by volume. The diets also provided 1% cellulose, 9% soy oil, 5% mineral and 1% vitamin fortification mixes. In the protein-free diet, corn-starch sucrose mixture replaced the test protein. The purpose of the protein-free diet was to estimate the endogenous nitrogen excretion of the rats. The dried ingredients for each of the diet were weighed into a bowl and mixed manually. Oil was added and mixed thoroughly with the other ingredients, to a smooth texture by hand. Water was added in small quantity at a time to reconstitute it until a homogenous mixture was obtained and pelleted manually. The amount of composite flour for the diet was calculated using the formula:

$$10\% \text{ protein content} = \frac{3.2 \times 100}{\% \text{ N of sample}} \quad [1]$$

2.3 Animals and Housing

Twenty weanling male Albino rats, from the same colony, weighing 40-60 g were purchased from the Veterinary Medicine Department of the University of Nigeria, Nsukka. The rats were housed in individual metabolism cages equipped to separate faeces and urine on a base tray. The rats had exactly 12 h of light and 12 h of darkness in a day. Temperature was maintained at 21-25°C. All procedures using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and approved by Industrial Animal Care Committee, University of Nigeria, Nsukka.

2.4 Growth and Maintenance Study

After a 7- day acclimatization period, the rats were weighed prior to access to their respective diets. The casein and the test diets were fed 15 g each to the rats daily for 21 day growth period. During this study, food intake was measured on daily basis. The other group of rats fed nitrogen free diet was fed normal rat chow during the growth studies and switched over to nitrogen free diet for maintenance studies. After the weight measurement on day 21, carmine red was added to each diet to mark the beginning of nitrogen balance. On day 22, all red faeces were retained and black ones discarded. Black faeces collected after day 23 was retained until day 28 when another carmine red was added to mark the end of N balance period. On day 29, all the red faeces were discarded and the black ones retained. The faeces were dried at room temperature for 48 h, weighed, ground and stored in the freezer. Urine collected was treated with 0.1N HCl to avoid microflora growth. The respective faecal and urinary samples were pooled together and stored in a deep freezer for analysis. The rats were sacrificed on day 29. The liver were removed, weighed and analysed for minerals.

2.5 Chemical Analyses

The weight of the rats was taken using an electronic balance and repeated on alternate days throughout the study. The faecal materials were pooled per group, sun-dried for 8 h, and then crashed using a mortar and pestle. The crude protein (N X 6.25) content of the faecal materials and dried diets were determined using the micro kjeldahl procedure [9]. The dried diets and faecal samples were analyzed for some mineral content using AAS (Atomic absorption spectrophotometer) methods. The same procedure was used for determination of urinary nitrogen and minerals. The values obtained were used for the calculation of food and nitrogen intake, faecal and urinary nitrogen. Faecal nitrogen from the rats fed the protein-free diet was used to calculate the endogenous nitrogen loss required for determining true digestibility (TD). N balance, net protein utilization (NPU), protein efficient ratio (PER) and biological value (BV), were also computed. The result from AAS was used for the consumed, retained and absorbed minerals.

Table 1. Composition of experimental diets (g/kg)

Diet Ingredient	CAS	SHD	FMS	NFD
Casein	306.32	0	0	0
SHD	0	510.34	0	0
FMS	0	0	554.60	0
Corn starch	250.34	146.33	126.20	403.50
Sucrose	250.34	146.33	126.20	403.50
Fibre	40	40	40	40
Soy bean oil	90	90	90	90
Mineral mix	50	50	50	50
Vitamin mix	10	10	10	10
L-cysteine	3	3	3	3
Total	**1000**	**1000**	**1000**	**1000**

CAS- Casein; SHD-Shade dried leaves; FMS-Fermented Moringa oleifera seed (96hrs); NFD-Nitrogen free diet

2.6 Experimental Design

The growth and digestibility studies were carried out using the Completely Randomized Design (CRD). Rats were randomly assigned to the treatments based on their weights. There were four treatments each replicated five times. The rats were the replicates while the different diets were the treatments.

2.7 Formulation of Diet

The diet was formulated using AIN 93G (American Institute of Nutrition) method for growth, pregnancy and lactating phases in laboratory rats [18]. The four experimental diets formulated (g/kg) are given in Table 1.

2.8 Crude Protein

Crude protein content of the samples was determined using the automated micro-Kjeldahl method as described by AOAC [19]. The method is generally used to determine N in substances which contain N as ammonium salts, nitrate or organic N compound. The quantity of N measured is the multiplied by 6.25 to calculate the protein content of the compound.

$$N \text{ content} = \frac{\text{ml acid (sample)} - \text{ml acid (blank)} \times N \text{ mol} \times 100}{\text{Dry weight of sample in grammes} \times 1000}$$

2.9 Mineral Determination

The mineral contents, namely: iron, zinc and iodine contents were determined by the method described by Pearson [20] using a Pye Unicam SP9 Atomic Absorption Spectrophotometer (AAS) [19].

2.10 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) version 22. Means was separated using Turkey's Least Significance Difference (LSD) test and probability judged at P=0.05. Results were represented as mean ± standard deviation.

3. RESULTS

Nitrogen balance of rats fed two mixed protein diet and casein was presented in Table 2. The maintenance body weight of rats fed casein diet had the highest value of 49.10 g which differed significantly ($p < 0.05$) from the rats fed shade dried leaves (23.76 g) and 69 h fermented seeds (34.28 g) of *Moringa oleifera* diets.

The food intake of rats fed casein diet had the highest intake (209.40 g) followed by that of the rats fed 96 h fermented *Moringa oleifera* seed diet (74.09 g), whereas rats fed shade dried leaves diet had the least value (60.02 g). The food intake of rats fed casein diet was significantly different ($p<0.05$) from that of the other test diets. There was also significant difference ($p<0.05$) between the food intake of rats fed 96 h fermented *Moringa oleifera* seed diet and that of the rats fed shade dried leaves diet.

The nitrogen intake of rats fed casein diet was highest (1.10 g) and differed significantly ($p < 0.05$) from those of the other test diets. There was no significant difference ($p < 0.05$) in nitrogen intake of the rats fed 96 h fermented *Moringa oleifera* seed diet (0.87 g) and rats fed shade dried leaves diet (0.72 g).

Table 2. Nitrogen balance of rats fed two mixed protein diets and the control casein

Variables	CAS	SHD	FMS
Maintenance body weight	$49.10^a \pm 1.24$	$23.16^c \pm 0.20$	$34.28^b \pm 0.07$
Food intake	$209.00^a \pm 10.14$	$60.02^c \pm 3.11$	$74.09^b \pm 6.41$
Nitrogen intake	$1.10^a \pm 0.52$	$0.72^b \pm 0.86$	$0.98^{ab} \pm 0.73$
Faecal nitrogen	$0.28^a \pm 0.13$	$0.03^b \pm 0.01$	0.00 ± 0.00
Absorbed N	$1.24^a \pm 0.82$	$0.94^b \pm 0.04$	$1.02^b \pm 0.67$
Urinary N	$0.06^c \pm 0.05$	$0.59^a \pm 0.20$	$0.40^b \pm 0.13$
Retained N	$1.10^a \pm 0.33$	$0.44^b \pm 0.10$	$0.55^b \pm 0.07$
Biological value	$1.09^a \pm 0.03$	$0.47^c \pm 0.17$	$0.64^b \pm 0.16$
NPU	$0.94^a \pm 0.06$	$0.35^c \pm 0.21$	$0.52^b \pm 0.08$
PER	$2.60^a \pm 0.40$	$-2.43^c \pm 0.11$	$-1.10^b \pm 0.02$
TD	$97.64^a \pm 0.01$	$89.75^b \pm 0.14$	$90.44^{ab} \pm 0.64$

Values are means from five rats ± standard deviation.
CAS- Casein; SHD-Shade dried leaves; FMS-Fermented Moringa oleifera seed (96 h)

Table 3. Nutrient composition of liver of rats fed two test diet and nitrogen free diet

Sample	SHD	FMS	NFD
Food intake (g)	$60.02^b \pm 3.11$	$74.09^a \pm 6.43$	$46.71^c \pm 3.62$
Nitrogen intake (g)	$0.72^a \pm 0.86$	$0.67^a \pm 0.08$	0.00 ± 0.00
Liver weight (g)	$5.79^a \pm 0.13$	$5.37^a \pm 0.02$	$4.22^a \pm 0.31$
Nitrogen (g)	$0.16^a \pm 0.02$	$0.15^a \pm 0.03$	$0.13^b \pm 0.07$
Fat (g)	$0.18^a \pm 0.03$	$0.17^a \pm 0.01$	$0.14^b \pm 0.05$
Fibre (g)	$0.25^a \pm 0.01$	$0.26^a \pm 0.06$	$0.22^a \pm 0.09$
Moisture (g)	$0.36^a \pm 0.05$	$0.36^a \pm 0.28$	$0.31^a \pm 0.01$
Iron (mg)	$0.83^a \pm 0.70$	$0.73^a \pm 0.10$	$0.53^b \pm 0.11$
Zinc (mg)	$0.08^a \pm 0.02$	$0.07^a \pm 0.00$	$0.06^a \pm 0.01$
Iodine (mg)	$1.00^a \pm 0.12$	$1.01^a \pm 0.45$	$0.80^a \pm 0.34$

Values are means from five rats ± standard deviation.
SHD-Shade dried leaves; FMS-Fermented Moringa oleifera seed (96 h); NFD-Nitrogen free diet

Table 4. Mineral metabolism of rats fed two mixed diet

Variables	SHD	FMS	CAS
Consumed zinc (mg)	$0.10^c \pm 0.06$	$6.82^b \pm 0.08$	$6.92^a \pm 1.08$
Faecal zinc (mg)	$9.00^a \pm 0.75$	$2.04^b \pm 0.21$	$1.22^b \pm 0.51$
Absorbed zinc (mg)	$-8.90^b \pm 0.03$	$4.78^a \pm 0.05$	$5.70^a \pm 0.96$
Urinary zinc (mg)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Retained zinc (mg)	$-8.90^b \pm 0.03$	$4.78^a \pm 0.05$	$5.70^a \pm 0.96$
Consumed iron (mg)	$1.02^c \pm 0.57$	$12.06^a \pm 0.32$	$8.24^b \pm 0.56$
Faecal iron (mg)	$0.27^b \pm 0.11$	$0.07^c \pm 0.03$	$0.98^a \pm 0.13$
Absorbed iron (mg)	$0.75^c \pm 0.15$	$11.99^a \pm 0.61$	$7.26^b \pm 0.61$
Urinary iron (mg)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Retained iron (mg)	$0.75^c \pm 0.15$	$11.99^a \pm 0.61$	$7.26^b \pm 0.61$
Consumed iodine (Ug)	$222.16^b \pm 0.06$	$569.43^a \pm 0.15$	$176.12^c \pm 0.31$
Faecal iodine (Ug)	$61.36^a \pm 0.38$	$16.34^c \pm 0.13$	$52.20^b \pm 0.06$
Absorbed iodine (Ug)	$160.80^b \pm 0.73$	$553.09^a \pm 0.07$	$123.92^c \pm 0.08$
Urinary iodine (Ug)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Retained iodine (Ug)	$160.80^b \pm 0.73$	$553.09^a \pm 0.07$	$123.92^c \pm 0.08$

The protein efficiency ratio of rats fed casein diet (2.60 g) was significantly different ($p < 0.05$) from those of the rats fed 96 h fermented *Moringa oleifera* seed diet (-1.10 g) and rats fed shade dried leaves diet (-2.43 g). There was no significant difference ($p < 0.05$) in protein efficiency ratio of the rats fed 96 h fermented *Moringa oleifera* seed diet and rats fed shade dried leaves diet (0.72 g).

The rats fed casein diet digested nitrogen (1.24 g) more than those rats fed the test diets. There was no significant difference (p < 0.05) in digested nitrogen between the rats fed casein diet and the group of rats fed 96 h fermented *Moringa oleifera* seed diet. There was also no significant difference (p < 0.05) in digested nitrogen between the rats fed 96h fermented *Moringa oleifera* seed diet and the rats fed shade dried leaves diet (p < 0.05).

The rats fed casein diet retained more nitrogen (1.10 g) which differed significantly (p < 0.05) from the groups of rats fed the test diets. The group of rats fed fermented *Moringa oleifera* seed diet retained more nitrogen (0.55 g) than the group fed shade dried *Moringa oleifera* leaves. There is no significant difference between the group of rats fed 96 h fermented *Moringa oleifera* seed diet and the group fed shade dried *Moringa oleifera* leaves diet (p< 0.05).

The biological value (BV) of the rats fed casein diet was higher (1.09 g) than those fed the test diet. There was significant difference in the BV of the group of rats fed casein diet and the group fed 96 h fermented *Moringa oleifera* seed diet. The BV of the rats fed 96 h fermented *Moringa oleifera* seed diet was higher (0.64 g) than the group fed shade dried leaves diet (0.47 g). There was no significant difference in the BV of rats fed 96 h fermented *Moringa oleifera* seed diet and the group fed shade dried *Moringa oleifera* leaves diet (p< 0.05).

The net protein utilization (NPU) value for casein was highest (0.94 g) and differed significantly (p < 0.05) from those of the rats fed test diets. The rats fed 96 h fermented *Moringa oleifera* seed diet group had the higher NPU (0.52 g) and the rats fed shade dried *Moringa oleifera* leaves diet had the least (0.35 g).

The true digestibility (TD) of rats fed casein diet had the highest intake (97.64 g) followed by that of the rats fed 96 h fermented *Moringa oleifera* seed diet (90.44 g) and rats fed shade dried leaves diet had the least value (89.75 g). The TD of rats fed casein diet was significantly difference (p< 0.05) from that of the other test diets. There was no significant difference (p< 0.05) between the TD of rats fed 96 h fermented *Moringa oleifera* seed diet and that of the rats fed shade dried leaves diet.

Nutrient composition of rats fed two mixed protein diet and protein free diet was presented in Table 3. The food intake of rats fed SHDL diet had the highest value of 60.02 g which differed significantly (p < 0.05) from the rats fed FMS leaves (54.09 g) and NFD diet (46.71 g).

The nitrogen intake of rats fed SHDL had the highest intake (0.72 g) followed by that of the rats fed 96 h fermented *Moringa oleifera* seed diet (0.67 g) and rats fed NFD diet had the least value (0.00 g). The food intake of rats fed SHDL diet was not significantly difference (p< 0.05) from that of the rats fed FMS diets. There was significant difference (p< 0.05) between the food intake of rats fed 96 h fermented *Moringa oleifera* seed diet and that of the rats fed NFD diet.

The liver weight of rats fed SHDL diet was highest (5.79 g) and has no significantly different (p < 0.05) from those of the other diets. There was no significant difference (p < 0.05) in liver weight of the rats fed 96 h fermented *Moringa oleifera* seed diet (5.39 g) and rats fed NFD diet (4.22 g).

The nitrogen of rats fed SHDL diet (0.16 g) was not significantly different (p < 0.05) from those of the rats fed 96 h fermented *Moringa oleifera* seed diet (0.15 g) and rats fed NFD diet (0.13 g). There was also no significance difference (p < 0.05) in nitrogen of the rats fed 96 h fermented *Moringa oleifera* seed diet and rats fed NFD diet.

The rats fed SHDL diet had more iron (0.83 mg) which differed significantly (p < 0.05) from the groups of rats fed NFD diets. The group of rats fed fermented *Moringa oleifera* seed diet had more iron (0.73 g) than the group fed NFD. There is no significant difference (p<0.05) between the group of rats fed 96 h fermented *Moringa oleifera* seed diet and the group fed shade dried *Moringa oleifera* leaves diet.

The zinc value of the rats fed SHDL diet was higher (0.08 mg) than those fed the other diets. There was no significant difference in the zinc value of the group of rats fed SHDL diet and the group fed 96h fermented *Moringa oleifera* seed diet. The zinc level of the rats fed 96 h fermented *Moringa oleifera* seed diet was higher (0.07 g) than the group fed NFD diet (0.06 g). There was no significant difference (p<0.05) in the BV of rats fed 96 h fermented *Moringa oleifera* seed diet and the group fed NFD diet.

Mineral metabolism of rats fed two mixed protein diet was presented in Table 4. The rats fed 69 h fermented seeds of *Moringa oleifera* consumed more zinc (6.82 mg) than the rats fed shade dried leaves of *Moringa oleifera* diet (0.10 mg).

The rats fed SHDL diet lost more zinc in faeces (9.00 mg) than the rats fed 96 h fermented *Moringa oleifera* seed diet (2.04 mg). Both the rats fed 96 h fermented *Moringa oleifera* seed diet and rats fed shade dried *Moringa oleifera* leaves diet lost no zinc in the urine (0.00). The rats fed 96 h fermented *Moringa oleifera* seed diet retained more zinc (4.78 mg) than the rats fed shade dried *Moringa oleifera* leaves diet (-8.90 mg).

The rats fed 96 h fermented *Moringa oleifera* seed diet consumed more iron (12.06 mg) than the rats fed shade dried *Moringa oleifera* leaves diet (1.02 mg) and lost 0.07 mg of iron in faeces, while the rats fed shade dried *Moringa oleifera* leaves diet lost 0.27 mg iron in faeces. The rats fed 96 h fermented *Moringa oleifera* seed diet retained more iron (11.99 mg) than the rats fed shade dried *Moringa oleifera* leaves diet (0.75 mg). Both the rats fed 96 h fermented *Moringa oleifera* seed diet and rats fed shade dried *Moringa oleifera* leaves diet lost no iron in the urine (0.00).

The rats fed 96 h fermented *Moringa oleifera* seed diet consumed more iodine (569.43 Ug) than the rats fed shade dried *Moringa oleifera* leaves diet (222.16 Ug) and lost 16.43 Ug of iodine in faeces, while the rats fed shade dried *Moringa oleifera* leaves diet lost 61.36 Ug of iodine in faeces. The rats fed 96 h fermented *Moringa oleifera* seed diet retained more iodine (553.09 Ug) than the rats fed shade dried *Moringa oleifera* leaves diet (160.80 Ug). Both the rats fed 96 h fermented *Moringa oleifera* seed diet and rats fed shade dried *Moringa oleifera* leaves diet lost no iodine in the urine (0.00).

4. DISCUSSION

4.1 Food Intake

Ene-obong and Obizoba [21] noted that feed intake can be influenced by palatability, source of nitrogen and essential amino acid. It also depend on its capacity to satisfy the needs for nitrogen and essential amino acid. The rats fed test diets had lower feed intake. This lower feed intake might be due to lack of palatability and flavor to increase their appetite. This findings is in line withObizoba [22], who observed a lower feed intake of test diets in a study.

4.2 Maintenance Body Weight

The rats fed casein diet had higher maintenance body weight (49.10 g) than the rats fed 96 h fermented *Moringa oleifera* seed diet (34.28 g), followed by the rats fed shade dried leaves diet (23.16 g). Casein has a better essential amino acid profile than the test diets. The lower maintenance body weight by the rats fed test diets might be due to lower feed intake. [23] observed that weight gain of animals is partly influenced by food intake and partly by the essential amino acid pattern of the dietary protein. Poor feed intake will translate to poor protein utilization.

4.3 PER (Protein Efficiency Ratio)

PER is one of the commonly used methods of assessing quality of protein [24]. The PER standard value is 2.7, which is the standard value of casein protein. The rats fed casein diets had highest PER of 2.60. The higher PER of rats fed casein diet might be due to its better EAA (essential amino acid) pattern. The lower PER value of rats fed test diets -1.10 and -2.43 than those fed casein diet might be due to low feed intake and weight gain. Wardlaw et al. [25] observed an inter-relationship between feed intake, weight gain and PER. Campbell et al. [26] opined that variation in PER may be due to the level of protein in the diet, different strains of rats, sex of the rats used for the test and assay period. The positive PER of the rats fed 96 h fermented *Moringa oleifera* seed diet as against those fed shade dried *Moringa oleifera* leaves diet suggested that rats fed 96 h fermented *Moringa oleifera* seed diet had a much more desirable EAA pattern than its counterparts.

4.4 Urinary Nitrogen

The low urinary N of the rats fed casein diets showed high digestibility and utilization of nitrogen in casein diet. [21] observed that feacal and urinary nitrogen levels influence nitrogen digestibility and utilization.

4.5 Faecal Nitrogen

The rats fed test diets had least faecal N (0.00 and 0.03 g). The low faecal N of the test diets could be attributed to low feed intake. It is a common observation/phenomenon that high feed intake would lead to high faecal deposit.

4.6 Retained/Absorbed Nitrogen

The rats fed casein diet had the highest retained and absorbed N. The more absorbed and retained N of casein diet showed that it had high

protein quality. The retained and absorbed N of the rats fed 96 h fermented *Moringa oleifera* seed diet is more than half of the retained and absorbed N for the rats fed casein diet which is the reference protein. This showed that the rats fed 96 h fermented *Moringa oleifera* seed diet had also high protein quality diet. [27] observed that the higher absorbed and retained nitrogen is, the better the dietary protein quality.

4.7 Biological Value (BV)

Biological value measures protein quality by calculating the nitrogen used for tissue formation divided by the nitrogen absorbed from food. This product is multiplied by 100 and expressed as a percentage of nitrogen utilized. The biological value provides a measurement of how efficient the body utilizes protein consumed in the diet [16]. The rats fed casein diets had BV value of (90.00%), while the rats fed 96 h fermented *Moringa oleifera* seed diet had (76.00%) and rats fed shade dried *Moringa oleifera* leaves diet had (64.00%). [27] reported that a protein with a BV of 70% or more can support human growth and tissue maintenance as long as energy intake is adequate. The high BV of rats fed 96 h fermented *Moringa oleifera* seed diet suggests that protein from these diets can support human growth and maintenance.

4.8 Net Protein Utilization (NPU)

Net protein utilization is similar to the biological value except that it involves a direct measure of retention of absorbed nitrogen. Net protein utilization and biological value both measure the same parameter of nitrogen retention, however, the difference lies in that the biological value is calculated from nitrogen absorbed whereas net protein utilization is from nitrogen ingested [16]. The high NPU of (0.94 and 0.92 g) for rats fed casein diet and rats fed 96 h fermented *Moringa oleifera* seed diet respectively were due to lower urinary output against those fed shade dried *Moringa oleifera* leaves diet and rats fed nitrogen free diet (NFD) (Table 2).

True digestibility (TD): The casein diet had the highest TD (97.64%). The higher TD of the rats fed casein diet is not a surprise because it is a reference protein. The TD of rats fed test diets (90.44%) and (89.75%) for rats fed 96 h fermented *Moringa oleifera* seed diet and rats fed shade dried *Moringa oleifera* leaves diet, respectively is of interest. TD gives information on the nitrogen intake absorbed by the body. [27]

observed that the most complete protein is worthless to the body if it is not digested and absorbed to yield desirable pattern of essential amino acids for new protein synthesis.

4.9 Liver Weight

The higher liver weight of rats fed shade dried *Moringa oleifera* leaves diet might be because they consumed more food (60.02 g) (Table 3) compared to the other groups. [28] observed that organ weights were influenced by food intake and body weight. There was no significant difference (p<0.05) in the liver weight of rats fed shade dried *Moringa oleifera* leaves diet and rats fed 96 h fermented *Moringa oleifera* seed diet. The increase in the liver weight of the rats fed tests diet showed that the diets supported growth as well as body weight.

4.10 Zinc

Fermentation had a better indices in the mineral metabolism in the rats. The increase observed in the zinc intake of rats fed 96 h fermented *Moringa oleifera* seed diet might be due to the fact that fermentation led to hydrolytic action of micro-flora enzymes. The enzymes hydrolyzed the zinc from their bound forms and make them available for use by the body. The result is in line with the observation of [29] that noted increased hydrolytic action of micro-flora enzymes in the minerals and other nutrients in the fermented samples of their study. [30] stated that zinc is a trace mineral element that plays a catalytic role in enzymes. Shankar and Prasad [31] observed that zinc enrichment may be beneficial for health, but excess zinc may interact with iron and copper metabolism. The rats fed shade dried *Moringa oleifera* leaves diet had little zinc intake (0.10 mg) and excreted more zinc 9.00 mg thereby having a negative zinc absorption of -8.90 mg. The negative zinc absorption might be due to poor zinc utilization in the rats fed shade dried *Moringa oleifera* leaves diet. The fermented seeds with high iron levels could be useful in the fight against iron deficiency anaemia. The rats fed shade dried *Moringa oleifera* leaves diet had an iron intake of (1.02 mg), faecal iron of 0.27 mg and retained 0.75 mg iron. The low level of iron in shade dried *Moringa oleifera* leaves diet might be due to low level of iron in the shade dried leaves. [28] reported that iron intake is controlled by many factors which include: seed type, food intake and treatment. These factors may have affected zinc intake and utilization of rats fed shade dried leaves diet.

5. CONCLUSION

Moringa oleifera seed and leaves could serve as a source of high quality protein and caloric supplement in foods. The *Moringa oleifera* seed and leaves protein induced positive nitrogen balance. More than 70% of nitrogen consumed from test diet were absorbed and retained. Low acceptability of the test diets was due to lack of palatability of the diets. The true digestibility (TD), biological value (BV) and net protein utilization (NPU) values for both fermented seed and shade dried leaves of *Moringa oleifera* nitrogen were utilized for growth and tissue maintenance.

Fermentation improve the nutrient quality of these foods especially protein and hence is useful for individuals that prefer natural enhancement of nutrient to enrichment.

ETHICAL APPROVAL

All procedures using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and approved by Industrial Animal Care Committee, University of Nigeria, Nsukka.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/51625