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Evaluation of nutritional and therapeutic effects of defatted *Moringa oleifera* seeds in protein energy malnourished rats

Raphael Eneji Jegede[1]*, Gideon Ayeni[1], Rose Mafo Abaniwo[1], Abdulquadri Funmbi Olutoye[2], Godwin Amoka Audu[1], Abdullahi Haruna[1] and Oluwabusayomi Stella Oluwole[1]

[1]Department of Biochemistry, Prince Abubakar Audu University, Anyigba, Nigeria.
[2]Department of Biochemistry, University of Ilorin, Nigeria.
*Corresponding author email: jegederaphael@gmail.com

ABSTRACT

Protein-energy malnutrition (PEM) is a significant global health problem, particularly in a developing country. This study investigates the nutritional potential of defatted Moringa oleifera seeds in ameliorating PEM in albino rats. Moringa oleifera seeds were defatted to enhance protein concentration. The seeds' proximate composition, mineral elements, amino acid profile, and in-vitro protein digestibility were analyzed. Additionally, the effects of Moringa oleifera seed based-diet on liver function indices of Protein energy malnourished rats were assessed. Results indicated that defatting significantly increased crude protein content and reduced crude fat level. Mineral analysis revealed high sodium and calcium concentrations, essential for electrolyte balance and bone health. Amino acid profiling showed a significant ($p < 0.05$) increase in essential amino acids, particularly arginine, leucine, and valine, which are crucial for tissue repair and muscle growth. In-vitro protein digestibility improved significantly after defatting, with the highest digestibility observed in seeds defatted with the solvent mixture. Protein energy malnourished rats exhibited significantly ($p< 0.05$) elevated serum level of alanine amino transferase, aspartate aminotransferase and alkaline phosphatase, conjugated and total bilirubin and significantly ($p <0.05$) reduced plasma protein levels (total protein, globulin and albumin), indicative of liver dysfunction. Treatment with the Moringa oleifera kernel-based diet resulted in significant reduction on these liver enzymes and significant increase in the serum protein compare to the malnourished control group. The diet normalized enzyme activities and increased plasma protein levels, suggesting liver recovery. The study concludes that defatted Moringa oleifera seeds is a viable, cost-effective alternative protein source for managing PEM, exhibiting promising nutritional and functional properties.

Keyword: Moringa, Malnutrition, bioactive compound, amino acid, proximate

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INTRODUCTION

Protein-energy malnutrition (PEM) is a severe health condition that arises due to inadequate intake of protein and energy, affecting millions, particularly in low-income and developing countries (Naheed *et al*., 2021). PEM is particularly common among children and can result in conditions such as kwashiorkor, marasmus, or marasmic-kwashiorkor, a combination of both. It leads to impaired growth, weakened immune function, and a higher susceptibility to infections, contributing significantly to child mortality (Michael *et al*., 2022). According to the World Health Organization (WHO), malnutrition effects nearly half of all child deaths globally (WHO, 2020). PEM can develop due to various factors, including poor dietary intake, infections, and socioeconomic conditions. While the primary cause is inadequate nutrient intake, the condition can also be aggravated by diseases like diarrhea, which reduces the body's capacity to absorb nutrients (Mathewson *et al*., 2021). Children, pregnant women, and the elderly are most vulnerable to PEM due to their increased nutritional needs (Rasheed *et al*., 2023).

The management of PEM involves dietary interventions aimed at restoring nutritional balance by providing energy

and protein in adequate amounts. Typically, treatment includes the use of ready-to-use therapeutic foods (RUTFs) which are energy-dense, micronutrient-enriched foods used in treating severe malnutrition (Okhiria, 2007). However, the high cost of RUTFs can limit their accessibility, particularly in resource-poor regions, highlighting the need for affordable and locally available alternatives.

Traditional therapies for PEM include dietary formulations based on animal proteins, such as milk and eggs, as well as plant-based protein sources. However, due to high cost of animal protein and soya bean, these options may not be accessible in some regions, prompting the search for alternative protein-rich foods, such as *Moringa oleifera* (Awuchi *et al*., 2020). *Moringa oleifera*, commonly known as the drumstick tree, is widely recognized for its exceptional nutritional and medicinal properties. Native to parts of South Asia and Africa, the tree has gained international recognition due to its ability to thrive in harsh climatic conditions and provide essential nutrients in regions with food insecurity (Gopalakrishnan *et al*., 2016)). *Moringa oleifera* seeds are especially rich in protein, vitamins, minerals, and bioactive compounds, making them a viable option for addressing malnutrition (Dhakar *et al*., 2011).

The seeds of *Moringa oleifera* are an excellent source of protein, containing all the essential amino acids required by the body. According to research, Moringa seeds contain approximately 30-35% protein, comparable to commonly consumed legumes like soybeans (Gopalakrishnan *et al*., 2016). In addition to its protein content, the seeds are rich in vitamins (A, C, E), minerals (calcium, magnesium, potassium), and antioxidants. These nutritional attributes make *Moringa oleifera* seeds an ideal supplement in diets for treating PEM (Ayodele *et al*., 2021). Moreover, Moringa seeds possess a balanced amino acid profile, which is essential for the growth and repair of tissues, making it particularly suitable for combating protein deficiency in PEM (Dhakar *et al*., 2011). Additionally, Moringa seeds are a good source of fats and essential fatty acids, which are crucial in providing energy and promoting cognitive development in malnourished children (Ashraf & Gilani, 2007).

MATERIALS AND METHODS

Chemicals and reagents

All reagents used are of analytical grade and were procured from British Drug House (BDH), United Kingdom (UK).

Plant materials and authentication

Moringa oleifera seeds were obtained from the premises of Faculty of Agriculture, Prince Abubakar Audu University, Anyigba, Kogi state. The seeds were identified and authenticated at the herbarium unit, Plant Biology Department, University of Ilorin, Ilorin, Nigeria with specimen voucher number (UILH/001/1585/2023) deposited.

Defatting of *Moringa oleifera* kernel

The seed *coat of M. oleifera* seeds was removed, the kernel oven dried at 40ºC to a constant weight, which was then pulverized with electric blender. 200g of the pulverized *M. oleifera* kernels was weighed into a dry conical flask. 800mls of n-hexane was added and the flask shaken vigorously. It was then left for 24hrs for the lipid to extract from the kernel. The solvent containing the extracted lipid was then decanted and filtered using Whatman filter paper no 1. The process was repeated for 2 more times to improve the efficiency of the defatting process (Jegede *et al*., 2020). After the solvent has been filtered, the residue was spread on aluminum foil at room temperature to evaporate the residual solvent and dried the residue to fine powder and packed into an air tight container for feed formulation and analyses.

Experimental animals

Twenty (20) White Albino rats of *Wistar* strain [average weight ±SEM (53.21±2.74g) were procured from animal holding unit of the department of biochemistry, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria for the research. The rats were housed in clean plastic cages located at a well-ventilated room, maintaining specific housing conditions: a 12-hour light-dark cycle, temperature ranging between 25–27°C, and humidity levels of 45%–55% (according to Weather.com). They were fed with pelletized diet from Chikun Feeds Limited, Ilorin, Nigeria, and they had continuous access to clean water and were acclimatized for a week before the commencement of the experiment.

Induction of protein energy malnutrition

Protein Energy Malnutrition (PEM) was induced in fifteen (15) rats by feeding them with low protein isocaloric diet *ad libitum* and clean water for 28days according to modified method of Adelusi & Olowokere, (1985). The composition of the diet is as shown in (Tables 1 and 2).

Animal grouping

Twenty (20) *Wistar* rats were randomly assigned into four groups each containing five (5) rats: A, B, C and D. group A was fed with rat feed (Chikun Feeds, Ilorin, Nigeria) and water *ad libitum* and group B, C and D, were fed with low protein isocaloric diet (Table 1) and clean water *ad libitum* for 28days to induce protein energy malnutrition. On the 29th day, animals in group A and B were sacrificed while

Table 1: Composition of low protein isocaloric diet.

Composition	Low protein Isocaloric diet (g/Kg)
Soya Bean	40g
Corn Starch	516g
Sucrose	300g
Cellulose	40g
Soya Bean oil	50g
DL-methionine	4g
* Vitaflash Amino WSP	50g
Total	1000g

*VITAFLASH AMINO WSP (vitamin-Amino Acids):vitamin A 10,000,000 IU; vitamin D3 2,000,000 IU; vitamin E 15,000 mg; vitamin K3 2,500mg; vitamin B1 1,000mg; vitamin B2 2,000mg; vitamin B6 2,000mg; vitamin B12 10,000mg; folic acid 300mg; Ca-d-pantothenate 7,500mg; nicotine acid 20,000mg; choline chloride 15,000mg; vitamin C 40,000mg;DL-Methionine 50,000mg; L-Lysine 50,000mg; amino acids 52,000mg (cysteine,tryptophan,arginine,threonine,isoleucine,leucine,valine,histidine,phenylanine,tyrosine and glycine).

Table 2: Composition of treatment diets.

Composition	15%Soya Bean Protein (g/Kg)	15% *M. oleifera kernel* Protein based-diet (g/Kg)
Soya Bean	150	-
M. oleifera	-	150
Corn Starch	356	356
Sucrose	300	300
Cellulose	40	40
Soya Bean oil	50	50
DL-methionine	4	4
* Vitaflash Amino WSP	50	50
Total	1000	1000

VITAFLASH AMINO WSP (vitamin-Amino Acids):vitamin A 10,000,000 IU; vitamin D3 2,000,000 IU; vitamin E 15,000 mg; vitamin K3 2,500mg; vitamin B1 1,000mg; vitamin B2 2,000mg; vitamin B6 2,000mg; vitamin B12 10,000mg; folic acid 300mg; Ca-d-pantothenate 7,500mg; nicotine acid 20,000mg; choline chloride 15,000mg; vitamin C 40,000mg; DL-Methionine 50,000mg; L-Lysine 50,000mg; amino acids 52,000mg (cysteine,tryptophan,arginine,threonine,isoleucine,leucine,valine,histidine,phenylanine,tyrosine and glycine).

animals in group C and D were fed with various treatment diets (Table 2) for another 28days and on the 29th day they were sacrificed.

A: Control Rats fed with commercial diet (Naïve)
B: Rats fed with Low Protein isocaloric diet only
C: Rats fed with Low Protein Isocaloric diet but treated with 15% soya bean Based Diet
D: Rats fed with Low Protein isocaloric diet but treated with 15% defatted *M. oleifera* kernel-based diet

Animal sacrifice

On the 29th day post treatment, animals were sacrificed according to the method described by Yakubu *et al*., (2005) after they were anaesthetized by diethyl ether and blood was collected by cardiac puncture.

Determination of body weight

Body weight measurement was done using 0.01g sensitive weighing balance.

Quantitative determination of nutritional composition of *m. oleifera* kernel determination

Mineral elements were determined following the standard procedures of the Association of Official Analytical Chemists (AOAC) (Poitevin, 2016), amino acids profile was determine following the method described by AOAC with modifications (Otemuyiwa & Adewusi, 2013). The proximate compositions were determined in accordance with AOAC (Horwitz & Latimer, 2005). The carbohydrate was determined by calculation using the formula: Carbohydrate = 100 - (Crude protein +Fat +Ash + Crude fiber + Moisture). *In-vitro* Protein digestibility was carried out following the method developed by Mertz *et al*., (1984) with modifications as reported by Gulati *et al*., (2017).

Biochemical and enzymes assays

Determination of plasma liver function indices

The plasma enzymes activity was determined spectrophotometrically using commercial assay kits, Aspartate amino transferase and Alanine amino

transferase was quantified following procedure outlined by Reitman & Frankel, (1957), Alkaline Phosphatase (Ahlers, 1975), total protein was determined by biuret method as (Gornall *et al*., 1949), Albumin was quantified as described by (Doumas, 1975), globulin (Goldenberg & Drewes, 1971), total and conjugated bilirubin (Otsuji *et al*., 1988).

Statistical analysis

All data are expressed as the mean of five replicates ± standard error of mean (SEM). Statistical evaluation of data was performed by Statistical Package for the Social Sciences (SPSS) version 23. Using one way analysis of variance (ANOVA), followed by Duncan's posthoc test for multiple comparisons. Values were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Proximate compositions

The result of proximate composition is presented in (Table 3). The crude protein content significantly ($p < 0.05$) increased across the samples after defatting, with the highest value observed in Sample L, which was defatted with a mixture of n-hexane and acetone (1:1). This suggests that the defatting process enhances protein concentration by removing the fat content. Sample I (undefatted *Moringa oleifera* seed) showed a lower protein value, as the presence of fat dilutes the protein content. Defatting with n-hexane (Sample J) and acetone (Sample K) also showed increased protein levels, with significant differences compared to the undefatted sample ($p < 0.05$). This suggests that the type of solvent used in defatting affects the protein yield, with acetone defatting showing slightly lower protein content than n-hexane. Increased protein content is vital in addressing protein-energy malnutrition (PEM), as dietary protein is essential for growth, tissue repair, and overall metabolism (Wu, 2016). The fat content was significantly ($p < 0.05$) reduced in all defatted samples compared to the undefatted Sample I, with Sample K (acetone-defatted) showing the lowest fat value ($p < 0.05$). Sample J (n-hexane) and Sample L (n-hexane + acetone) also had low fat content but were slightly higher than Sample K. The significant reduction in fat after defatting enhances the utility of Moringa seeds for protein supplementation in PEM treatment without contributing excess calories from fat, which can lead to metabolic disturbances (Lambe, 2019).

Ash content, which represents the mineral composition, was highest in Sample L (4.96±0.04%), indicating that the mixed solvent of n-hexane and acetone might extract fat more effectively, preserving and even enhancing the mineral content. Sample I (undefatted) and Sample K (acetone-defatted) showed similar ash content, while Sample J (n-hexane) showed a slight increase. Minerals play a significant role in addressing malnutrition, particularly in improving bone health, electrolyte balance, and enzymatic processes (Shankar, 2020).

The crude fiber content was significantly ($p < 0.05$) higher in Sample I compared to the defatted samples. This is expected, as defatting processes concentrate other macronutrients while reducing fiber content. Among the defatted samples, Sample J (n-hexane-defatted) had a higher fiber content than Sample K (acetone-defatted) and Sample L (mixed solvent), showing that solvent choice also influences fiber retention. Dietary fiber is essential in the management of malnutrition-related gastrointestinal issues, aiding digestion and improving nutrient absorption (Shozib *et al*., 2018).

Sample J (n-hexane) had the highest moisture content, followed by Sample L and Sample K. Sample I (undefatted) had the lowest moisture content, suggesting that defatting increases the moisture retention capacity of Moringa seeds. Higher moisture content may affect the shelf life and storage of the defatted seeds, but it can also improve the texture and palatability of food products formulated from these seeds (Anselm *et al*., 2023).

The nitrogen-free extract, representing the carbohydrate content, was highest in Sample, followed by Sample L and Sample J. Sample I (undefatted) had a similar NFE value to the defatted samples. Carbohydrates, while not as critical in addressing PEM as proteins, still provide essential energy for individuals suffering from malnutrition (Siva Kiran *et al*., 2015).

The energy values for each sample show that Sample I (undefatted) had the highest caloric content due to its higher fat content, which is more energy-dense than protein or carbohydrates. Sample J (n-hexane) had the lowest energy value, while Samples K and L had moderate energy values. The reduced caloric content in the defatted samples makes them ideal for protein supplementation in malnutrition without providing excess calories that could potentially lead to obesity or other metabolic conditions, especially in the management of protein-energy malnutrition (Siva Kiran *et al*., 2015).

Mineral element compositions

The mineral element analysis of *Moringa oleifera* seeds is shown in (Figure 1). The result revealed Sodium (Na) as the most abundant element. Sodium is an essential mineral that plays a vital role in maintaining fluid balance, nerve signaling, and muscle function (Ali, 2023). Calcium (Ca) is the second most abundant mineral. Calcium is crucial for bone health, muscle contractions, and enzymatic processes. The relatively high concentration of calcium in *Moringa* seeds makes it an important contributor to nutritional intake, particularly in populations at risk of calcium deficiency (Kamran *et al*., 2020; Saa *et al*., 2019). On the lower end, zinc (Zn) was the least abundant element. Despite its low concentration zinc

Table 3: Proximate compositions of *M. oleifera* kernel and the defatted kernels.

Sample	Crude Protein (%)	Fat (%)	Ash (%)	Crude Fiber (%)	Moisture (%)	NFE (%)	Energy Value (kcal/100g)
I	35.66±0.20^{a}	14.29±0.05^{a}	3.84±0.04^{a}	10.06±0.06^{a}	3.81±0.03^{a}	32.36±0.02^{a}	346.98±0.11^{a}
J	46.33±0.13^{b}	3.13±0.04^{b}	4.42±0.04^{b}	5.45±0.08^{b}	6.96±0.03^{b}	33.72±0.07^{b}	299.93±0.10^{b}
K	45.92±0.02^{b}	2.33±0.03^{c}	3.84±0.02^{a}	3.39±0.01^{c}	5.41±0.01^{c}	39.13±0.06^{c}	309.81±0.08^{c}
L	50.58±0.17^{c}	2.63±0.02^{d}	4.96±0.04^{c}	3.16±0.03^{d}	5.77±0.05^{d}	32.92±0.18^{ab}	312.43±0.14^{c}

Values are expressed as means of three (3) replicates ± SEM (Standard Error of Mean). Values with different superscripts down the column are statistically significant ($p < 0.05$). I= undefatted *Moringa oleifera* seed, J= *Moringa oleifera* seed defatted with n-hexane, K= *Moringa oleifera* seed defatted with acetone, L= *Moringa oleifera* seed defatted with a mixture of n-hexane and acetone in ratio 1:1

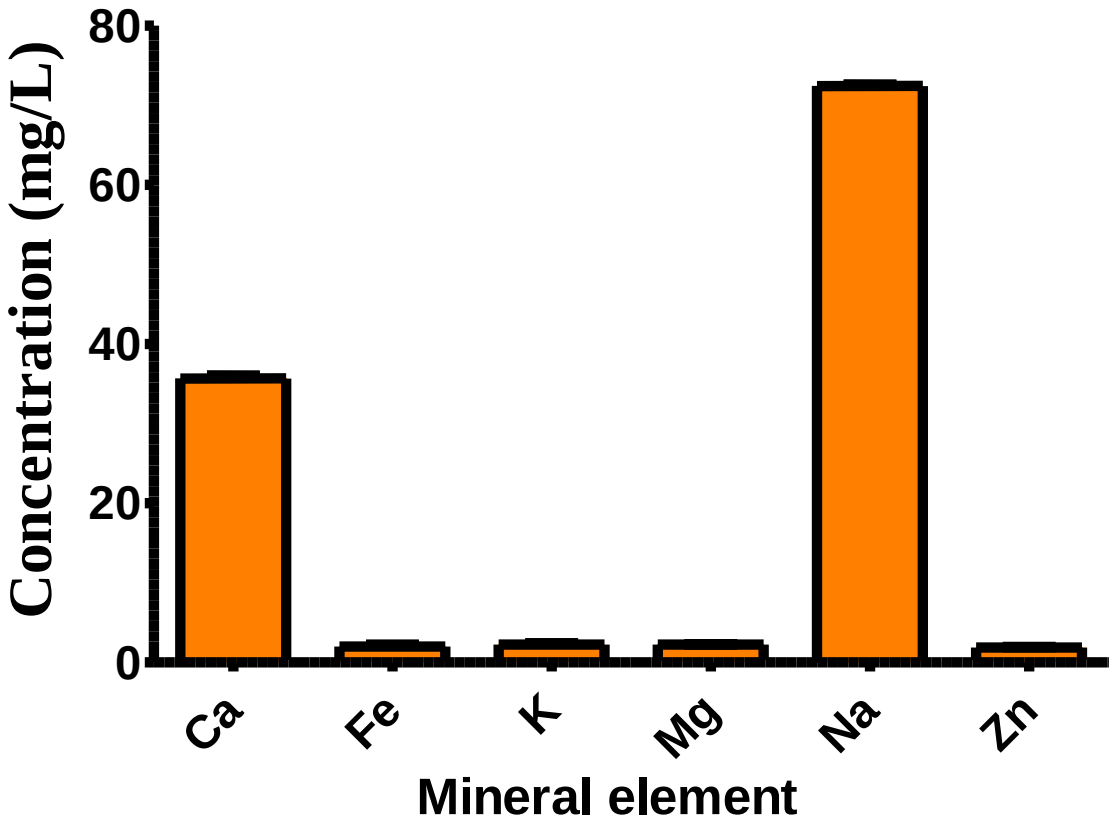

Figure 1: Mineral Elements of *M. oleifera* kernel and the defatted kernels
Values are expressed as means of three (3) replicates ± SEM (Standard Error of Mean). I= undefatted *Moringa oleifera* seed, J= *Moringa oleifera* seed defatted with n-hexane, K= *Moringa oleifera* seed defatted with acetone, L= *Moringa oleifera* seed defatted with a mixture of n-hexane and acetone in ratio 1:1

Table 4: Amino acids profile of *M. oleifera* kernel and the defatted kernels.

	Sample			
	I	J	K	L
Essential Amino Acids (EAA) g/100g protein				
Arginine	6.76±0.02^{a}	7.44±0.03^{b}	7.18±0.02^{c}	7.66±0.03^{b}
Histidine	2.63±0.01^{a}	3.07±0.02^{b}	2.94±0.02^{b}	3.07±0.02^{b}
Isoleucine	3.20±0.02^{a}	3.72±0.01^{b}	3.42±0.01^{a}	3.72±0.01^{c}
Leucine	6.39±0.02^{a}	7.25±0.03^{b}	6.61±0.06^{a}	7.84±0.03^{c}
Lysine	3.75±0.01^{a}	4.06±0.02^{b}	3.88±0.04^{ab}	4.27±0.02^{c}
Methionine	1.22±0.00^{a}	1.29±0.01^{a}	1.27±0.01^{a}	1.33±0.02^{a}
Phenylalanine	4.21±0.02^{a}	4.85±0.01^{b}	4.74±0.02^{b}	5.03±0.04^{b}
Threonine	2.49±0.01^{a}	3.18±0.01^{b}	2.78±0.01^{c}	3.27±0.01^{b}
Tryptophan	0.74±0.00^{a}	0.85±0.01^{bc}	0.78±0.02^{ab}	0.95±0.01^{c}
Valine	4.08±0.01^{a}	4.28±0.01^{b}	4.40±0.01^{c}	4.70±0.01^{d}
Total of EAA (g/100g protein)	35.46±0.04^{a}	39.97±0.05^{b}	37.97±0.12^{c}	41.81±0.15^{d}

Table 4 Cond: Amino acids profile of *M. oleifera* kernel and the defatted kernels.

Non-Essential Amino Acids (NAA) g/100g protein				
Alanine	3.44±0.01^{a}	4.07±0.03^{b}	3.63±0.02^{c}	4.17±0.02^{b}
Aspartic acid	8.19±0.02^{a}	9.18±0.04^{b}	8.59±0.02^{c}	9.47±0.04^{b}
Cysteine	0.60±0.00^{a}	0.94±0.02^{b}	0.82±0.02^{b}	1.20±0.00^{c}
Glutamic acid	10.39±0.08^{a}	11.54±0.02^{b}	10.66±0.03^{a}	12.20±0.08^{c}
Glycine	3.40±0.01^{a}	3.57±0.01^{b}	3.64±0.02^{bc}	3.83±0.02^{c}
Proline	3.06±0.01^{a}	3.43±0.01^{b}	3.24±0.0^{c}	3.47±0.01^{b}
Serine	3.19±0.01^{a}	4.06±0.02^{b}	3.68±0.01^{c}	4.06±0.02^{b}
Tyrosine	3.25±0.01^{a}	3.51±0.04^{ab}	3.37±0.04^{ab}	3.59±0.01^{b}
Total of NAA (g/100g protein)	35.50±0.04^{a}	40.29±0.08^{b}	37.61±0.02^{c}	41.87±0.10^{d}
Total	70.95±0.08^{a}	80.25±0.13^{b}	75.58±0.10^{c}	83.68±0.04^{d}

Values are expressed as means of three (3) replicates ± SEM (Standard Error of Mean). Values with different superscripts across the row are statistically ($p < 0.05$) significant. I= undefatted *Moringa oleifera* seed, J= *Moringa oleifera* seed defatted with n-hexane, K= *Moringa oleifera* seed defatted with acetone, L= *Moringa oleifera* seed defatted with a mixture of n-hexane and acetone in ratio 1:1.

remains essential for immune function, protein synthesis, and DNA synthesis. Even small amounts of zinc are vital for maintaining a variety of biological processes (Bonaventura *et al*., 2015; Wessels *et al*., 2017). Other minerals such as potassium (K), magnesium (Mg), and iron (Fe) contribute additional nutritional benefits. Potassium is key in regulating blood pressure and heart function, magnesium supports enzymatic reactions and muscle health, and iron is critical for hemoglobin production and oxygen transport in the blood (Jomova *et al*., 2022).

Amino acid profile

The result of the amino acid profile of the *M. oleifera* seed is presented in (Table 4). The result showed that the most abundant essential amino acids is arginine while tryptophan is the least amino acid in the kernel and its defatted form. The defatting process significantly ($p < 0.05$) increase the overall essential amino acids. The highest arginine content was observed in the sample L, kernel defatted with mixture of solvents followed by Sample J, kernel defatted with n-hexane though not statistically significant ($p > 0.05$), while the lowest was in the undefatted seed, Sample I. Arginine is known to promote wound healing and immune function, which are crucial in combating the negative impacts of PEM (Stechmiller, 2010). The increase in arginine after defatting suggests enhanced bioavailability of this essential amino acid.

Defatted samples J and L showed a significant increase ($p < 0.05$) in Histidine content compared to Sample I ($p < 0.05$), with Sample L having the highest value compared to undefatted and acetone defatted variants. Histidine is vital for growth and tissue repair, making it essential for recovery in PEM conditions (Siva Kiran *et al*., 2015).

Leucine and isoleucine are branched-chain amino acids (BCAAs) that play a key role in muscle protein synthesis. Leucine concentration significantly increased in mixture of solvent and n-hexane defatted variants, compared to undefatted and acetone defatted samples. This is particularly beneficial in addressing muscle wasting in PEM cases, where muscle breakdown exceeds muscle synthesis (Gorissen & Phillips, 2019).

Lysine, an essential amino acid important for collagen formation and protein utilization, increased significantly ($p < 0.05$) in defatted samples, with mixture of solvents defatted Sample showing the highest value followed by n-hexane defatted sample. In malnourished individuals, lysine deficiency can impair growth and immune responses (Morales *et al*., 2023).

The defatted process also significantly improves the Valine concentration with mixture of solvents defatted sample Sample L also showed the highest Valine value followed by n-hexane defatted sample, making them strong sources for improving the protein quality of diets for PEM treatment (Wei *et al*., 2021).

In general, the total Essential Amino Acids (EAA) concentration increased with defatting, which was significantly higher than the undefatted sample ($p < 0.05$). This improvement suggests that defatting *Moringa oleifera* seeds can enhance the nutritional quality of the seeds by concentrating the EAAs. This is crucial for addressing PEM, as EAAs are critical for muscle protein synthesis and metabolic recovery in malnourished individuals (Hoffer, 2016).

Non-essential amino acids, though synthesized by the body, contribute significantly to protein metabolism and overall health, especially in recovery from malnutrition. The result of the amino acids profile revealed that, glutamic acid and Cysteine were the most abundant and least abundant non-essential amino acids respectively. The defatted process significantly improves the overall non-essential amino acids. Alanine and aspartic acid are involved in glucose metabolism and energy production, which are critical in providing fuel for malnourished individuals (Remesar & Alemany, 2020). Cysteine is important for antioxidant production, particularly glutathione, which helps combat oxidative stress—a condition prevalent in PEM (Raj Rai *et al*., 2021). Glutamic acid serves as a precursor for glutamine, a vital nutrient for

intestinal health, and is essential in PEM recovery due to its role in maintaining gut integrity and promoting immune function (Aanandhi & John, 2017). Proline and glycine are elevated after defatting, enhances the structural protein content, crucial for rebuilding tissues in malnourished individuals (Albaugh *et al*., 2017).

Protein-energy malnutrition is characterized by a significant deficiency of both protein and energy in the diet, leading to muscle wasting, weakened immunity, and poor growth, particularly in children. The data from this study demonstrates that defatting *Moringa oleifera* seeds significantly enhances the protein quality by increasing the concentration of both essential and non-essential amino acids, making it a potent nutritional supplement for individuals suffering from PEM. The increase in branched-chain amino acids (leucine, isoleucine, Valine) is particularly beneficial for promoting muscle protein synthesis and preventing muscle wasting in PEM (Rajendram *et al*., 2015). The higher levels of glutamic acid and cysteine also suggest that defatted *Moringa oleifera* seeds can help restore immune function and combat oxidative stress, which is critical in malnourished individuals (Oladele *et al*., 2022).

In-vitro protein digestibility

Protein digestibility is an important metric, especially in the context of protein-energy malnutrition (PEM), as it determines how well proteins are broken down and absorbed, which is crucial for combating malnutrition. Figure 2 shows the result of the in-vitro protein digestibility.

The *In-invitro* protein digestibility (IVPD) of the undefatted *Moringa oleifera* seed (Sample I) was significantly lower than the defatted samples ($p < 0.05$). This lower digestibility may be attributed to the presence of higher fat content, which can interfere with protein breakdown and enzymatic action during digestion (Orlien *et al*., 2023). Undefatted seeds likely retain anti-nutritional factors, such as phytates and saponins, which are known to reduce protein digestibility by binding to proteins and digestive enzymes, limiting their functionality (Ohanenye *et al*., 2022). In malnourished populations, consuming foods with low protein digestibility exacerbates protein deficiencies, making it difficult to meet dietary protein needs (Schönfeldt & Hall, 2012).

The IVPD of seeds defatted with n-hexane (Sample J) was significantly higher indicating that defatting with n-hexane improves protein digestibility by removing fats that interfere with enzymatic action ($p < 0.05$). The removal of fats through solvent extraction enhances the access of proteolytic enzymes to protein molecules (Dzuvor *et al*., 2022). N-Hexane has been shown to effectively extract lipids without significantly denaturing proteins, preserving their structure and improving digestibility. In the context of PEM, diets formulated with defatted seeds could provide more bioavailable protein, which is crucial for restoring nitrogen balance and promoting growth (Chandran *et al*., 2023).

Sample K, defatted with acetone, showed a significantly ($p < 0.05$) lower digestibility than Sample J (defatted with n-hexane), though still significantly higher than the undefatted sample ($p < 0.05$). Acetone is a polar solvent and may have partially denatured some proteins during extraction, reducing their digestibility compared to n-hexane (Rose, 2019). Despite this, acetone extraction still improves digestibility by reducing the fat content. In terms of dietary application for protein-energy malnutrition, this sample would still be beneficial, though slightly less effective compared to n-hexane-defatted seeds in enhancing protein utilization (Ahmed *et al*., 2022; Elsebaie *et al*., 2023).

The highest protein digestibility was observed in Sample L, where a mixture of n-hexane and acetone (1:1 ratio) was used for defatting ($p < 0.05$). The combined use of these solvents likely maximized lipid removal without significant protein denaturation, providing the most accessible protein for digestion (Dzuvor *et al*., 2022).

Defatted Moringa seeds provide a lower fat content, allowing the digestive system to focus on protein breakdown. Additionally, removing fats can reduce the inhibitory effects of anti-nutritional factors on protein digestibility (Sardabi *et al*., 2022). For malnourished populations, consuming defatted Moringa seeds processed with optimal solvent extraction techniques could enhance protein utilization and improve nutritional outcomes (Gopalakrishnan *et al*., 2016).

Body weight

Figure 3 shows the effects of soya bean and defatted *Moringa oleifera* seed-based diets, on the weekly mean body weight of protein-energy malnourished rats over a four-week period. As shown, the control group, which was fed a commercial diet, experienced a steady and significant weight gain. This is expected, as the control group had access to a nutritionally balanced diet that supported normal growth and weight gain in the rats (Sousa *et al*., 2018). In contrast, the PEU (Protein Energy Undernourished) group, which was fed a low-protein isocaloric diet, showed little to no weight gain, maintaining an almost flat line throughout the study. This result is typical of protein-energy malnutrition, where a lack of adequate protein leads to stunted growth and muscle wasting. This supports the understanding that an insufficient protein supply directly affects weight maintenance in animals (de França *et al*., 2009). The groups treated with 15% soybean and 15% defatted *Moringa oleifera* seed (MO) showed significant ($p < 0.05$) increases in body weight compared to protein energy undernourished group. Previous studies indicate that soybeans and *Moringa oleifera* seeds are rich in high-quality protein and essential amino acids, which promote

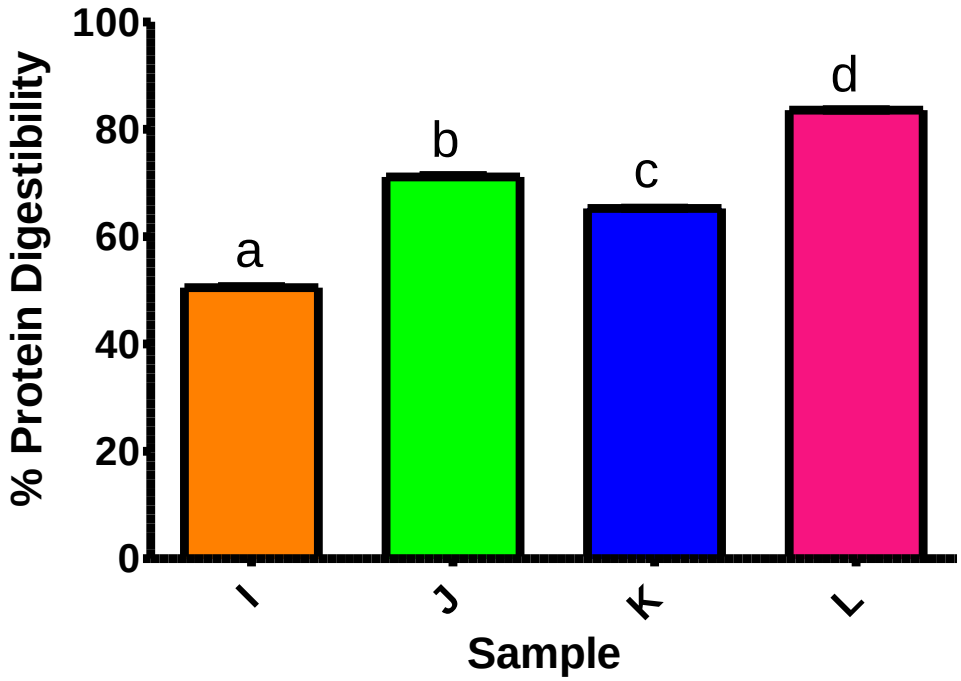

Figure 2: I*n-vitro* Protein Digestibility
Values are expressed as means of three (3) replicates ± SEM (Standard Error of Mean). Bars with different alphabets are statistically significant ($p < 0.05$) I= undefatted *Moringa oleifera* seed, J= *Moringa oleifera* seed defatted with n-hexane, K= *Moringa oleifera* seed defatted with acetone, L= *Moringa oleifera* seed defatted with a mixture of n-hexane and acetone in ratio 1:1

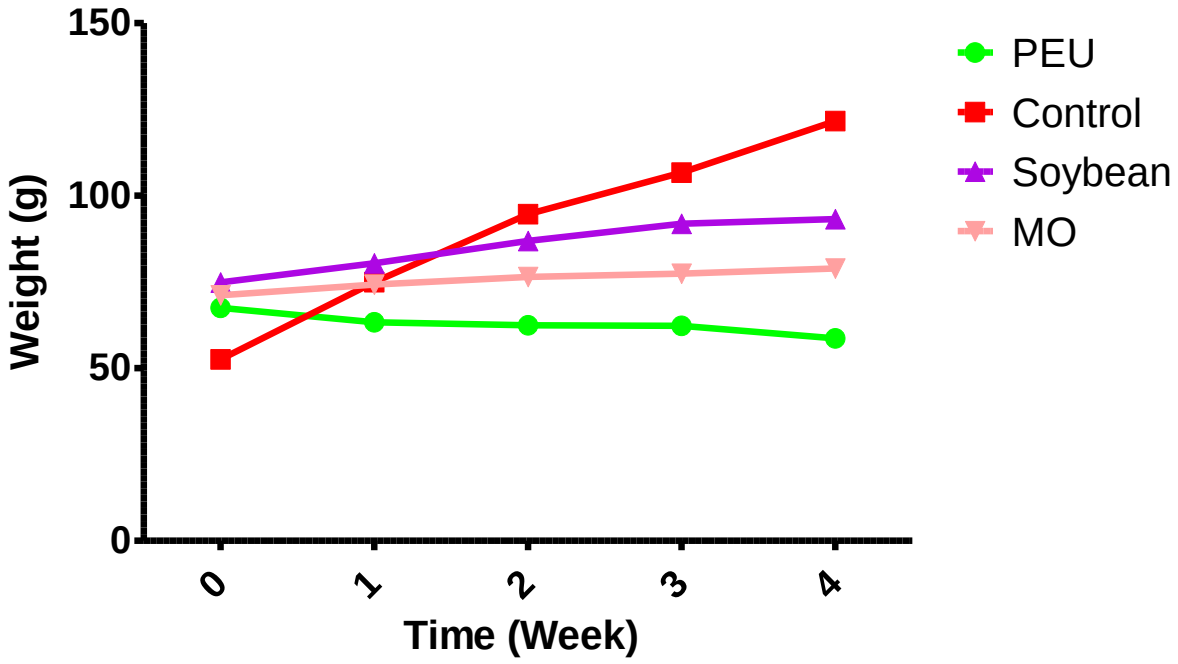

Figure 3: Effects of *M. oleifera* kernel based- diet on mean weekly body weight of protein energy malnourished rats
Values are expressed as means of five (5) replicates ± SEM (Standard Error of Mean). Control= Control Animals fed with commercial diet, PEU= Animals malnourished with low protein isocaloric based diet, Soya bean = undernourished animals treated with 15% soya been, MO= undernourished animals treated with 15% defatted *Moringa oleifera* seed.

Table 6: Effects of *Moringa oleifera* kernel based-diet on liver function indices of protein energy malnourished rats.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dL)	CB (mg/dL)	TP (g/dL)	ALB (g/dL)	GLO (g/dL)
A	249.75±7.05^{a}	105.50±9.15^{a}	254.50±13.01^{a}	6.48±1.44^{a}	2.90±0.80^{a}	71.00±4.80^{a}	37.75±1.80^{a}	33.25±3.07^{a}
B	296.75±20.70^{b}	121.00±14.34^{b}	741.25±40.44^{b}	15.60±3.15^{b}	6.20±1.28^{b}	60.00±1.08^{b}	31.50±0.65^{b}	28.50±0.50^{b}
C	267.00±13.89^{ab}	106.25±12.32^{a}	491.50±7.60^{b}	13.80±1.21^{b}	5.93±0.27^{b}	73.25±2.87^{a}	36.50±1.04^{a}	36.75±2.02^{a}
D	213.75±2.63^{c}	92.75±3.15^{c}	318.25±9.00^{ac}	6.15±0.46^{a}	3.08±0.287^{a}	77.00±2.08^{a}	39.75±1.11^{a}	37.25±1.18^{a}

Values are expressed as means of five (5) replicates ± SEM (Standard Error of Mean). Values with different superscripts down the column are statistically ($p < 0.05$) significant. AST= aspartate amino transferase, ALT= alanine amino transferase, ALP = Alkaline Phosphatase, TB = total bilirubin CB= conjugated bilirubin, TP= total protein, ALB= albumin, GLO= globulin: A= Control Animals fed with commercial diet, B= Animals malnourished with low protein isocaloric based diet, C= undernourished animals treated with 15% soya been, D= undernourished animals treated with 15% defatted *Moringa oleifera* seed.

muscle mass and overall growth (Abdel-Latif *et al*., 2022). However, *Moringa oleifera* seeds, though rich in nutrients, may have some anti-nutritional factors that reduce their efficacy compared to soybean, as noted in other studies. The partial defatting of *Moringa oleifera* may improve its protein content but could also reduce some lipid-based nutrients necessary for optimal growth (Soetan & Aiyelaagbe, 2016).

Biochemical analyses

Liver function

Table 6 shows the results of the effects of defatted *Moringa oleifera* kernel based-diet on liver function parameters of protein energy malnourished rats. The parameters measured—AST, ALT, ALP, TB, CB, TP, ALB, and GLO—are key indicators of liver health and metabolic function. The results show significant variations across the experimental groups, indicating both the effects of malnutrition and the efficacy of the treatments administered.

AST and ALT are liver enzymes that serve as markers of hepatocellular injury. Elevated levels typically indicate liver damage or inflammation. In this study, group B (malnourished rats fed with a low-protein isocaloric diet) had significantly ($p < 0.05$) elevated AST and ALT levels compared to the control (group A), reflecting hepatic stress induced by protein-energy malnutrition (PEM).

The rats treated with 15% soybean (group C) exhibited moderate AST and ALT levels compared to group B, suggesting partial recovery of liver function. However, the *Moringa oleifera* kernel-based diet-treated group (D) demonstrated the most significant ($p < 0.05$) reduction in AST and ALT, approaching normal levels. This indicates the potential hepatoprotective effects of *Moringa oleifera*, possibly due to its antioxidant and anti-inflammatory properties, which have been reported in previous studies (Atta *et al*., 2017). ALP is another marker of liver function, often elevated in conditions involving bile duct obstruction or hepatobiliary disease. In group B, malnourished group, ALP levels were significantly ($p < 0.05$) elevated, indicating severe hepatobiliary dysfunction due to malnutrition. However, treatment with both soybeans based-diet (group C) and defatted *Moringa oleifera* kernel based- diet (group D) significantly ($p < 0.05$) lowered ALP levels, with group D showing the greatest improvement. The reduction in ALP suggests that both diets improved bile flow and overall liver function, with *Moringa oleifera* being more effective in restoring liver enzyme levels to near-normal values (Ekong *et al*., 2022).

Total bilirubin (TB) and conjugated bilirubin (CB) are markers of liver excretory function. Elevated levels of bilirubin, as observed in group B (malnourished group), indicate impaired liver function and bile excretion. These elevated bilirubin levels confirm the presence of cholestasis or hepatic damage due to malnutrition. In contrast, groups C and D showed significant ($p < 0.05$) reductions in both total and conjugated bilirubin levels. The values of Group D are not significant ($p > 0.05$) different compared to the control values, further indicating that *Moringa oleifera* seed has a restorative effect on liver excretory function (Mhlomi *et al*., 2022). Total protein, albumin, and globulin levels are crucial indicators of nutritional status and liver synthetic function. Protein-energy malnutrition leads to a decline in protein synthesis, reflected in the significant ($p < 0.05$) reductions in Total Protein, Albumin, and Globulin in group B (malnourished rats). The decrease in these proteins compromises immune function and fluid balance, as albumin helps maintain osmotic pressure (Amir, 2003). Rats in group C (treated with 15% soybean) and group D (treated with Moringa oleifera) showed improved protein levels. Group D displayed higher Total protein, albumin and globulin compared to group C, suggesting that *Moringa oleifera* was more effective in promoting protein synthesis and liver function restoration. This could be due to the high nutritional content and bioactive compounds found in *Moringa oleifera*, including essential amino acids and phytochemicals that support protein metabolism (Ayodele *et al*., 2021).

Conclusion

Moringa oleifera seeds, when defatted, present a cost-effective and nutritionally rich option for the management of protein energy malnutrition. Given the accessibility and

affordability of *Moringa oleifera* in regions prone to malnutrition, its incorporation into dietary interventions could have significant impacts on reducing the prevalence of PEM and associated health complications. Further research and clinical trials are recommended to confirm its efficacy in human populations and explore its broader applications in nutritional therapies.

Conflict of interest

The authors declared that the research has been thoroughly carried out and no conflict of interest

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