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# Assessment of Rats Fed Protein-Deficient Diets Supplemented with Moringa Oleifera Leaf Meal

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# Abstract

This report is part of a larger study on bioavailability of nutrients in Moringa oleifera leaf meal (MOLM) grown in South Africa. Albino male rats were assigned into five groups each having a replicate of five; PD-protein deficient diet, PD3, PD5 and PD10 had protein insufficient diets augmented with MOLM at 3, 5 and 10% respectively, while control (C)- was fed normal rat chow, for 28 days. Growth performances were recorded weekly, blood biochemistry and histopathology of the animals were evaluated in the serum and selected organs respectively. Severe loss in body weight caused by PD was slightly ameliorated by MOLM supplementation; improved protein quality indicated by higher red blood cells count was noticed in animals fed the augmented diets, while decrease in lymphocytes count reflected possible leucopoiesis and immunomodulatory effect of MOLM. High platelet counts (807.5 x109/L-1011.27x109/L) could indicate secondary anaemia caused by PD, while decrease in calcium and total protein was improved by MOLM. Increase in creatinine for all treatments could imply that kidney function was compromised. Serum ALP, ALT and AST activities were down regulated significantly in animals fed MOLM diets, suggesting organ-protective properties of Moringa. Histopathological evaluation of organs supported the biochemical findings and confirmed the negative effect of dietary protein deficiency. Although MOLM is nutrient-rich, unprocessed MOLM impacted negatively on physiology of the rats and could not completely replace protein in the diet.



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### **Keywords**

Bioavailability; Histopathology; Haematology; *Moringa Oleifera*; Protein Deficiency.

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### Introduction

*Moringa oleifera* Lam. plant originated in South Asia, though now found in most parts of the world.<sup>1</sup> It is a fast growing plant widely distributed in the tropics and subtropics with numerous important uses for food, industrial and medicinal applications. The leaves of the plant are very rich sources of macro and micro nutrients.<sup>2,3</sup>

Globally, protein, vitamin A, iodine and iron insufficiencies abound especially among children and account for health concerns. These deficiencies including stunting in children and young women are endemic in South Africa.<sup>4</sup> This condition has not changed much despite economic growth and national nutrition programs, as stunting remains persistent and prevalent in South Africa.<sup>5</sup>

The use of *M. oleifera* in many parts of the world as a nutritious addition to the diet is on the increase. For instance, leaves of the plant are added to meals in most African nations like Nigeria, Malawi, and Ethiopia because of easy availability, high nutritional and nutraceautical values.<sup>6</sup> The current use of *M. oleifera* leaves in soups, weaning foods, breads, cakes, cookies and yoghurt<sup>7-9</sup> have been reported. Dietary contents usually have an effect on the blood profile of healthy individuals;<sup>10</sup>haematological components are useful in measuring toxicity, physiological and health status of farm animals and are often used to determine stress caused by nutrient deficiencies.<sup>11-13</sup>

*Moringa oleifera* has recently been introduced to South Africa for nutritional purposes. While the leaves have been shown to be high in proteins and other nutrients,<sup>14</sup> the accessibility of these nutrients and the impact of using MOLM as a sole protein source in diets are still not well documented.

Therefore, this study investigates the bioavailability of nutrients in MOLM using protein-deficient rat models.

### Materials and Methods Plant Materials

The plant materials were obtained from Limpopo Province of South Africa in the Tooseng village of Ga-Maphahlela with coordinates of (24°26'57".10S, 29°33'47".02E). The dried leaves were pulverized into powder and kept in an airtight container at 4 °C until needed.

## **Experimental Diets**

Five diets were formulated using the basic ingredients [wheat bran, maize, fish meal (which served as a protein source), vegetable oil, vitamins and mineral premixes] for normal rat chow. The control diet contained all the required nutrients, while the protein deficient diet (PD-no fish meal) which served as negative control, was deficient in protein. PD3, PD5 & PD10 were deficient in protein but with inclusion of MOLM at 3, 5 and 10% respectively (Table 1).

### **Experimental Rats and Treatments**

Twenty five healthy, male Wistar rats weighing between 220-290 g were randomly allocated and replicated into 5 groups of 5 animals each. They were kept under 12 h light and dark cycle; temp of 22±2°C, with no restrictions on feed and water. Ethical approval for the study was granted by the Animal Research Ethics Committee: AREC: OTU011SMHL01 University of Fort Hare, South Africa.

| Ingredients (%)                   | Control (C) | PD   | PD3  | PD5  | PD10 |  |
|-----------------------------------|-------------|------|------|------|------|--|
| Maize                             | 57.7        | 57.7 | 57.7 | 57.7 | 57.7 |  |
| Wheat bran                        | 7.3         | 30.9 | 27.9 | 25.9 | 20.9 |  |
| Sunflower oil                     | 7.5         | 7.5  | 7.5  | 7.5  | 7.5  |  |
| Fishmeal                          | 23.6        | -    | -    | -    | -    |  |
| Vitamin premix                    | 2.5         | 1.4  | 1.4  | 1.4  | 1.4  |  |
| Mineral premix                    | 1.4         | 2.5  | 2.5  | 2.5  | 2.5  |  |
| Moringa oleifera leaf meal (MOLM) | -           | -    | 3    | 5    | 10   |  |
|                                   |             |      |      |      |      |  |

### Table 1: Composition of Experimental diets

## **Performance Evaluations**

The experimental animals were monitored for mortality, morbidity or any abnormal behavioral and physical changes two times a day. The weights of all the animals were recorded on the first day of the experiment, then weekly and feed intake daily. Growth parameters were measured as previously described.<sup>15</sup>

Feed consumption was calculated by subtracting the amount of left-over feed from feed offered after 24 hours.

Body weight gain (%) =  $\frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$ 

Feed efficiency ratio (FER) = Body weight gain/ feed intake

Protein efficiency ratio (PER) = Body weight gain/ protein consumed

Rats refrained from feeding overnight were euthanized under mild anesthesia on the 29<sup>th</sup> day and both haematology and serum biochemistry respectively were evaluated in blood collected by cardiac puncture.

### Histopathological examination

Liver, kidney and heart already fixed in 10 % formalin were thinly sliced, coated with paraffin wax and treated with haematoxylin/eosin, then assessed with the light microscope for anomalies.

### **Data Analysis**

All data were analyzed by ANOVA, followed by Fisher's least significant difference test and values were considered significant at P < 0.05 using MINITAB version 17 for windows.

# Results

# **Proximal Content**

Table 2 shows that MOLM had 28% crude protein and the highest total ash content. Moisture content was higher in MOLM supplemented diets and a statistically insignificant decrease (P>0.05) in moisture, ash, ADF and NDF with increasing levels of MOLM. Crude protein was lowest in PD at P<0.05, crude fiber was highest in PD3 (12.59%) and least in PD (8.26%) and total carbohydrate was highest in PD followed by PD10, PD3, PD5 and control. Energy value ranged from 277.16 in MOLM to 337.89 in PD.

| Parameters    | MOLM        | Control                   | PD                       | PD3                      | PD5                      | PD10                    |
|---------------|-------------|---------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Moisture      | 8.36±0.15   | 7.7±0.04ª                 | 7.91±0.30ª               | 7.97±0.15ª               | 7.76±0.05ª               | 6.52±0.33ª              |
| Ash           | 8.72±0.01   | 8.05±0.15ª                | 5.54±0.47⁵               | 6.39±0.18 <sup>₅</sup>   | 5.97±0.54 <sup>b</sup>   | 5.96±0.63 <sup>b</sup>  |
| Fat           | 5.22±0.20   | 2.53±0.11ª                | 2.53±0.01ª               | 1.58±0.04 <sup>♭</sup>   | 2.37±0.29ª               | 2.31±0.06ª              |
| ADF           | 25.19±0.87  | 10.51±11.96ª              | 10.33±0.57ª              | 15.74±0.42ª              | 14.98±0.27ª              | 13.70±1.10ª             |
| NDF           | 37.55±1.29  | 35.42±0.57 <sup>abc</sup> | 32.93±0.37 <sup>bc</sup> | 36.97±2.11ª              | 35.61±0.46 <sup>ab</sup> | 32.31±1.5°              |
| Protein       | 28.72±0.18  | 24.81±1.31ª               | 9.95±0.54°               | 10.95±0.03bc             | 11.30±0.08 <sup>bc</sup> | 12.2±0.20 <sup>b</sup>  |
| Crude fibre   | 20.15±0.69  | 8.41±9.57ª                | 8.26±0.45ª               | 12.59±0.3 ª4             | 11.99±0.22ª              | 10.96±0.88ª             |
| Carbohydrates | 28.83±0.73  | 48.51±10.88ª              | 65.82±1.76 <sup>₅</sup>  | 60.53±0.31 <sup>ab</sup> | 60.64±0.35 <sup>ab</sup> | 62.06±0.21 <sup>b</sup> |
| Energy        | 277.16±1.96 | 316.03±39.29ª             | 337.89±21.95ª            | 300.10±1.04ª             | 308.96±4.24ª             | 317.79±0.63ª            |

| Table 2: Proximal | content of Moringa | oleifera leaf meal | (MOLM) | and feeds |
|-------------------|--------------------|--------------------|--------|-----------|
|                   |                    |                    |        |           |

Values are means  $\pm$ SD, n=5. Values with different superscripts along a row are considered significantly different at p<0.05. The same superscript letters on values in the same row indicate statistically insignificant differences (p<0.05); two or more superscripts on a value indicates numerical difference but no statistical significant difference.

Micronutrient contents of the experimental diets (Table 3), showed that no significant difference was observed for all the experimental diets compared to control, although numerical increase was present in MOLM supplemented diets and the control had significantly higher Na, Ca, K and P contents.

| Elements<br>(mg/100g) | MOLM       | Control      | PD          | PD3          | PD5         | PD10        |
|-----------------------|------------|--------------|-------------|--------------|-------------|-------------|
| Ca                    | 1.60±0.02  | 1.79±0.01a   | 0.66±0.00b  | 0.86±0.19b   | 0.79±0.01b  | 0.82±0.08b  |
| Mg                    | 0.44±0.00  | 0.16±0.01 a  | 0.18±0.00b  | 0.18±0.01b   | 0.2±0.00c   | 0.20±0.01bc |
| к                     | 1.69±0.02  | 0.51±0.01 a  | 0.59±0.01b  | 0.61±0.04b   | 0.66±0.01c  | 0.68±0.01c  |
| Na                    | ND         | 0.19±0.01 a  | 0.01±0.00b  | 0.01±0.01 b  | 0.01±0.00 b | 0.00 b      |
| K/Ca+Mg               | 0.27±0.16  | 0.13± 0.00 a | 0.32±0.01 b | 0.28±0.04 b  | 0.30±0.01 b | 0.31±0.02 b |
| P                     | ND         | 0.85±0.01 a  | 0.39±0.01 b | 0.37±0.02bc  | 0.38±0.01bc | 0.34±0.01 c |
| Zn                    | 2.77±0.05  | 239.95±1.34  | 232.4±2.97  | 257.5±17.82  | 256.25±3.61 | 248.5±7.35  |
| Cu                    | 0.9±0.00   | 27.2±0.00    | 29.35±0.07  | 31.15±1.91   | 30.4±0.00   | 29.45±0.92  |
| Mn                    | 22.83±3.01 | 242.8±7.21   | 283.95±6.72 | 334.5±44.69  | 303.8±9.48  | 298.15±3.89 |
| Fe                    | 21.13±0.84 | 157.05±13.36 | 156.1±5.52  | 176.85±21.28 | 167.45±3.32 | 167.75±1.34 |

Table 3: Mineral composition of Moringa oleifera leaf meal (MOLM) and feeds

Values are means ± SD, n=5. Values with different superscripts along a row are considered significantly different at p<0.05. The same superscript letters on values in the same row indicate statistically insignificant differences (p<0.05); two or more superscripts on a value indicates numerical difference but no statistical significant difference. Ca: Calcium; Mg: Magnesium; K: Potasium; Na: Sodium; P: Phosphorus; Zn: zinc; Cu: copper; Mn: Manganese; Fe: Iron. ND-not detected.

#### **Performance Indices**

Table 4 presents the growth parameters of the animals and reveals that though feed intake was similar for all treatments, feed consumption and weight gain was greatly reduced with MOLM augmentation. Rats fed PD and PD5 consumed significantly less feed than those fed PD10, PD3 diets respectively. Feed efficiency ratio also decreased as MOLM increased, with rats fed PD diet having lowest value of -2.04. kidney was observed but liver showed significant reduction (P<0.05) as MOLM inclusion increased. Rats fed control and PD diets showed higher (P<0.05) liver weights, but these organ weights decreased significantly with increase in MOLM supplementation.

Besides the difference in feed intake, animals showed significantly reduced weekly weight gain (Figure 1).

No significant differences among treatment means of the organ weights including heart and

| Parameters           | Control   | PD          | PD3          | PD5          | PD10         |
|----------------------|-----------|-------------|--------------|--------------|--------------|
| Initial weight (g)   | 294.78    | 285.64±2.01 | 224.43±1.40  | 249.02±0.87  | 256.38±3.41  |
| Final weight (g)     | 318.51    | 224.65±8.94 | 209.29±30.02 | 210.41±21.46 | 213.19±31.53 |
| Weight gain/loss (g) | 8.05      | -21.35      | -6.75        | -15.50       | -16.85       |
| Feed intake (g)      | 15.95     | 10.42       | 12.63        | 10.66        | 11.36        |
| FER                  | 0.50      | -2.04       | -0.53        | -1.45        | -1.48        |
| PER                  | 0.06      | 2.73        | 1.38         | 3.42         | 3.54         |
| Liver weight (g)     | 12.04     | 8.35        | 7.1          | 6.77         | 5.33         |
| Kidney weight (g)    | 2.58      | 1.82        | 1.63         | 1.55         | 1.68         |
| Heart weight (g)     | 1.03±0.07 | 0.84±0.07   | 0.73±0.12    | 0.74±0.09    | 0.72±0.12    |
|                      |           |             |              |              |              |

### Table 4: Performance indices of rats fed on the experimental diets

#### **Haematological Parameters**

Haematological and WBC differentials are presented in Table 5. White blood cells were significantly higher

in PD3 rats compared to the other diets; and the white blood differentials followed the same trend, but with no significant (P > 0.05) difference. A gradual

non-significant increase was observed in red blood cells with increase in MOLM in diets when compared to the control and PD, though a noteworthy (P < 0.05) reduction in RBC count was evident in animas on the PD diet.

Table 6 shows that no notable difference (P>0.05) was observed in the calcium, chloride and magnesium content for all treatments including control; however a significant increase in Na with

an increase in MOLM was noted. Significant increase in serum creatinine of rats fed protein deficient diets compared to control was also observed, while total protein and albumin were lowest in animals on PD diet. Animals fed on the PD3, PD5 and PD10 diets exhibited increased serum total protein. On the contrary total and conjugated bilirubin decreased with increasing MOLM supplementation, though the values were within the normal range for healthy albino rats



Fig. 1: a) Weekly body weight (g); (b) weekly body weight gain/loss of rats fed on experimental diets

| Table 5: Blood | parameters and | white blood cells | differentials of | rats fed on | experimental diets |
|----------------|----------------|-------------------|------------------|-------------|--------------------|
|----------------|----------------|-------------------|------------------|-------------|--------------------|

| Parameters        | Control     | PD                       | PD3                    | PD5                       | PD10                     |
|-------------------|-------------|--------------------------|------------------------|---------------------------|--------------------------|
| WBC (x109/L)      | 10.23±5.62ª | 11.42±1.76ª              | 12.10±1.33ªb           | 9.84±2.40 <sup>ab</sup>   | 6.39±0.91⁵               |
| Neutro (X 109/L)  | 0.74±0.58ª  | 0.69±0.11ª               | 0.5±0.03               | 0.31±0.11                 | 0.21±0.04                |
| Lymph (X109/L)    | 5.39±2.09ª  | 6.24±1.69ª               | 5.78±0.35ª             | 4.31±1.68ª                | 3.41±0.82ª               |
| Mono (X109/L)     | 2.83±2.15ª  | 2.54±0.13ª               | 3.28±0.38ª             | 1.36±1.09ª                | 1.88±0.78ª               |
| Eosino (X109/L)   | 0.08±0.04ª  | 0.12±0.03ª               | 0.05±0.01 <sup>b</sup> | 0.07±0.03 <sup>ab</sup>   | 0.05±0.02 <sup>ab</sup>  |
| Basophil (X109/L) | 0.09±0.08ª  | 0.07±0.02ª               | 0.05±0.01ª             | 0.04±0.02ª                | 0.03±0.01 <sup>a</sup>   |
| RBC (X1012/L)     | 9.08±0.25ª  | 8.63±0.56ª               | 9.46±0.29ª             | 9.52±0.38ª                | 9.42±0.22ª               |
| Hb (g/dL)         | 16.9±0.42ª  | 16.47±0.21 <sup>ab</sup> | 16.47±0.46°            | 16.33±0.15 <sup>abc</sup> | 15.63±0.23 <sup>bc</sup> |
| He (L/L)          | 0.53±0.01ª  | 0.52±0.01ª               | 0.53±0.01ª             | 0.51±0.02ª                | 0.52±0.01ª               |

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|--|--------------|------------|--------------------|-----------|-----------|------|
|--|--------------|------------|--------------------|-----------|-----------|------|

| MCV (fL)           | 58.2±0.14ª              | 57.57±0.89 <sup>a</sup> | 55.1±0.5 <sup>b</sup>    | 53.4±0.52 <sup>b</sup>  | 54.67±0.74 <sup>b</sup> |
|--------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|
| MCH (pg)           | 18.6±0.99ª <sup>b</sup> | 18.33±0.41 <sup>a</sup> | 17.57±1.42 <sup>ab</sup> | 17.17±0.75 <sup>b</sup> | 16.63±0.47 <sup>b</sup> |
| MCHC (g/dL)        | 32+1.56ª                | 32 17+0 97 <sup>a</sup> | 30.8+0.92ª               | 31.9+1.21ª              | 30.37±0.96 <sup>a</sup> |
| MPV (fL)           | 9.6±0.28ª               | 9.2±0.61 <sup>ab</sup>  | 8.37±0.23°               | 8.5±0.2°                | 8.6±0.2 <sup>bc</sup>   |
| RDW%               | 13.05±0.21ªb            | 13.5±0.26 <sup>a</sup>  | 12.6±0.26 <sup>b</sup>   | 12.83±0.23ªb            | 12.5±0.7 <sup>b</sup>   |
| Platelets (X109/L) | 807.5±176.07ª           | 898±113.53ª             | 1011.27±83.79ª           | 896±131.64ª             | 906±160.48ª             |

Values are means ±SD, n=5. Values with different superscriptsS along a row are considered significantly different at p<0.05. The same superscript letters on values in the same row indicate statistically insignificant differences (p<0.05); two or more superscripts on a value indicates numerical difference but no statistical significant difference. Lymph: Lymphocyte, WBC: white blood cell, Neutro: Neutrophils, Mono: Monocytes, Eosino: Eosinophils RBC: Red blood cells; Hb: Hemoglobin, He: Haematocrit, MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentrate; MPV=; RDW= red cell width.

Serum enzymes activities for liver, heart and kidney functions (Figure 2) of the experimental animals revealed that animals on the control and PD diets had elevated ALT, AST and ALP activities, which were notably (P < 0.05) and dose-dependently reduced as a result of MOLM augmentation. ALP did not follow a particular trend but was lowest in rats fed the PD5 diet.

| Parameters              | Control     | PD           | PD3          | PD5          | PD10         |
|-------------------------|-------------|--------------|--------------|--------------|--------------|
| Na (mmol/dL)            | 139±1.73a   | 140.67±1.53  | 141.67±1.15b | 142±0b       | 144.33±0.58c |
| Chloride (mmol/dL)      | 101.5±0.71a | 105.67±2.31b | 105.33±1.15b | 107.33±1.15b | 106.67±1.53b |
| Calcium (mg/dL)         | 2.52±0.07a  | 2.53±0.02a   | 2.47±0.05a   | 2.49±0.06a   | 2.47±0.04a   |
| Magnesium (mmol/dL)     | 1.13±0.09a  | 1.12±0.07    | 1.05±0.07ab  | 0.97±0.01b   | 1.04±0.03ab  |
| Creatinine (mg/dL)      | 34.67±3.21a | 42.33±4.51b  | 38±3ab       | 43±2.65b     | 43.33±4.04b  |
| Total protein (g/dL)    | 57±3.61a    | 52±1.73a     | 53±3.61a     | 53.33±3.21a  | 53±1.53a     |
| Albumin (g/dL)          | 18.67±1.15a | 18±1a        | 18.33±0.58a  | 18±1a        | 18.67±0.58a  |
| Total bilirubin (mg/dL) | 23±5.29a    | 31±10.54b    | 21±2.65ab    | 21±1.73ab    | 20.33±2.31b  |
| C. bilirubin (mg/dL)    | 9.67±1.53a  | 13.67±4.04b  | 10.33±1.15a  | 9.33±2.52a   | 9.33±1.53a   |
| Glucose (mg/dL)         | 4.53±0.23a  | 4.6±0.26a    | 4.93±0.38a   | 3.73±0.21b   | 4.5±0.53a    |
|                         |             |              |              |              |              |

### Table 6: Biochemical indices of rats fed on experimental diets

Values are means  $\pm$ SD, n=5. Values with different superscripts along a row are considered significantly different at p<0.05. The same superscript letters on values in the same row indicate statistically insignificant differences (p<0.05); two or more superscripts on a value indicates numerical difference but no statistical significant difference. Na: Sodium, C. bilirubin: Conjugated bilirubin



Fig. 2: Serum enzyme activities of rats fed on experimental diets

Figure 3 shows the effect of protein deficient diets and MOLM supplementation on selected organs of rats. No abnormalities were observed in the architecture of the kidney, heart and liver and of rats fed on the control diet, though mild congestion in liver and heart was present in rats fed the PD diet. No visible abnormalities were observed in the heart, kidney and liver of animals on the PD 10 diet, but cardiac muscles of PD and PD3 rats exhibited myocardial fibers showing intense eosinophilia.



Fig. 3: Photomicrographs of heart, kidney and liver of rats fed control; (A1, A2, A3) protein deficient
(PD): (B1, B2, B3) and MOLM supplemented (PD3, (C1, C2, C3): PD5 (D1, D2, D3) and PD10 (E1, E2, E3)) diets. (A1, B1, C1, D1, E1): heart; (A2, B2, C2, D2, E2): kidney; (A3, B3, C3, D3, E3): liver

### Discussion

Although the high nutritional and pharmacological attributes of *Moringa oleifera* leaves makes it a potential dietary supplement for both humans and animals, lots of inconsistencies on feeding trials and not much evidence on nutrient bioavailability reports are available in literature. According to <sup>16</sup>foods with low moisture content have longer shelf life. Generally, any food that can provide 12% and more is considered a good source of protein. Thus, all the diets except the PD contained sufficient amount of protein required by the rats. Crude fiber, fat, carbohydrates and ash were all sufficient in the diets and mineral content of the diets was increased with MOLM in diets.

Total removal of dietary protein (PD) affected growth and nutrient utilization in the rats which was reflected through the significantly retarded performance indices ratios of rats. The unexpected growth retardation in rats fed MOLM could be attributed to the presence of anti-nutrients, although some studies have reported that M. oleifera leaf extract has no adverse effects in humans, rats, rabbits or poultry.17,18 In addition, the depressed growth could be linked with unpalatability of the diet which caused reduced feed intake at higher substitution level. Saponins and tannins present in the MOLM are known to have a bitter taste that might have impacted on the feed<sup>19</sup> resulting in reduced intake most especially as the MOLM supplementation increased. The study of<sup>20</sup> reported that taste and texture influence the feed intake of animals, moreover, reduced feed consumption could be attributed to bad taste of the feed caused by bitterness of the leaf meal which reduced palatability. Also, reduction in feed intake observed in rats fed the PD5 and PD10 diets may be due to high crude fiber content which invariably reduced palatability.21,22 The observed decrease in organ weights with increase in MOLM may be a response to the body weight changes since there was a decrease in weight as inclusion levels of MOLM increased.

The loss in body weight of PD and MOLM supplemented diets agree with<sup>23</sup> who reported that protein-deficiency leads to great loss in body mass. Also<sup>24</sup> showed that MOLM substitution up to 20% decreased growth in broilers, while<sup>25</sup> reported that MOLM enhanced broiler chicks' growth at up to 3%. In addition<sup>26</sup> also reported that including MOLM as a dietary supplement in West African dwarf goats diets had no significant impact on body weight gain and dry matter intake.

These results suggest that, although *Moringa oleifera* leaves are nutrient-rich, availability and utilization of the nutrients may be limited, thus it cannot completely replace protein in the diet in the raw unprocessed form as was used in this study.

No notable difference was observed for haematological indices among animals fed the experimental diets, except for decreased red blood cells in PD rats. Higher RBC values recorded for rats fed on MOLM diets indicated blood quality ascribed to improved protein content and quality. This is similar to the report of<sup>27</sup> that high protein quality feeds results in increased RBC and healthy animals. These observations agree with<sup>28</sup> when MOLM was used as a substitute for antibiotics in broiler chicks.

The insignificant decrease in the PCV and Hb values of rats were within range of values for normal rats, though on the contrary to the report of<sup>29</sup> of general dose dependent increase in haematological parameters when Moringa leaf extract was administered to rats in a sub-toxicity study. The decrease in haemoglobin and haematocrit observed is an indication that the protein content of *Moringa oleifera* leaf meal alone was not sufficient to compensate for the total deficiency in diet, as well as the result of residual anti-nutrients present in Moringa leaves. This is in accordance with the findings of<sup>30</sup> who reported that deficiency of protein had no negative effect on platelet and red cell counts in rats fed cassava peel-based diets.

The change in lymphocytes count with increasing MOLM indicates that moringa has possible leukopoietic and immunodulatory effect. The bioactive components in MOLM are responsible for these biological functions and the capacity of the rats to avoid diseases.<sup>31</sup> Eosinophils are responsible

for allergic reactions and disorders and increase with allergic conditions, stress or infection. The decrease in eosinophils observed with increasing MOLM compared to positive and negative controls (PD) might be an indication that MOLM had a protective effect against infections. Neutrophils carry out phagocytosis of pathogenic microorganisms in the first few hours after their entry into tissue.32 The significant decline noticed for neutrophils in this study as a result of leucopoiesis suppression was similar to that reported by.33 Defense of tissues against microbial agents is the responsibility of monocytes, while basophils counts play an important role in immune responses. According to<sup>34</sup> the normal values range from 0-1% while the values detected in this study ranged from 0.03-0.09 in all the treatments. Platelets are implicated in blood clotting and play a vital role in reducing blood loss and vital in cellular repair and a range of 702-796 or 720-746 t/mm have been reported for healthy rats.<sup>35</sup> Long-term reduction in platelet count results in internal and external haemorrhage and finally death.<sup>36</sup> However, the observed higher platelet values in this study may indicate secondary anemia attributed to protein deficiency. According to<sup>37</sup> even when caloric intake is sufficient, protein insufficiency will cause anaemia and inadequate production of red blood cells. Sodium is important in regulating the water needed by the body, excess sodium can cause cell malfunction and too little can be fatal.38 Reduced calcium levels observed is attributed to protein deficiency and indicates that supplementation with MOLM improved availability of protein and agrees with the findings of.23

Creatinine is important in assessing kidney function, increase in creatinine is indicative of reduced kidney function<sup>37</sup> the observed increase in creatinine in all the treatments compared to control could imply that kidney function was compromised by protein deficiency.

Physicians often use albumin levels to determine the nutritional status of patients.<sup>39</sup> Increase in albumin above normal range indicates stress on the liver, but as observed in this study, all the treatments and control exhibited similar serum albumin concentration. This agrees with the findings of<sup>40</sup> and<sup>36</sup> for rats fed diets supplemented with Spirulina. The increased bilirubin levels in rats fed on PD diet is however, indicative of decreased hepatic clearance caused by protein deficiency.<sup>41</sup>

Addition of MOLM to protein-deficient diets significantly lowered the activities of ALT and AST which are marker enzymes of liver function in a dose-responsive manner and could mean that Moringa has hepato protective properties, while ALP activity suggests hepato and nephroprotective properties of Moringa. These protective actions of MOLM on tissues of the body are attributed to phytochemicals and bioactive components.<sup>42</sup>

Histopathology evaluation supported the biochemical findings and confirmed further, the negative effects of protein deficient diet, while supporting the ameliorative effect of MOLM as a food supplement and protein source as well as its protective properties against tissue and organ damage.

# Conclusion

Moringa leaves are highly rich in macro and micronutrients and thus can be used to alleviate related problems such as protein malnutrition in vulnerable communities. Based on the results of proximate assays MOLM could be used as a supplement for protein in food formulation. The present study revealed that unprocessed MOLM was detrimental to feed intake, well-being and performance in the animals. However, haematology, serum biochemistry and enzyme function suggest that MOLM has the potential to defend the body against infection and also has hematinic and blood enhancing qualities. Nevertheless, more investigations, including human trials are essential before conclusions on the availability of nutrients especially protein in MOLM or its use as a protein substitute can be drawn.

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### **Conflict of Interest**

The authors declare no conflicting interest.

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