OPEN ACCESS
Scholars International Journal of Biochemistry
Abbreviated Key Title: Sch Int J Biochem
ISSN 2616-8650 (Print) |ISSN 2617-3476 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Effects of Weaning Diets Supplemented with *Moringa oleifera* Leaf Powder on the Biochemical and Hematological Indices of Weanling Wistar Rats

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DOI: 10.36348/sijb.2022.v05i03.001 | **Received:** 03.02.2022 | **Accepted:** 07.03.2022 | **Published:** 22.03.2022

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Abstract

The effect of weaning diets formulated locally were assessed on weanling wistar rats and compared with a commercial brand to ascertain its use as weaning diets for infants in resource-poor nations. The raw ingredients were processed using local methods into different diets and fed to weanling rats weighing between 45-60 g. Thirty weanling wistar rats were divided into six groups of five animals each and were allowed access to food and water ad-libitum for 28 days following standard procedures. Results of the antinutritive components of the diets revealed that phytate recorded the least amount of 0.29±0.00 mg/110g while oxalate was highest (18.12±0.07) in diet formulated majorly with plant materials. Organ weights of the experimental animals showed that animals fed the locally formulated diets compared favourably with the commercial diet while the least weights observed in animals fed protein-free diets. The Biochemical and hematological assessment of the serum of experimental animals did not show any marked difference in all the experimental groups. From the result, it can be deduced that the locally formulated diets did not pose any adverse health effect on rats and therefore is safe for use as infant diets.

Keywords: weanling wistar rats, experimental animals, oxalate, diet.

1.0 INTRODUCTION

Malnutrition among children aged under the age of 5 years has been a major public health and wellness concern in developing countries. Malnutrition a state of nutrition where there is excess, deficiency or imbalance of protein, energy or other nutrients in the body causing observable adverse effects on body function and clinical outcome [1] is a leading cause of childhood mortality in Nigeria [2]. Several studies have examined childhood malnutrition in Africa, and especially in Nigeria, and have identified risk factors for malnutrition that are not limited to maternal health status, inappropriate care, socioeconomic status of parents, frequent illness, and the geographical location of the children [3,4,5]. Malnutrition has three indicators including stunting, wasting and underweight (underweight for age) [6]. It has been reported globally that malnutrition is a major cause of child mortality with nearly half of all deaths in children under the age of 5 years attributed to under-nutrition [1]. The United Nations International Children's Fund (UNICEF) data on malnutrition in 2019 estimated that 149 million children were stunted in 2018, down from 198.2 million children in the year 2000 with Africa and Asia bearing the greatest share of all forms of malnutrition [7]. Children who are malnourished are exposed to adverse health consequences which are both short- and long-term, including poor mental development and increased risk of mortality arising from all causes, including diarrhoea, pneumonia and measles [8]. The degree of cognitive impairment is directly related to the severity of stunting; mental impairment due to iodine deficiency is permanent and is directly linked to a decline in economic productivity [9]. Chronic malnutrition has been linked with impaired development of the thymus, leading to a decline in the peripheral lymphocyte count, which can result to increased susceptibility to infections [10]. Severe malnutrition has also been shown to impair the innate host defence mechanism, further weakening the body's immune system [11].

The biochemical and haematological parameters of blood can be used to monitor and or evaluate health, nutritional and physiological status of animals [12]. Furthermore, Mohammed *et al*. [13] reported that blood constituents are biomarkers employed in determining the efficacy of nutrient composition and diet utilization. It was observed by Al-Seaf and AlHarbi [14] that biochemical and haematology profiles can also be used to assess the immunity status of animals while they can also be an index of transportation stress [15]. Several other factors among which are, nutrition, stress, reproductive status, age, sex, genetics, management, housing, and other environmental determinants have been reported to have profound effects on haematological and biochemical profiles [13]. Therefore, in line with the Sustainable Development Goal (SDG) 2 (end hunger, achieve food security and improved nutrition and promote sustainable agriculture) and SDG 3 (ensure healthy lives and promote wellbeing for all at all ages), there is a need to establish the dietary effects of consuming locally formulated foods supplemented with *Moringa oleifera* leaf powder to tackle the problem of malnutrition in Nigeria. Because biochemical and haematological parameters provide clear indication of non-observable human health; the objective of this study was to evaluate the effects of weaning diets on growth, haematological and biochemical parameters of weanling wistar rats as a basis for infant nutrition.

2.0 MATERIALS AND METHOD

2.1 Diets Ingredients

Maize, guinea corn, millet, soyabean, crayfish, dates, tiger nuts were bought from a local market in Uyo, Akwa Ibom State, Moringa leaves were obtained from a vegetable farm while the commercial food and corn starch was obtained from a reputable shopping outlet in Port Harcourt, Rivers State Nigeria.

2.2 Ingredients Processing

Maize, guinea corn, millet was cleaned during which stones and other foreign materials were sorted. They were washed with clean water, sun dried for 6 hours and roasted until they turned golden brown. Soyabeans were sorted, cleaned, sun-dried and roasted until it turned golden brown Date palms were cleaned using a muslin cloth and sun-dried while tiger nuts were sorted, washed with clean water and over dried at 60^{o}C for 4 hours. Crayfish was cleaned, sorted and dried for 4 hours in an oven at 60^{o}C. All the food materials were ground to powder using attrition mill. Moringa leaves were cleaned, sorted, washed and air-dried under shade. The leaves were allowed to dry under shade for 3 days before it was ground to powder with the aid of a blender. All the ingredients were sieved with a 60 mm mesh sieve and stored in airtight containers.

2.3 Diets Formulation

The diets were formulated as follows: as shown in Table 1 where the different food ingredients were mixed in their different proportions to obtain the different diet mixes. Corn starch (containing 2% protein) and cerelac (a commercial weaning diet) were used as the control food samples.

Table 1

Diets	Food Ingredients
Diet 1	Maize + Guinea Corn + Millet + Soyabean + Tiger nut + Dates (22:22:21:20:10:5)
Diet 2	Maize + Guinea Corn + Millet + Soyabean + Tiger nut + Dates (22:22:21:20:10:5) + 10% *Moringa oleifera* leaves
Diet 3	Maize + Guinea Corn + Millet + Crayfish + Tiger nut + Dates (22:22:21:20:10:5)
Diet 4	Maize + Guinea Corn + Millet + Crayfish + Tiger nut + Dates (22:22:21:20:10:5) + 10% *Moringa oleifera* leaves
Diet 5	Corn Starch
Diet 6	Cerelac

2.4 Animal Feed Studies

Thirty weanling albino rats (*Rattus norvegicus*) aged between 21- 30 days weighing between (45-60 g) were obtained from the Animal House of the Department of Anatomy, University of Port Harcourt, Rivers State, Nigeria. The rats were separated into 6 groups of five rats and acclimatized for 7 days. At the end of acclimatization, their initial weights were taken after which they were fed the test and control diets. Rats were kept one per cage with facilities for faecal, spilled food and urine collection. Water was provided from rubber water bottles and food in specially fabricated metallic vessels and they were allowed to feed on the different diets (Diets 1-6) ad libitum for 28 days. At the end of the feeding experiments, their final weights were taken after which the rats were euthanized with chloroform. The carcass was dissected, and the lungs, liver, kidney and heart were removed, weighed and returned to each individual carcass. Ethical approval for this study was obtained from the University of Port Harcourt, Ethical Committee.

2.5 Determination of Biochemical Parameters

Plasma total bilirubin assay content of experimental animals was performed based on the reaction principle of Freitag [16]. Serum alanine transaminase (ALT) activity was done based on the method of Deneke and Rittersdorf [17]. Aspratate amino transferase (AST) activity was done based on the reaction principle of Deneke *et al*., [18]. Alkaline phosphatase (ALP) activity was according to the

method described by Heins [19] using the Reflotron dry chemistry spectrophotometric system. Total Protein in serum was investigated according to the method of Doumas *et al.,* [20] while the albumin content of the serum was determined according to the method of Rodykey [21]. The globulin content of the serum of experimental animals was according to the method of Kingsley [22].

2.6 Determination of hematological parameters and indices

Hematological parameters and indices of blood samples were determined following standard protocols [23]. Erythrocytes, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, were determined using the Coulter Counter System (Beckman Coulter®, ThermoFisher, UK). Air-dried blood films stained with Giemsa stain were microscopically examined using magnification x200 and x400 for differential white blood cell counts.

2.7 Determination of anti-nutritional components

Phytic acid was extracted from 3g of each food sample with 3% trichloroacetic acid by shaking at room temperature which was followed by centrifugation as described by Wheeler & Ferrel [24]. Oxalate was determined according to the method of Franco and Krinitz [25] while tannin contents was determined according to the modified vanillin-HCl methods [26] (Burns 1971). Trypsin inhibitory activity was determined as described by Smith *et al*., [27].

2.8 Statistical Analyses

The results obtained were expressed as mean ± standard error of mean of triplicate readings. Analysis of variance (for multiple comparisons) was used. Statistical Package for Social Science (SPSS) version 22.0 was used for the analysis. The significance level was kept at ($p < 0.05$).

3.0 RESULTS

Results of the antinutrient content of locally formulated diets are as presented in Table 2. Showed that all the anti-nutritive components except phytate were significantly higher ($p<0.05$) in diet 1 when compared to the other diets. Oxalate ranged from 8.70±0.01 to 18.13±0.07 (mg/100g), phytate was in the range of 0.29±0.00 to 0.51±0.00 while trypsin inhibitory activity at 50mg/ml ranged from -2.43±0.02 to 4.14±0.01.

The organ weights of animals fed the different diets as presented in Table 3 showed that the liver weight ranged from 2.03±0.07 to 6.20±0.38 while the lungs recorded values ranging from 0.63±0.09 to 1.40±0.23. The kidney and heart recorded values ranging from 0.50±0.12 to 1.30±0.06 and 0.47±0.09 to 0.67±0.03 respectively.

The biochemical parameters of animals fed the experimental diets (Table 4) showed that the values for aspartate amino transferase (AST) and alanine amino transferase (ALT) ranged from 30.33±2.03 to 43.33±1.45 and 6.90±1.14 to 10.73±0.93 respectively.

Table 2: Antinutrients content of Formulated Diets

Parameters	Diets			
	1	2	3	4
Oxalate (mg/100g)	$18.13±0.07^{d}$	$17.40±0.00^{c}$	$14.51±0.00^{b}$	$8.70±0.01^{a}$
Phytate (mg/100g)	$0.49±0.02^{bc}$	$0.44±0.00^{b}$	$0.51±0.00^{c}$	$0.29±0.00^{a}$
Trypsin Inhibition (50mg/ml)	$8.18±0.01^{d}$	$0.88±0.01^{b}$	$1.69±0.01^{c}$	$0.78±0.01^{a}$
Trypsin Inhibition (40mg/ml)	$4.14±0.01^{c}$	$-1.46±0.02^{b}$	$-1.15±0.02^{a}$	$-2.43±0.02^{a}$
Trypsin Inhibition (30mg/ml)	$0.85±0.02^{d}$	$-2.84±0.02^{b}$	$-2.18±0.01^{c}$	$-2.99±0.02^{a}$
Trypsin Inhibition (20mg/ml)	$-0.05±0.02^{c}$	$-2.79±0.05^{b}$	$-2.75±0.02^{b}$	$-3.20±0.02^{a}$
Trypsin Inhibition (10mg/ml)	$-0.28±0.02^{d}$	$-3.06±0.01^{b}$	$-2.52±0.02^{c}$	$-3.31±0.01^{a}$

Values are represented as Mean ± SEM of triplicate determinations. Values with different superscript across rows are statistically significant ($p\leq0.05$).

Table 3: Weight Gain, Food Intake and Organ weights of Experimental Animals Fed the Diets

	Groups					
	1	2	3	4	5	6
Weight Gain (g)	$33.37±0.55^{b}$	$30.33±2.31^{b}$	$52.33±1.76^{c}$	$72.10±2.57^{d}$	$-22.67±3.21^{a}$	$59.67±2.85^{c}$
Food Intake	$143.72±0.14^{b}$	$147.66±0.38^{b}$	$175.87±0.76^{c}$	$176.78±0.76^{c}$	$132.12±0.02^{a}$	$178.80±4.97^{c}$
Liver (g)	$3.17±0.03^{ab}$	$3.67±0.43^{bc}$	$4.70±0.20^{cd}$	$5.57±0.24^{de}$	$2.03±0.28^{a}$	$6.20±0.38^{e}$
Lungs (g)	$0.83±0.07^{ab}$	$1.20±0.12^{ab}$	$1.40±0.23^{b}$	$1.30±0.12^{b}$	$0.63±0.09^{a}$	$1.33±0.03^{b}$
Kidney (g)	$0.83±0.03^{ab}$	$0.90±0.10^{b}$	$1.00±0.06^{bc}$	$1.30±0.06^{c}$	$0.50±0.12^{a}$	$1.13±0.03^{bc}$
Heart (g)	$0.40±0.00^{a}$	$0.50±0.00^{ab}$	$0.63±0.03^{b}$	$0.63±0.03^{b}$	$0.47±0.09^{ab}$	$0.67±0.03^{b}$

Values are represented as Mean ± SEM of triplicate determinations. Values with different superscript across rows are statistically significant ($p\leq0.05$).

Table 4: Biochemical Parameters of Experimental Animals fed the Diets

Parameters	Diets					
	1	2	3	4	5	6
AST (µ/L)	43.33±1.45^{a}	31.67±1.20^{a}	30.33±2.03^{a}	33.33±5.24^{a}	37.00±4.00^{a}	33.00±2.00^{a}
ALT (µ/L)	10.67±0.77^{a}	8.53±1.07^{a}	7.50±0.23^{a}	6.90±1.14^{a}	10.73±0.93^{a}	9.77±0.74^{a}
ALP (µ/L)	33.00±2.52^{b}	26.67±3.2^{ab}	21.33±0.88^{a}	18.00±1.15^{a}	21.00±3.00^{a}	16.00±2.52^{a}
Total Protein (g/L)	63.00±2.65^{ab}	60.00±2.89^{ab}	68.67±2.84^{b}	68.67±3.76^{b}	52.00±2.00^{a}	61.33±1.20^{ab}
Albumin (g/L)	37.67±1.45^{bc}	34.67±1.45^{ab}	44.00±2.08^{c}	40.33±1.45^{bc}	30.67±0.67^{a}	36.67±0.88^{ab}
Globulin (g/L)	25.33±2.40^{a}	25.33±2.33^{a}	24.67±0.88^{a}	28.33±2.33^{a}	21.33±1.33^{a}	24.67±0.67^{a}
Total Bilirubin (µmol/L)	8.70±0.29^{a}	6.33±0.24^{a}	6.23±0.34^{a}	6.60±1.03^{a}	7.40±0.80^{a}	6.70±0.36^{a}
Conjugated Bilirubin (µmol/L)	6.40±0.46^{b}	4.47±0.15^{ab}	3.43±0.18^{a}	4.00±0.72^{ab}	4.17±0.97^{ab}	4.17±0.71^{ab}

Values are represented as Mean ± SEM of triplicate determinations. Values with different superscript across rows are statistically significant ($p \leq 0.05$). AST- Aspartate Amino Transferase; ALT- Alanine Amino Transferase; ALP- Alkaline Phosphatase.

Table 5: Hematological Parameters of Experimental Animals Fed the Diets

Diets						
	1	2	3	4	5	6
PCV (%)	37.33±1.20^{a}	36.67±0.67^{a}	38.33±0.88^{a}	35.33±2.67^{a}	32.00±2.00^{a}	32.67±0.33^{a}
Hb (g/dL)	12.33±0.52^{a}	12.00±0.17^{a}	12.77±0.29^{a}	11.80±0.90^{a}	10.67±0.67^{a}	10.13±0.72^{a}
RBC(x10^{12}/L)	5.50±0.40^{a}	5.47±0.26^{a}	5.80±0.29^{a}	5.40±0.35^{a}	4.33±0.24^{a}	4.77±0.39^{a}
WBC (x10^{9}/L)	7.40±0.21^{bc}	7.33±0.24^{bc}	7.90±0.21^{c}	6.73±0.37^{b}	5.57±0.15^{a}	6.13±0.23^{ab}
Platelets (x10^{9}/L)	229.00±8.33^{a}	252.00±9.82^{a}	229.67±7.22^{a}	269.00±13.0^{a}	241.67±5.84^{a}	263.33±8.41^{a}
MCHC (g/L)	32.67±0.33^{a}	32.33±0.33^{a}	33.00±0.58^{a}	32.33±0.67^{a}	30.67±0.33^{a}	31.67±0.88^{a}
MCH (pg)	22.67±0.67^{b}	21.33±0.88^{ab}	22.00±0.58^{ab}	21.67±0.33^{ab}	19.67±0.33^{a}	21.33±0.33^{ab}
MCV (fL)	68.33±2.85^{a}	66.33±1.86^{a}	66.33±2.03^{a}	65.00±1.15^{a}	61.67±0.88^{a}	63.67±0.88^{a}
Neutrophils (%)	27.33±1.76^{a}	28.67±3.28^{a}	33.33±2.40^{a}	36.67±0.88^{a}	30.00±0.58^{a}	35.00±1.73^{a}
Lymphocytes (%)	61.67±1.67^{b}	62.33±1.45^{b}	56.33±2.33^{ab}	51.67±1.67^{a}	58.00±1.73^{ab}	54.33±2.33^{ab}
Eosinophils (%)	4.00±0.58^{a}	4.00±0.58^{a}	3.33±0.33^{a}	3.33±0.33^{a}	2.67±0.67^{a}	3.00±0.58^{a}
Monocytes (%)	7.00±0.58^{a}	7.33±0.67^{a}	7.00±0.57^{a}	8.33±0.88^{a}	6.67±0.67^{a}	7.67±0.33^{a}

Values are represented as Mean ± SEM of triplicate determinations. Values with different superscript across rows are statistically significant ($p \leq 0.05$). PCV-- Packed Cell Volume, Hb--Hemoglobin; MCHC—Mean Corpuscular Hemoglobin Concentration; MCH – Mean Corpuscular Hemoglobin; MCV – Mean Corpuscular Volume.

4.0 DISCUSSION

The antinutrient composition of locally prepared diets in this study showed that phytate was the lowest of all the antinutrients and was slightly higher than the study of Uzo-Peters and Akinola [28] who observed a phytate level of 0.10 – 0.31 mg/100g in weaning diets but lower than 29.60 mg/100g reported by Obiakor-Okeke *et al*., [29]. Oxalate in this study was highest in diets formulated entirely with plant materials as compared with the diet where animal materials were added. This is corroborated by Holmes and Kenedy [30] who observed that dietary oxalate sources are mainly from plants and plant products but negligible levels are found in foods of animal origin. This same author reported an oxalate level of up to 524 mg/100g in wheat bran which was lower than the values in current study. Antinutrients have been implicated in interference with proper mineral elements absorption by cells of the body. It has been reported that high oxalate levels may pose an adverse by binding calcium and other minerals in the body. Trypsin inhibition in present study was lower than the value stated by Kakade *et al*., [31] who observed a trypsin inhibition of 1.9 and72.8 TIU (trypsin inhibition unit) in unprocessed and processed soybean. Nonetheless, it is known that processing of foods through cooking, frying and soaking can reduce the antinutritive constituents of foods [32]. The low levels of antinutritive contents in this study may be as result of the processing methods employed in preparing the diets.

The relative organ weights of the experimental models fed the locally produced complementary diets, Cerelac and corn starch in this study showed that the locally prepared diets influenced the weight of organs of the experimental animals as the organ weights of the animals fed corn starch decreased significantly when compared to animals fed Cerelac. Rats fed the commercial diet gave the highest liver weight which is an indication of better feed utilization which is supported by the work of Essien *et al*., [33]. Animals fed the locally prepared diets especially the Group 4 animals compared favorably with the group fed the commercial brand (Group 6). This may be attributed to the inclusion of protein-rich ingredients in the formulated diets. Similar results have been reported by several authors [34, 35].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the major markers employed in the assessment of liver health of organisms [36]. In present study, the biochemical assessment of animals fed the different diets revealed that AST and ALP level of Group 1 was significantly high ($p<0.05$) when compared with other groups whereas ALT did not show any significant difference in all the experimental groups. As stated by Kaplan *et al*., [37], a normal liver is known to contain AST and ALT but when in a diseased state or injured it releases these enzymes to the blood stream. Increased level of serum AST and ALT can ensue as a result of damage to hepatocellular membrane occasioned by hepatocyte proliferation, exposure to toxins, hypoxia and inflammation and metabolic disorders [38]. Therefore, the absence of significant differences in serum ALT, AST and ALP of most of the formulated foods especially those with 10% fortification with moringa leaf powder with those fed the commercial diets in present study may reflect the normal liver function of the animals fed the diets. This result is in line with the research of Olugbemi *et al*., [39] who reported that moringa leaves have beneficial effect on intestinal health and immune response of broilers. Total protein, albumin and globulin in present study were seen to be high in rats fed the local diets as compared with the protein-free diet. This suggest that the total protein and albumin of serum may be influenced by the protein content of a diet. It also reveals that the albumin production was stimulated more than the globulin content of the total protein in Groups where animal protein was used in the preparation of the diets which is similar to the research of Agbede *et al*., [40]. Total and conjugated bilirubin in this study showed that there was no significant difference in their levels but was slightly higher in Group 1 animals. This may mean that the locally formulated weaning foods did not cause haemolysis in animals fed the diets as compared with the commercial food.

Hematological assessment in this study showed that there was no significant difference in the packed cell volume, hemoglobin, red blood cells, white blood cells, platelets, MCHC, MCV, Neutrophils, Lymphocytes, Eosinophils and monocyte levels of all the experimental groups and this suggest that the weaning foods did not pose any blood-related adverse health outcome in the experimental animals. Values in present study were lower than the study of Agbede *et al*., [40] who reported a PCV, Hb, RBC and WBC range of 38.20 - 45.80 %, 12.74 – 15.24 g/100ml, 9.33 – 12.98 $x10^6$ mm^3 and 3.59 – 4.28 $x10^6$ mm^3 respectively. Togun *et al.*, [41] asserted that when hematological values fall within the standard range reported for an animal, it is an indication that the food administered did not pose adverse effect on the hematological parameters during the experimental period, but when the values fall below the normal range, it is an indication of a diseased-state which could be as a result of altered blood production.

CONCLUSION

This study has shown that formulating weaning diets from locally available raw materials did not pose any deleterious health outcome to the liver of experimental animals fed the diets. It showed that such diets can be used as weaning diets to infants to fight against protein-energy malnutrition. This study shows that locally formulated diets can enhance hematopoietic conditions necessary for blood production in infants who are placed on these diets.

ACKNOWLEDGMENT

The authors wish to thank the management of the Africa Centre of Excellence for Public Health and Toxicological Research, University of Port Harcourt, Nigeria for the assistance offered for this research to be possible.

Funding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors Contributions

Archibong, Idongesit E: Conceptualization, Methodology, Investigation, Formal Analysis, Writing-Original draft presentation.
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Competing Interests: Authors have declared that no competing interests exist.

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