

ISSN: 2437-1955

IJMNR

International Journal of Moringa and Nutraceutical
Research

Volume 1 | Number 2 | June 2015



International Journal of Moringa and Nutraceutical Research

Volume 1 | Number 2 | June, 2015

Published by the International Moringa and Nutraceutical Research Society

INTERNATIONAL JOURNAL OF MORINGA AND NUTRACEUTICAL RE- SEARCH

International Journal of Moringa and Nutraceutical Research highlights new developments and perspectives on Moringa and Nutraceutical Research, including roles in health, wellness, sustainable development, environmental protection and global food security.

Aims and Scope

The International Journal of Moringa and Nutraceutical Research (IJMNR) aims to provide an effective medium for dissemination and publication of scientific information emanating from research on Moringa and Nutraceuticals.

The Journal publishes original research papers, review articles, technical notes and book reviews on Moringa and Nutraceutical Research and related subjects.

Key areas covered by the journal are: Human and Animal Health and Nutrition; Processing, Value Addition and Food Science; Industrial Chemistry and Engineering; Economics and Management, Carbon Balance, Water Economics, Environmental Footprint management; Science of Human Behaviour and Demographics; Historic, Contemporary and Projected Associations in Language, Religion, Arts and Culture; Agronomy, Crop Protection and Ecology and all other original research and Data-driven perspectives on Moringa and Nutraceutical Research.

The Journal is published by the International Moringa and Nutraceutical Research Society (IMNRS). The Editorial Board and IMNRS wish to make it clear that statements or views expressed in papers published in this journal are those of the authors and no responsibility is assumed for the accuracy of such statements or views. In the interest of factual recording, occasional reference to manufacturers, trade names and proprietary products may be inevitable. No endorsement of a named product is intended nor is any criticism implied of similar products that are not mentioned.

Submission of an article for publication implies that it has not been previously published and is not being considered for publication elsewhere. The Journal's peer review policy demands that at least two reviewers give positive recommendations before the paper is accepted for publication. Prospective authors are advised to consult the Guide for Authors which is available in each volume of the Journal. Manuscripts should be sent to:

Editor-In-Chief: Dr. Ayokunle Afolabi Toyé

Regular Mail:

Functional Biology of Health and Disease Group, and
Quantitative Functional Genetics, Genomics and Cell Biology Group,
Department of Animal Production,
Faculty of Agriculture,
University of Ilorin,
P.M.B. 1515, Ilorin, Kwara State,
Nigeria

Electronic mail address: ijmnrjournal@gmail.com; ayo.toye@btinternet.com,

Phone: +234(0)8131011392

All correspondence with respect to status of manuscripts should be sent to the Technical Assistant to the Editor-In-Chief at ijmnrjournal@gmail.com.

**INTERNATIONAL JOURNAL OF MORINGA AND NUTRACEU-
TICAL RESEARCH**

EDITOR-IN-CHIEF

Dr. Ayokunle Afolabi Teye
Functional Biology of Health
and Disease Group, and
Quantitative Functional Genetics,
Genomics and Cell Biology Group,
Department of Animal Production,
Faculty of Agriculture,
University of Ilorin, Kwara State, Nigeria.

EDITORS

Dr. (Mrs.) Ayinde, Opeyemi Eyitayo
Department of Agricultural Economics and
Farm Management, University of Ilorin,
Ilorin, Kwara State, Nigeria.

Dr. Bolu, Stephen Abiodun
Department of Animal Production, University
of Ilorin, Ilorin, Kwara State, Nigeria.

ADVISORS

Prof. Olaoye, G.
Department of Agronomy and Soil Science,
University of Ilorin, Ilorin, Kwara State,
Nigeria.

Prof. Atteh, J. O.
Department of Animal Production,
University of Ilorin, Ilorin, Kwara State,
Nigeria.

Prof. Ogunlela, A. O.
Department of Agricultural and Biosystems
Engineering, University of Ilorin, Ilorin,
Kwara State, Nigeria.

Prof. (Mrs.) Malomo, S. O.
Biochemistry Department, University of
Ilorin, Ilorin, Kwara State, Nigeria.

Prof. (Mrs.) Odunola Bakare
Department of Pharmaceutical and Medicinal
Chemistry, University of Ilorin, Ilorin, Kwara
State, Nigeria.

Prof. (Mrs.) Ibrahim Hajara
Pharmaceutical Sciences, University of Ilorin,
Ilorin, Kwara State, Nigeria

Prof. Omotesho, O. A.
Department of Agricultural Economics and
Farm Management, University of Ilorin,
Ilorin, Kwara State, Nigeria

Prof. Joseph, J. K.
Department of Home Economics and Food
Science, University of Ilorin, Ilorin, Kwara
State, Nigeria.

Prof. Ayodele Jimoh
University of Ilorin

Prof. Ayorinde, K. L.
Department of Animal Production,
University of Ilorin, Ilorin, Kwara State,
Nigeria.

Prof. (Mrs.) Oladiji, A. T.
Centre for Research Development and In-
house Training CREDIT, University of Ilorin,
Ilorin, Kwara State, Nigeria.

Prof. Mokuolu, O. A.
Centre for International Education CIE,
University of Ilorin, Ilorin, Kwara State,
Nigeria.

INTERNATIONAL JOURNAL OF MORINGA AND NEUTRACEUTICAL RESEARCH

Volume 1 | Number 2 | December, 2014

CONTENTS

Evaluation Of Blood Parameters And Total Cholesterol Of Exotic Chickens Fed Diet Supplemented With Moringa Oleifera Seed Products.

Daramola, O.T., Sola-Ojo, F.E., Annongu, A.A., Ogunsola, F.O and Toye, A.A.....1

Development Of Moringa Value Added Product As Nutritional And Medicinal Supplements For Healthy Living In Nigeria

Abolaji, G. T 10

Amino and fatty acid profiles of Moringa oleifera seed flour

Abiodun O.A., Adegbite, J.A., Ojo, A. and Amanyunose, A.A.....
24

Performance Of Rabbits Fed *Moringa Oleifera* Lam. Based Diets

Oyedele O. J. and Odeyinka S. M 31

Moringa germplasm collection and screening

Getachew Mulugeta.....
39

Evaluation of the Effect of *Moringa oleifera* Leaf Powder on Digestion of some Nutrients in Weanling Albino Rats

Oladiji, A.T., Omole, O. M., Oloyede, H. O. B. M. T. Yakubu. 51

β ETA-Lactamase Production and Plasmid Profiling of Penicillins Resistant *Staphylococcus Aureus* Isolated From Food Handlers

Kayode, R.M.O., Adedeji, B.S., Oladoye, C.O., Laba, S.A., and Salami, K.O. 70

The Economics of *Moringa oleifera* use by people living with HIV/AIDS (PLWHA) in Kwara State, Nigeria

Animashaun, J.O., Williams, F.E., Ibrahim, H. and Toye, A.A.....80

EVALUATION OF BLOOD PARAMETERS AND TOTAL CHOLESTEROL OF EXOTIC CHICKENS FED DIET SUPPLEMENTED WITH *MORINGA OLEIFERA* SEED PRODUCTS.

Daramola, O.T., *Sola-Ojo, F.E., Annongu, A.A., Ogunsola, F.O. and *Toye, A.A.

Department of Animal Production University of Ilorin

Corresponding Author's e-mail Addresses: ayo.toye@btinternet.com,
mofesola1@yahoo.com

ABSTRACT

This study was carried out to evaluate the effect of the inclusion of level of Moringa oleifera seed products in the diet of pullet and broiler strains. Sixty four birds [32 broilers (Marshall) and 32 pullets (Harco)] were used for the experiment. At four weeks of age, blood samples were taken through the brachial venipuncture, and then analysed for total cholesterol, lipid, protein, Pack Cell Volume (PCV), White Blood Cell (WBC), Red Blood Cell (RBC) and haemoglobin. Neutrophil, lymphocytes, glucose, uric acid, serum protein, albumin, creatinine, alkaline phosphate, Glutamic oxaloacetate Transaminase (GOT), Glutamic phosphate transaminase (GPT) were also determined from the blood samples. There was a significant ($p < 0.05$) difference in the cholesterol level between genotypes (Marshall vs. Harco) irrespective of diet, with the Marshall Group showing higher cholesterol level than Harco breed. There was no significant difference ($p > 0.05$) in cholesterol between groups fed different components of Moringa oleifera seed. The seed cake (6% defatted seed) and whole meal (10% Moringa oleifera seed) diets groups showed significantly higher cholesterol than control diet and seed oil diet. Cholesterol significantly ($p < 0.05$) determined by an interaction between genotype and dietary levels of Moringa oleifera component (Genotype $\times$ diet interaction). It can therefore be concluded that the inclusion of Moringa oleifera seed oil from 0 - 4 weeks of age in the diet of broilers does have any detrimental effect on broiler performance, and Moringa oleifera seed oil shows a beneficial effect in reduction of Cholesterol in broiler and pullets but in pullet the control diet shows lower cholesterol than the Moringa oleifera seed group. The response of broilers and pullets to Moringa oleifera seed cake and seed oil diets shows that genotype should be taken into consideration when an animal is recommended for evaluation of non-conventional feedstuffs.

Keywords: Moringa Seeds, Broiler, Pullets Blood parameters and Total Cholesterol

INTRODUCTION

Escalating cost of conventional feedstuff has compelled animal Nutritionist to incorporate non- conventional feedstuff to poultry diets and ingestion of numerous dietary components has measurable effects on blood constituents (Bhatti *et al.*, 2009). Long term Nutritional status of animal can be proximately measured through nutrient levels in the blood and body fluids, although this might not be a valid indication status (Animasahun *et al.*, 2006). Blood parameters are important in assessing the quality and suitability of feed ingredients in farm animals, Maxwell *et al.* (1990) and

hematological constituents reflects the physiological responsiveness of the animal to its internal and external environments which include feed and feeding.

Moringa oleifera Seed Oil (MOSO) contains 75% Oleic acid, a mono-unsaturated fatty acid that is less vulnerable to oxidative stress than poly-unsaturated fat. *Moringa oleifera* Seed Cake (MOSC) is Rich in quality and 100% free from any contamination. Moringa seed cake (defatted seed cake) is used as fertilizer (Fahey *et al.*, 2001). Faizi *et al.* (1988) and Lalas and Tsaknis (2002) reported that the seed extracts exerts its protective effect by decreasing liver lipid peroxide, and it has been found to be highly effective on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice (Bharali *et al.*, 2003). This study was aimed at comparing blood chemistry profile of broilers and pullets strain fed *Moringa oleifera* seeds and its byproducts.

MATERIALS AND METHOD

Sources and Management of Experimental Chickens

The experiment was carried out in the Animal Pavilion of the Animal Production Department, Faculty of Agriculture, University of Ilorin. Day-old broilers and pullet chicks (Marshall and Harco Black Breeds) were purchased from Obasanjo Farm, Ota, Ogun state. A total of 64 chickens were used for the experiment comprising 32 broiler and 32 pullet chicks. Within each breed, the birds were randomly assigned to four dietary treatments of control diet, 10% *Moringa oleifera* seed meal, 6% *Moringa oleifera* seedcake and 4% *Moringa oleifera* oil. Such that there were eight birds in each of the four treatment groups in each breed. On arrival the chicks were counted and observed for health status. They were then weighed and randomly distributed to treatment groups. The birds were vaccinated in order to prevent mortality and morbidity from infectious diseases (MVM, 1986).

Data Collection

At four weeks of age, blood samples were taken from the birds through brachial venipuncture and taken to the laboratory for analysis. Serum total cholesterol, lipids and protein, Packed Cell Volume, White blood cells, Red Blood Cells, Hemoglobin, Neutrophils, Lymphocytes, Glucose, Uric Acid, Serum Protein, Albumin, Creatinine, Alkaline phosphatase, Glutamic – Oxaloacetate Transaminase (GOT), Glutamic Phosphate Transaminase (GPT) were determined from the blood samples.

RESULTS AND DISCUSSION

Effects of Different Components of Moringa seed on Total Cholesterol

There was a significant ($p < 0.05$) difference in Cholesterol level between genotypes (Marshall vs Harco irrespective of diet), with the Marshall broiler group showing higher Cholesterol level than Harco layer breed. When diet was considered irrespec-

tive of genotype, significant ($p < 0.05$) differences in cholesterol were not observed between groups fed different components of Moringa. The seed cake diet (6% defatted seed) and whole meal (10% *Moringa oleifera* seed) diet groups showed significantly higher cholesterol than control diet and seed oil diet.

Cholesterol was not significantly ($p < 0.05$) determined by an interaction between genotype, and dietary level of Moringa components (Genotype x Diet interaction Table 1).

Within the Broiler breed (Marshall), significant difference in cholesterol was observed between diet groups' Whole meal and Seed Cake significantly has higher cholesterol than Control and Seed oil groups. In the Harco pullet breed, Control and Seed Oil has significantly ($p < 0.05$) lower cholesterol than other diet groups (Table 2).

Effects of Different Components of Moringa seed on Haematological Parameters

There was a significant ($p < 0.05$) difference in Lymphocyte, between genotypes (Marshall vs Harco irrespective of diet), with the Harco layer group showing higher level than Marshall broiler breed while in Monocyte, Eosinophil and Basophil, Neutrophil, the Marshall broiler shows higher level than the Harco layer (Table 2). When diet was considered irrespective of genotype, significant ($p < 0.05$) differences were not observed between groups fed different components of Moringa (Table 1 and 2).

Parameters was not significantly ($p < 0.05$) determined by an interaction between genotype, and dietary level of Moringa components (Genotype x Diet interaction) (Table 1.) There was no significant difference between all groups both within broiler and pullet breeds in blood parameters.

There was a significant ($p < 0.05$) difference in PCV, HB, WBC and RBC between genotypes (Marshall vs Harco irrespective of diet), with the Harco layer group showing higher level than the broiler group when diet was considered irrespective of genotype, significant ($p < 0.05$) differences were observed between groups fed different components of Moringa. The Seed Cake diet (6% defatted seed) and Whole meal (10% *Moringa oleifera* seed) diet groups showing significantly higher PCV, Hb, RBC, and WBC than Control diet and Seed oil diet. In the Harco pullet breed, Control and Seed oil has significantly ($p < 0.05$) lower PCV, Hb, WBC and RBC than other diet groups (Table 2). Within the Broiler breed (Marshall), significant difference in PCV, Hb, WBC and RBC was observed between diet groups, whole seed meal and Seed Cake group has significantly ($p < 0.05$) higher level of PCV, Hb, WBC and RBC than the Control and Seed oil diet groups.

Effects of different components of moringa seed on Total Cholesterol

Total cholesterol was considerably higher in the broiler than in the pullet birds. High cholesterol is associated with obesity and is a risk factor for metabolic syndrome and heart disease. Since broilers have been genetically modified for fast growth and this has increased their tendency for increased fat deposition, the higher blood cholesterol level in broilers' is indicative of the tendency for broiler to accumulate more body fat than the pullet chickens and a higher risk of metabolic syndrome and heart disease.

Within Broiler breed

The result shows that seed cake and whole meal had higher cholesterol level than the control and seed oil groups, the seed cake having the highest while the seed oil has the lowest, this indicates that Cholesterolaemia was highest in the Seed cake diet and lowest in the Seed oil diet. The data suggests that *Moringa oleifera* seed components when fed as seed cake supplement contribute or produce adverse metabolic outcomes reflected in increased Cholesterolaemia.

Within Pullet breed

The results show that seed cake and whole meal have higher cholesterol levels than the control group and seed oil group, the seed cake having the highest cholesterol level, while the control group has the lowest in contrast to the observation in the broiler breed. This may be due to the difference in genetic composition between the broiler and the pullet breed.

Effects of different components of moringa seed on Blood haematology

From the results reported in the present work, it could be concluded that irrespective of diet, pullet birds have higher levels of RBC, Neutrophil and Lymphocytes than the broilers.

Packed cell volume (PCV), haemoglobin (Hb), Red blood cell (RBC) and White blood cell (WBC) counts differ significantly with diet treatment. Diets 4 (seed oil) has the lowest level of Hb which could imply that protein in diet 4 were not of high quality. Same standard ingredients were used to supply a bulk of protein in all diets (other than protein supplemented through *Moringa* seed and Seed cake in Whole meal and Seed cake groups). It seems likely that oil which is calorie dense may have reduced feed intake of the MOSO group in a way that satisfied energy requirement of the birds while compromising protein intake, which may explain their reduction in Hb. In general, a reduction in the concentration of PCV in a diet suggests the presence of toxic factor and traces of anaemia in the diets.

Also, high WBC is associated with microbial infection or the presence of foreign bodies or antigen in the circulating system. The range of WBC count can be 5000-15000 cells/cu mm depending on species according to Bentricks (1974), in this study all diet group has a normal WBC which suggest that birds were not fighting a disease at the time of blood collection. Reductions in RBC value are indicative of

low protein intake or mild anaemia Lindsay (1977), level of RBC in both the broiler and pullet breed is higher than lower limit of the normal range of (2.1-3.69) which indicates that birds were not anaemic.

As the level Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil was not significantly affected by diet in the study, it is suggested that birds are not suffering from any infection.

Effects of different components of moringa seed on Total Cholesterol

Total cholesterol was considerably higher in the broiler than in the pullet birds. High cholesterol is associated with obesity and is a risk factor for metabolic syndrome and heart disease. This higher broiler cholesterol level is indicative of the tendency for broiler to accumulate more body fat than the pullet chickens and a higher risk of metabolic syndrome and heart disease. Broilers have been genetically modified for fast growth and this has increased their tendency for increased fat deposition

Within Broiler breed

The result shows that seed cake and whole meal had higher cholesterol level than the control and seed oil groups, the seed cake having the highest while the seed oil has the lowest, this indicates that Cholesterolaemia was highest in the seed cake diet and lowest in the Seed oil diet. The data suggests that components of Seed cake when fed as seed cake supplement or contributed by Whole seed produce adverse metabolic outcomes reflected in increased Cholesterolaemia.

Within Pullet breed

The results show that seed cake and whole meal have higher cholesterol levels than the control group and seed oil group, the seed cake having the highest cholesterol level while the control group has the lowest in contrast to the observation in the broiler breed. This may be due to the difference in genetic composition between the broiler and the pullet breed.

Within the broiler group, there was significant difference in the cholesterol was observed between diet groups wholemeal and seed cake than control and seed oil groups. In the harco pullet breed, control and seed oil has significantly ($p < 0.05$) lower cholesterol than other diet groups. There was significant ($p < 0.05$) difference in lymphocyte between genotypes. The harco layer group showed a higher level than Marshall Broiler breed. In monocyte, eosinophil, basophil and neutrophil he Marshall Broiler shows a higher level than the harco group. When diet was considered irrespective of genotype, significant ($p, 0.05$) were not observed between groups fed different components of *Moringa oleifera*. Parameters was not significantly ($p < 0.05$) determined by an interaction between genotype and dietary level of *Moringa oleifera components* (genotype and dietary interaction). There was no significant

difference between all groups both within broiler and pullet breeds in blood parameters. There was a significant difference in PCV, Hb, WBC, and RBC between genotypes (Marshalls' Harco irrespective of diet), with harco layer group showing higher level than the broiler group when diet was considered irrespective of genotype. Significant differences were observed between groups fed different components of Moringa. The seed cake diet (6% defatted seed) and wholemeal (10% *Moringa oleifera*) diets groups showing significantly higher PCV, Hb, WBC, and RBC than control diet and seed oil diet. In the harco pullet breed, control and seed oil has significantly ($p, 0.05$) lower PCV, Hb, WBC and RBC than other diet groups. Within the broiler breed (Marshall) significant difference in PCV, Hb, WBC and RBC was observed between diet groups. Wholemeal and Seed Cake has significantly higher level than control and seed oil groups.

Total cholesterol was considerably higher in the broiler than in the pullet birds. High cholesterol is indicative of the tendency for broiler to accumulate more body fat than pullet chicken and a higher risk of metabolic syndrome and heart diseases

Within the broiler group, the seed cake and Whole meal had a higher cholesterol level than the control and seed oil groups, the seed cake having the highest while the seed oil has the lowest; this indicates that cholesterolaemia was highest in the seed cake diet and lowest in the seed oil diet. Within seed cake and whole meal have higher level of cholesterol levels than the control group and seed oil group, the seed cake having the highest cholesterol, while the control group have the lowest in contrast to the observation in the broiler breed.

CONCLUSION

From the result obtained, it is concluded that inclusion of *Moringa oleifera* seed oil from 0-4 weeks of age in the diet of broilers has a positive effect on Broiler performance as measured by body weight at 7 weeks age. Seed oil also shows a beneficial effect in reduction of Cholesterol in broilers.

Inclusion of *Moringa oleifera* seed oil in pullet diet improves body weight compared with the whole meal and seed cake but the control diet has more effect on the performance of pullet as measured by body weight at 7 weeks age. Control diet shows beneficial effect in reduction of Cholesterol in pullets.

The response of broilers and pullets to *Moringa oleifera* seed cake and seed oil diet shows the value of specifically accounting genotype during evaluation of feed stuffs (Nutrigenetics).

Food with low Cholesterol is said to be best for the heart, low cholesterolaemia is associated with low body cholesterol levels. Therefore it is recommended that broiler should be fed with *Moringa oleifera* seed oil to improve quality (Cholesterol) for human to reduce the risk of arterial disease.

Because the use of *Moringa oleifera* seed oil is highly uneconomical, poultry farmer may not be encouraged to use it because consumers may not be ready to pay high amount of money especially in developing country like ours. So it is recommended that further studies should be done on the comparison between the use of MOSO and other less expensive oil which are rich in mono and poly-unsaturated and phenolic components e.g. Olive oil, Canola oil.

Pullet birds are egg laying birds, so further studies should be carried out on the future effects of these components of Moringa on the egg laying capacity and egg quality when such birds are fed a diet appropriate for the laying type birds from the onset.

It is recommended that care should be taken in including MOSC or Whole seed broiler diet because of its adverse effects on total cholesterol which may promote metabolic disease in the birds and subsequently the human consumers.

Further studies should be carried out to determine how (active components, mechanism of action) MOSC produces a negative effect on cholesteromia.

Studies should examine the value of Phytase supplement in MOSC containing diets to determine whether Phytase neutralisation can increase the feeding value of MOSC. Supplementation with inorganic Phosphate and extra Calcium may also help to counteract the effect of endogenous Phytate in MOSC. Additional studies should examine the effect of other antinutrients in MOSC on performance of broiler. It is recommended that feedstuffs validation should be based on studies in multiple genotypes because not all genotypes respond equally to the same feed stuff.

Table 1: EFFECTS OF GENOTYPE (GENOTYPE, HARCO and MARSHALL), DIETARY DCMOS LEVEL FROM 0-4 WEEKS AGE, AND THEIR INTERACTION ON TOTAL CHOLESTEROL AND HEAMATOLOGY PARAMETERS

Trait	Marshall ALL	Harco ALL	Diet-1 ALL	Diet-2 ALL	Diet-3 ALL	Diet-4 ALL	sig. (*, **, *** , NS)
TC	269.59 ± 20.29 (20)*	239.13 ± 20.29 (20)*	229.43 ± 27.06 (10)	267.35 ± 12.17 (10)	288.6 ± 21.42 (10)	232.08 ± 31.22 (10)	NS
PCV (%)	29.66 ± 1.0 (20)	33.45 ± 1.0 (20)*	32.1 ± 0.82 (10) ^b	33.11 ± 1.46 (10) ^b	33.8 ± 1.31 (10) ^b	27 ± 0.94 (10) ^a	NS
HB (g/100ml)	9.86 ± 0.32 (20)	11.11 ± 0.32 (20)*	10.7 ± 0.27 (10) ^b	11.05 ± 0.49 (10) ^b	11.21 ± 0.42 (10) ^b	8.99 ± 0.31 (10) ^a	NS
WBC (×10 ³ /ml)	5.94 ± 0.17 (20)	6.29 ± 0.17 (20)*	5.8 ± 0.16 (10) ^{ab}	6.42 ± 0.26 (10) ^{bc}	6.58 ± 0.29 (10) ^c	5.67 ± 0.24 (10) ^a	NS
RBC (×10 ³ /ml)	5.50 ± 0.13 (20)	5.3 ± 0.13 (20)*	5.02 ± 0.12 (10) ^{ab}	5.59 ± 0.2 (10) ^b	5.23 ± 0.23 (10) ^{ab}	4.77 ± 0.19 (10) ^a	NS
NEUTROP HIL (%)	54.1 ± 0.87 (20)*	53.4 ± 0.87 (20)	54.7 ± 1.26 (10)	54.1 ± 1.47 (10)	53.7 ± 1.16 (10)	52.6 ± 1.1 (10)	NS
LYMPHO CYTE (%)	43.8 ± 0.83 (20)	44.8 ± 0.83 (20)*	43.4 ± 1.24 (10)	44.1 ± 1.48 (10)	44.4 ± 1.02 (10)	45.3 ± 0.83 (10)	NS

MONOCYTE (%)	1.89 ± 0.32 (19)*	1.42 ± 0.32 (19)	1.50 ± 0.4 (10)	1.67 ± 0.37 (9)	1.5 ± 0.37 (10)	2.23 ± 0.47 (9)	NS
EOSINOPHIL (%)	0.32 ± 0.11 (19)*	0.26 ± 0.11 (19)	0.3 ± 0.15 (10)	0.33 ± 0.17 (9)	0.2 ± 0.13 (10)	0.33 ± 0.17 (9)	NS
BASOPHIL (%)	0 ± 0 (19)	0.05 ± 0 (19)*	0.1 ± 0.1 (10)	0 ± 0 (9)	0 ± 0 (10)	0 ± 0 (9)	NS

*Group mean, standard error and count are presented as; means ± sem (n)
a, b means with different superscripts within genotype are significantly different

Table 2. CHOLESTEROL AND HAEMATOLOGY PARAMETERS IN MARSHALL AND HARCO CHICKS FED DIFFERENT COMPONENTS OF MORINGA OLEIFERA SEED (DCMOS) FROM HATCH TO 4 WEEKS AGE.

Trait	Marshall				Harco			
	Control diet	10% Unde fatted Seed	6% Defatted Seed Meal	4% Seed Oil	Control diet	10% Unde fatted Seed	6% Defatted Seed Meal	4% Seed Oil
TC	201.19 ± 8.46 (5) ^a	270.32 ± 15.13 (5) ^a	285.06 ± 35.21 (5) ^a	199.96 ± 17.35 (5) ^a	257.67 ± 53.16 (5) ^a	264.37 ± 20.82 (5) ^a	292.14 ± 28.61 (5) ^a	264.19 ± 59.75 (5) ^a
PCV (%)	34 ± 0.32 (5) ^{cd}	35.4 ± 1.47 (5) ^d	35.4 ± 1.44 (5) ^{ab}	28.6 ± 0.81 (5) ^d	30.2 ± 1.07 (5) ^{bc}	30.82 ± 2.2 (5) ^{bcd}	32.2 ± 2.08 (5) ^{bcd}	25.4 ± 1.44 (5) ^a
HB (g/100ml)	11.32 ± 0.11 (5) ^{cd}	11.82 ± 0.5 (5) ^d	11.8 ± 0.47 (5) ^d	9.52 ± 0.27 (5) ^{ab}	10.08 ± 0.36 (5) ^{bc}	10.28 ± 0.73 (5) ^{bc}	10.62 ± 0.62 (5) ^{bcd}	8.46 ± 0.48 (5) ^a
WBC (×10 ³ /ml)	5.64 ± 0.21 (5) ^a	6.78 ± 0.4 (5) ^b	6.78 ± 0.47 (5) ^b	5.98 ± 0.27 (5) ^{ab}	5.96 ± 0.23 (5) ^{ab}	6.06 ± 0.27 (5) ^{ab}	6.38 ± 0.38 (5) ^{ab}	5.36 ± 0.38 (5) ^a
RBC (×10 ³ /ml)	5.06 ± 0.22 (5) ^{ab}	5.76 ± 0.27 (5) ^b	5.42 ± 0.45 (5) ^{ab}	4.98 ± 0.22 (5) ^{ab}	4.98 ± 0.13 (5) ^{ab}	5.42 ± 0.29 (5) ^{ab}	5.04 ± 0.16 (5) ^{ab}	4.56 ± 0.31 (5) ^a
NEUTROPHIL (%)	54 ± 1.76 (5) ^a	55.8 ± 2.52 (5) ^a	52.4 ± 1.33 (5) ^a	51.6 ± 1.08 (5) ^a	55.4 ± 1.94 (5) ^a	52.4 ± 1.4 (5) ^a	55.0 ± 1.84 (5) ^a	53.6 ± 1.94 (5) ^a
LYMPHOCYTE (%)	44.5 ± 1.3 (5) ^a	43.5 ± 2.65 (5) ^a	45.6 ± 0.93 (5) ^a	46.6 ± 0.75 (5) ^a	42.8 ± 2.24 (5) ^a	45.2 ± 1.5 (5) ^a	43.2 ± 1.77 (5) ^a	44.2 ± 1.3 (5) ^a
MONOCYTE (%)	1.6 ± 0.68 (5) ^a	1.25 ± 0.25 (4) ^a	1.4 ± 0.4 (5) ^a	1.4 ± 0.42 (5) ^a	1.4 ± 0.51 (5) ^a	2.1 ± 0.63 (5) ^a	1.6 ± 0.68 (5) ^a	2.75 ± 0.85 (4) ^a
EOSINOPHIL (%)	0.2 ± 0.20 (5) ^a	0.25 ± 0.25 (4) ^a	0.2 ± 0.20 (5) ^a	0.4 ± 0.24 (5) ^a	0.4 ± 0.24 (5) ^a	0.4 ± 0.24 (5) ^a	0.2 ± 0.20 (5) ^a	0.25 ± 0.25 (4) ^a
BASOPHIL (%)	0.2 ± 0.2 (5) ^a	0 ± 0 (4) ^a	0 ± 0 (5) ^a	0 ± 0 (5) ^a	0 ± 0 (5) ^a	0 ± 0 (5) ^a	0 ± 0 (5) ^a	0 ± 0 (4) ^a

*Group mean, standard error and count are presented as; means ± sem (n)
a, b means with different superscripts within genotype are significantly different

REFERENCES

Abuye C., Onwega, A.M., Imungi, J.K., (1999). Familial tendency and dietary association of goitre in Gamo- Gofa, Ethiopia. East African Medical Journal 76: 449-457.

- Animasahun, R.A., Omoikoye, S.O. and Bamgbose, A.M (2006).** Heamatological and Biochemical indices of weaner rabbits fed concentrates and *Syndrella rodiflora* forage supplement. Proc. 11th Annual Conference of Animal Science Association of Nigeria. Institute of Agricultural Research and Training, Ibadan, Nigeria. Pp 29-32.
- Bentrick, S. (1974).** Haematology, Textbook of Veterinary Pathology. Williams and Co., Baltimore, pp 217-224.
- Bhatti, J.A., Younas, M., Abdullah, M., Babar, M.E. and Nawaz, H (2009).** Feed Intake weight gain and heamatology in Nili Ravi Buffalo heifers fed on Mot grass and Barseen fodder substitutional with saltbush (A triplex an Nicola). Pakistan Veterinary Journal, 29 (3): 133- 137.
- Bharali, R.J., Jabassum, Azad, M.R.H. (2003):** Chemotherapy effect of *Moringa Oleifera*, Lan, on hepatic carcinogen metabolising enzymes antioxidant parameters and skin papilogenesis in mice. Asain Pacific Journal of Cancer Prevention 4: 131-139.
- Faizi, S., Siddiqui, B.S., Saleem, R., Siddiqui, S., Aftab, K., and Gilani, A.H. (1998).** Hypotensive constituents from the pods of *Moringa Oleifera* plant. Med 64:225-228.
- Fahey J.W., Zakman and Talalay O. (2001).** The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. (Corrigendum: Phtyochemistry, (59) :200-237.
- Lalas S, Tsakinis J. 2002.** Extraction and identification of natural antioxidants from the seeds of *Moringa oleifera* Tree variety of Malawi J Animoil Chem Soc. 79; 677-683.
- Lindsay D.B. (1977).** The effect of feeding patterns and sampling on blood parameters. Occasional publication Lister (ed.) No. 1. Br. Soc. Anim. Prod., pp 49-120
- Maxwell M.H. Robertson, W. Spener, S. And Maclorquadale, C.C. (1990).** Comparison of haematological parameters in restricted and adlibitum fed domestic fowls. British Poultry Science 31: 407-413.
- Oyawoye, E.O and Ogunkunle, M. (1998).** Chemicals analysis and biocemical effects of raw Jack benas on broilers. Proc. Nig. Soc. Animal Production 23: 141-142.

DEVELOPMENT OF MORINGA VALUE ADDED PRODUCT AS NUTRITIONAL AND MEDICINAL SUPPLEMENTS FOR HEALTHY LIVING IN NIGERIA

Abolaji, G.T.

Department of Agricultural Economics and Extension Service, College of Agriculture, Kwara State University, Malete, P.M.B. 1530, Ilorin, Kwara State, Nigeria.

Corresponding Author's e-mail address: *drgracea@hotmail.com, grace.abolaji@kwasu.edu.ng*

Phone number: *2348160786999 and 07041937758*

ABSTRACT

All over the world, literature has indicated that moringa is considered to be the most nutrient rich plant on earth; virtually every part of the tree can be used; nutritional and medicinal properties have the potential to end malnutrition, starvation, as well as prevent and heal many diseases and maladies; and it is truly a miracle plant and a divine gift for the nourishing and healing of man. Moringa oleifera holds promise as a sustainable crop and the future looks very promising especially in the formulation of food and medicine supplements; and in the manufacture of perfumes and cosmetics. However, moringa is perishable commodity and the ambient high temperature and humidity obtained in the tropical countries like Nigeria makes it more susceptible for rapid development of senescence and decay. There is therefore the need to add value to fresh moringa leaves such that the products could be stored, transported and used anywhere. Value addition means converting the fresh produce into products which could be utilized for immediate purpose or stored for future use by applying various indigenous and high techniques suitable for each produce. The results of the study indicated that: a) There was better understanding on moringa oleifera plant parts and their nutritional and medicinal benefits for healthy living; b) Through standardized harvesting and postharvest processing, moringa leaf powder, tea and capsules were developed by collaborating with Noble Icon Company and Kwara State Chapter of National Moringa Development Association; c) The nutritional values of Protein, Calcium, Iron, Vitamin A and Vitamin C obtained in this study were higher than those obtained by Armelle de S. S. and Broin, M. (2010) in a similar study, and met the daily recommended values for NAFDAC certification; and d) The laboratory analysis of the value added moringa products indicated their potential use as nutritional and medicinal supplements. And

these are supported by Armelle de S. S. and Broin, M. (2010), Anamed International (2011), and REAP (2013) who indicated that dried moringa leaf powder and tea are useful for treating malaria, strengthening the immune system of AIDS patients, and treating several other diseases such as blood pressure, diabetes, pile, nasal congestion and etc.

Keywords: *Anamed International, Artemisia annua, Artemisinin, Hemorrhoid, Malaria*

INTRODUCTION

The high concentration of essential amino acids, mineral ions, and vitamins makes moringa an ideal nutritional supplement. As a nutritional additive, 2 or 3 spoonfuls of powder are usually added to rice, soups and sauces just before serving. Regular intake of moringa in the form of relish made out of fresh leaves and dried leaf powder which can be added to porridge or other relishes prevent anemia and most forms of malnutrition. For medicinal uses, the flowers, leaves and roots are widely used as remedies for several ailments. The bark of the moringa root should be scraped off because of its toxicity and the flesh of the root should be eaten sparingly (Oliver-Bever 1986).

Moringa seeds are effective against skin-infecting bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They contain the potent antibiotic and fungicide terygospemin. The root powder could be used as an aphrodisiac and when it is mixed with milk, it is considered useful against asthma, gout, rheumatism and enlarged spleen or liver. It also helps in the removal of wind from the stomach and as a snuff can be used to alleviate ear and tooth ache. The leaf juice has a stabilizing effect on blood pressure. The leaf juice controls glucose levels in diabetic patients. Fresh leaves and leaf powder are recommended for tuberculosis patients because of the availability of vitamin A that boosts the immune system. If leaf juice is used as diuretic, it increases urine flow and cures gonorrhea. Leaf juice mixed with honey treats diarrhea, dysentery and colitis (colon inflammation). Fresh leaves are good for pregnant and lactating mothers; they improve milk production and are prescribed for anemia. Paste made from bark treats boils. Paste from ground bark can be applied to relieve pain caused by snake, scorpion and insect bites. Oil is sometimes applied externally for skin diseases (Council of Scientific and Industrial Research 1962; Oliver-Bever 1986).

Three non-governmental organizations in particular—Trees for Life, Church World Service and Educational Concerns for Hunger Organization—have advocated moringa as “natural nutrition for the tropics.” Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. The nutritional properties of Moringa are now so well known that there seems to be little doubt of the substantial health benefit to be real-

ized by consumption of Moringa leaf powder in situations where starvation is imminent (Jed W. Fahey, Sc.D., 2005).

Problem Statement

In Nigeria, moringa grows mainly in the wild for a long time with little effort to harness the limitless opportunities which can be used in tackling some of the nation's nutritional, medicinal and economic challenges. The first and second National Summits on Moringa development held at Abuja the Federal Capital City in year 2010 and Ilorin Kwara State in 2012 respectively created the awareness that made people to start growing and using moringa leaves for nutritional, medicinal and economic purposes. However, moringa leaves is a perishable commodity and the ambient high temperature and humidity obtained in the tropical countries like Nigeria make it more susceptible for rapid development of senescence and decay. Thus the purpose of this study is therefore to solve this problem through adoption of proper post-harvest practices and processing of fresh moringa into value added products such as leaf powder, tea, capsules, oil extracts/ointments, facial/body creams and etc.

Literature Review

According to Ramachandran, *et. al.*, (1980), moringa is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce. Analyses of the leaf composition have revealed them to have significant quantities of vitamins A, B and C, calcium, iron and protein. They further indicated that scientific research confirms that gram for gram, moringa leaves contain: SEVEN times the vitamin C in oranges, FOUR times the Calcium in milk, FOUR times the vitamin A in carrots, TWO times of the protein in milk and THREE times the Potassium in bananas. They also indicated that moringa tree has great use medicinally both as preventative and treatment and much of the India's ancient tradition of Ayurveda says the leaves of the moringa tree prevent 300 diseases. Field reports and ecological studies form part of a rich traditional medicine history claiming efficacy of leaf, seed, root, bark, and flowers against a variety of dermal and internal infections. Moringa species have long been recognized by folk medicine practitioners as having value in the treatment of tumors.

The nutritional and natural products characterization of Moringa conducted in this study show that the leaves of this plant can contribute significantly to the daily recommended allowance needed for many vitamins and mineral needs as well as serve as a rich source of polyphenols, confirming the importance and role that Moringa can play to improve the health and nutrition particularly in malnourished populations (Copin, J., 2008).

Busani, *et. al.*, (2011) in his study concluded that the data derived from nutrient characterization of moringa were clear indications that the plant leaves are rich in nutrients and has potential to be used as a feed additive with multiple purposes. These included serving as a protein, fatty acid, mineral and vitamin resource for animal and human feed formulations. High nutritional content found in the dried leaves are important nutritional indicators of the usefulness of the plant as a likely feed resource. Drying the leaves assisted to concentrate the nutrients, facilitated conservation and consumption, as such; it could be used during the time when feed is scarce or could be transported to areas where it is not cultivated. It was suggested that moringa should be consumed in the powder form.

Fahey, (2005) indicated that moringa possess some medicinal properties and its inclusion in the diets could function as curative and therapeutic therapy. As such, it could be used to improve health and nutrition in sub-Saharan countries.

Quoting Cheryl Laures, RN, *Personal Wellness Consultant* in 2009, the following testimonials were reported from her clients:

“I have used Zija extensively over the past 8 months (on myself and clients). I can honestly say there is nothing like it! After 25 years as a Clinical Nutritionist, I know this is a product I can endorse without reservation.

- 1) No more sleep apnea
- 2) No more sinus congestion
- 3) No more bloating after eating
- 4) Need only 5 to 6 hours of sleep and not 8 or 9 hours
- 5) Don't go to the bathroom in the middle of the night
- 6) Less muscle cramping and less leg fatigue
- 7) Much more calm under stress—“cured my road rage!”
- 8) No more hypoglycemia
- 9) Better flexibility and almost no joint pain
- 10) No more adrenal exhaustion
- 11) No Irritable Bowel Syndrome
- 12) No more food and air allergies
- 13) Greater lung capacity, from 68% to 85%
- 14) More stamina during, and faster recovery after, tennis and basketball
- 15) Better mental clarity and focus”

“One of my customers started Zija for insomnia, and within two days, was sleeping like a baby. After 5 weeks, she came out of the doctor's office testing for her high blood pressure and it had dropped down to 120/80, and her cholesterol was normal. She was able to come off of her meds for these, and she lost five lbs. in the process!”

“I was fighting with cancer, now my chemistries have all come back normal. After a year of consuming a can of Zija a day, my wife's really huge bunions are gone!”

“After 15 years of fighting fatigue and bad constipation, I could tell something was going from bad to worse. The doctors tried everything and could not find anything. I was totally laid up. Two weeks ago, a 3-4 lb. tumor excreted itself from my body—unbelievably huge, like a purple-black placenta. I am getting my life back!”

The following are testimonials from moringa users globally as reported on the website <http://moringawealthandhealth.blogspot.com/p/moringa-testimonials.html>: “I will start with myself. I used to have severe lower back ache. After I started taking Moringa, I did not quite notice the pain disappeared until one day, I just noticed there was no form of discomfort around my waist area. And that was it. It never surfaced till today!

Two women supposedly through with child bearing became pregnant within 4 months of using moringa seeds. A young mother of three, with chilly feeling in the chest was relived with the use of moringa seeds. Her child’s severe ear ache stopped after moringa leaf-juice was squeezed into her ear.

Two men that suffered and were weakened by malaria attacks regained vitality after the use of moringa leaves and seeds.

A woman from Anambra State, involved in a vehicle accident, had her fair skin bruised which resulted dark marks on her skin. After chewing moringa seeds consistently from then till now, she noticed that her skin has also been healed.

A man, diabetic since December 2002, also has chronic peptic ulcer, but after chewing a total of 300 moringa seeds within 10 weeks, he regained his weight, looks and vitality.

A lady who had recurrent malaria, stomach ailment, dental caries with hole got healed from all these after using moringa leaves and seeds.

A woman that had agalactia regained full breast-milk production and let-down after using moringa leaf powder.

A hair-dresser that had peptic ulcer and filarial skin disease was healed after consuming a total of 100 moringa seeds.

A lady with phobia for small dark rooms, marked by feeling of suffocation, got over this feeling with the use of moringa seeds.

Two young ladies reported having recovered from dysmenorrhea (painful menstruation) and amenorrhea (cessation of menstrual flow) respectively, after using moringa leaf powder.

Body fat was drastically reduced when a woman used moringa seed and leaves.

Some women used moringa seeds and leaves to improve and recondition their skin and hair.

Sore throat, cough, skin boils, worm infestation were reported to have been healed using moringa seeds and leaves.

Improved libido in men and women was also achieved by the use of moringa seeds and leaves

A woman was scheduled for surgical removal of a skin tumor at the cost of N30,000.00 got healed after the use of moringa seeds!

A middle aged man on the surgical list with pile also had the pile handled after using 100 moringa seeds.

A poor-sighted 60- year old woman who had used eye glasses for many years, could now see clearly (without eye glasses) after consistently chewing of moringa leaves.

A civil servant that could not go to the office due to disease health condition resumed office work within 6 weeks of taking a glass of fresh moringa water squeezed from moringa fresh leaves.

A lady who had severe back pain after delivery recovered after weeks of taking moringa leaf powder

A 90-year old diabetic had his sugar level miraculously lowered by consuming powdered moringa seeds.

An elderly man with diabetic sore hospitalized for 8 weeks whose legs was to be amputated to avoid whole leg ulceration used about 100ml of moringa oil got healed with skin restored and discharged within two weeks of use.

A middle aged lady with hair loss challenge had fuller hair after using 33mls of moringa oil as hair cream.

A man, over 40 years with non-healing reddish sores had the sore 50% healed within 4 weeks applying crushed seeds once a week.

A 50 year old lady had a toe cut by stone chewed 3 seeds made a paste out of it and applied on the bleeding wound, ten stuck to the surface of the wound, killed all the pain healing the wound within 4 days. She also used the moringa body cleanser (liquid supplement) to relieve her from HBP and cramps.

A young lady married for over 2 years with challenges that could not allow conception became a proud mother within 2 years of chewing about 6 moringa seeds daily!

"I normally didn't have a good sleep. But after using moringa for some time now I sleep like a baby. The moringa tree is very good. Thank God for such a good and miracle tree"

Anamed International (a German based humanitarian network of development workers, doctors, nurses and healers for the exchange of medical knowledge and experience in the Tropics) use moringa leave powder in conjunction with Artemisia tea for treating malaria, strengthening the immune system of AIDS patients, and treating several other diseases. Rural Extension for Africa Poor (REAP) in Kenya in collaboration with Anamed International offers a one week international seminar on various medicinal plants including moringa for nutritional and medicinal uses. And according to REAP (2013), moringa is a natural medicine that can be grown at home. Their workshops, seminars and other training programs are making tremendous impact on the awareness and dissemination of moringa growing, processing

and use as nutritional and medicinal supplement in rural communities of many East Africa countries, South Sudan, and Ethiopia.

According to Anamed International (2013), for nutrition, the leaves of moringa are outstanding as a source of vitamins A, B, and C. They contain many minerals, particularly calcium, iron and phosphorus. They are also rich in protein, and contain many essential amino acids required for good health, which the body cannot produce itself. Fresh leaves of moringa have 4 times the calcium content of milk and 4 times the vitamin A content of carrots – and these become concentrated in moringa leaf powder. For medicine, *M. oleifera* has constituents with antibiotic properties (isothiocyanates) which inhibits the growth of cancerous tumors.

Goals and Objectives

The primary goal of this study is to develop moringa value added products as nutritional and medicinal supplements for healthy living in Nigeria. And the specific objectives of this study were:

1. To acquire the knowledge and skills required to harvest, process and use *Moringa oleifera* as nutritional supplement and medicine.
2. To develop value added products from fresh moringa leaves and flowers (i.e., leaf powder, tea and capsules).
3. To evaluate the use of moringa value added products as nutritional and medicinal supplements for healthy living.

Justification of the Study

It is evident from literature that *Moringa oleifera* (especially the leaves) has remarkable nutritional and health benefits. The first and second International Moringa Development Summits held in Nigeria between year 2010 and 2012 created great awareness and sensitized people to start growing moringa mostly at their backyards. For sustainable production of moringa, groups of farmers in different states established National Moringa Development Associations. After interacting with individuals and Kwara State moringa development farmers association, it was identified that much of the moringa grown were either left to waste on the trees or harvested and dried to brownish or yellowish leaf powder. Also, moringa leaves are perishable commodity and the ambient high temperature and humidity obtained in the tropical countries like Nigeria makes it more susceptible for rapid development of senescence and decay. Thus there is therefore the need to solve these problems through adoption of proper post-harvest practices and processing of fresh moringa into value added products. The value added products could be in form of leaf powder, tea, capsules, facial/body creams, and oil extracts/ointments. These could be preserved and used for nutritional and medicinal purposes.

MATERIALS AND METHODS

Acquisition of Knowledge and Skills on Harvesting, Processing and Adding value to Fresh Moringa Leaves and Flowers.

In order to acquire some knowledge and skills on growing, harvesting and processing moringa, the researcher attended a one week (May 18th – 25th, 2013) Agricultural medicinal plant seminar offered by REAP in Nairobi, Kenya. Through classroom sessions, demonstrations and practical hands on experiences; the harvesting and processing of fresh moringa leaves were taught and the researcher acquired the knowledge and skills of producing value added products such as moringa leave powder, tea, herbal oil extracts, ointments, creams, lotions, and soaps.

Harvesting and Processing of *Moringa oleifera* to Value Added Products

The researcher collaborated with the Kwara State Chapter of National Moringa Development Association and Noble Icon Living Healthy (a commercial NAFDAC certified moringa processing company located in Ilorin metropolis in Kwara State) for materials, equipment and procedures used for this study. Fresh moringa leaves and flowers were harvested early in the morning from three association farmers. The harvested leaves and flowers were transported to Noble Icon Living Healthy within the hour of harvesting. The leaves and flowers were removed from the stalks, cleaned, sorted, arranged in the solar drying trays, and allowed to dry in the commercial solar dryer within 4 hours of the sunny day. The dried leaves and flowers were further transferred to the different grinders that processed them to leave powder and tea respectively. The capsules were produced using the empty gelatin capsules and manual capsule making machine (purchased from NOW Foods Company in United States of America); and the dried moringa leaf powder produced at the Noble Icon Company. The dried moringa leave powder, tea, and capsules were packaged according to NAFDAC certification requirements.

Evaluation and Use of Moringa Value Added Products as Nutritional and Medicinal Supplements

Following NAFDAC standard measures, the dried moringa leave powder and tea were analyzed in the laboratory for their nutritional and medicinal contents. The results of the laboratory analysis were compared with those obtained from other studies and daily recommended values. The NAFDAC suggested daily recommended nutritional value, dosage and directions for use were indicated on the NAFDAC certified packages respectively.

RESULTS AND DISCUSSIONS

Acquisition of Knowledge and Skills on Nutritional and Medicinal Use; Harvesting; Processing; and Adding value to Fresh Moringa Leaves and Flowers.

The knowledge and skills acquisition on use, harvesting, processing and adding value to moringa were obtained in a series of class sessions as shown in Fig. 1. Key information obtained is presented in outlines as follows:



Fig. 1: REAP Training Sessions in Nairobi, Kenya, May 18th – 25th, 2013

Characteristics of *Moringa oleifera*

- *Moringa oleifera* is a small, fast-growing deciduous tree or shrub that reaches 12m in height when mature.
- The tree grows rapidly from seeds or cuttings, even in poor soils.
- As the tree grows, the more it is cut back, the more leaves it will produce
- For nutrition, the leaves are outstanding as a source of vitamins A, B, C, and contains many minerals particularly calcium, iron and phosphorus.
- The leaves are also rich in protein and contain many essential amino acids required for good health.
- Fresh leaves are nutritious and eaten as a vegetable and wherever Anamed has introduced moringa, its leaf powder is being used very successfully to combat malnutrition, particularly amongst children.
- The leaves are harvested when they are at their best, and processed to leaf powder for use in the dry season when few other vegetables are available.

Harvesting and Processing Fresh Moringa Leaves to Dried Leaf Powder

- Harvest the leaves by breaking the stalks from the main stem.
- Remove the leaves from the stalks, sort out yellow or brown leaves and wash if necessary.
- Dry within 3 days if possible in the shade as they lose their vitamin A in strong sunlight.
- Dried leaves obtained must be dark green color. If the color changes to brownish or yellowish, the products become ineffective.
- Pound, sieve to remove the stems and store in airtight jars in the dark.

- If possible, check the moisture content with a hydrometer so that you know exactly how long you can store the leaf powder.

Nutritional Use

- Fresh leaves have 4 times the calcium content of milk and 4 times the vitamin A content of carrots – and these become concentrated in moringa leaf powder.
- The leaf powder can be added to rice, grains, sauces, in fact anything at all.
- For children under five, suffering from malnutrition and other health problems, a teaspoonful should be to their food three times a day.
- For children over five, add 2 or 3 tea spoonful, depending on their size and age.
- The pods when they are very young could be cut into short pieces and cooked and eaten as green beans.
- The flowers can be eaten as they are or used to make tea.
- Moringa seed oil is used for cooking like olive oil, lubrication and soap making.
- The seed cake can be used for water purification or fertilizer.

Medicinal Use

- Eating moringa leaves helps in cases of diarrhea, increases the amount of breast milk, and treats anemia and ulcers.
- For diabetes, drink tea made from the leaves several times a day
- Gout, stomach disorders and rheumatism, moringa seed oil is beneficial.
- The bitter roots serves as a tonic to the body and lungs; laxative; diuretic; enrich the blood; cure inflammations, chest wounds, bronchitis, piles (hemorrhoids), and loss of appetite.
- Squeezed leave juice, boiled root and bark, and paste of pounded seeds are great for skin infections and scabies.
- Pounded fresh root is used to treat toothache.
- Boiled pounded fresh leaves or scraped bark are used to treat wounds.
- Swollen lymph glands or bruises, pound the fresh leaves or bark, wrap in a banana leaf, place on hot charcoal, and place on the affected area.
- For colds, steep some flowers in boiling water for about 5 minutes and drink as needed
- Rheumatism, pound fresh roots, apply to the affected area or roast some seeds, pound to a powder, mix with vegetable oil and apply to the affected area.
- Oils and ointments are conventional herbal medicines for treating skin complaints of all sorts – rashes, cuts, bruises, sprains, burns, wounds, and even

rheumatic complaints. These could be stored for several years and very useful when herbs are not available the whole year round.

N.B. Root bark should not be eaten, nor the roots be used in any way by pregnant women.

Harvesting, Processing and Development of *Moringa oleifera* Value Added Products

After early morning harvesting, transporting, destalking, sorting, washing, and spreading of fresh moringa leaves on the drying trays; dark green dried leaves was obtained using the Noble Icon commercial solar dryer as shown in Fig. 2 and 3 below:



Fig. 2: Commercial Solar Dryer: Used for processing fresh Moringa leaves and flowers



Fig. 3: Farmer, Kwara State National Moringa Development Association and CEO, Noble Icon of the factory and solar dryer

As shown in Fig. 4, dark green colored and very fine (0.2mm – 0.5mm) moringa leaf powder was obtained after grinding with grinding machine and sieving the dried moringa leaves. Also a mixture of dark green colored leaves and cream colored flowers of coarse size (1.0 mm – 1.5 mm) was obtained and this was further processed to tea bags using the tea bag machine (Fig. 5). Using the NOW Foods Manual capsule making machine and the dark green fine moringa leaf powder, twenty four (24) dark green colored capsules were obtained per batch (Fig. 6). The value added products of leaf powder, tea and capsules obtained in this study were packaged as shown in Fig. 6 and 7. Thus, the harvesting, processing, value addition products, and their packaging met the recommended quality standard obtained by Anamed International (2011), REAP, (2013), and the NAFDAC certification requirements.



Fig. 3: Dark Green Colored Moringa Leaf Powder NAFDAC Certified



Fig. 4: Dark Green Colored Moringa Tea NAFDAC Certified



Fig. 5: Moringa Capsule Made from NAFDAC Certified Dried Moringa Leaf Powder



Fig. 6: NAFDAC Certified Packaged Moringa Leaf Pow-



Fig. 7: NAFDAC Certified Packaged Moringa Tea

Evaluation and Use of Moringa Value Added Products as Nutritional and Medicinal Supplement

As shown in Table 1, the protein content of 27.1; calcium of 2,003; Iron of 28.2; and vitamin A (Beta carotene) of 16.3; and vitamin C of 17.3 were obtained from the laboratory analysis of dried moringa leaf powder developed in this study.

Table 1: Nutritional Value of Dried Moringa oleifera Leaf Powder (Analysis per 100g)

Nutrients	Value per 100g
-----------	----------------

Moisture (%)	7.5
Calories	205
Protein (g)	27.1
Fat (g)	2.3
Carbohydrate (g)	38.2
Fiber (g)	19.2
Minerals (g)	
Ca (mg)	2,003
Mg (mg)	368
P (mg)	204
K (mg)	1,324
Cu (mg)	0.57
Fe (mg)	28.2
S(mg)	870
Oxalic acid (mg)	1.6%
Vitamin A-B carotene (mg)	16.3
Vitamin B- choline (mg)	-
Vitamin B1- thiamine (mg)	2.64
Vitamin B2- riboflavin (mg)	20.5
Vitamin B3- nicotinic acid (mg)	8.2
Vitamin C- ascorbic acid (mg)	17.3
Vitamin E- tocepherol acetate (mg)	113
Arginine (g/16g N)	1.33%
Histidine (g/16g N)	0.61%
Lysine (g/16g N)	1.32%
Tryptophan(g/16g N)	0.43%
Phenylalanine (g/16g N)	1.39%
Methionine (g/16g N)	0.35%
Threonine (g/16g N)	1.19%
Leucine (g/16g N)	1.95%
Isoleucine (g/16g N)	0.83%
Valine (g/16g N)	1.06%

As shown in Table 2 a similar results obtained by Armelle de S. S. and Broin, M. (2010) study, the mean values of protein content of 20-26; Calcium of 1600-2200; Iron of 18-28; Vitamin A of 4-8; and Vitamin C of 15-100 per 100 grams of *Moringa oleifera* leaf powder were lower than values obtained in this study. Also, the nutritional and medicinal properties obtained for the dried leaf powder and tea as listed on the packages met the NAFDAC daily recommended values for certification. Thus, the results of the laboratory analysis of the dried moringa leave powder and tea obtained in this study indicated their use as nutritional and medicinal supplements. And these are supported by several other studies and testimonials from Armelle de S. S. and Broin, M. (2010), Anamed International (2011), and REAP (2013).

Table 2: Mean nutritional values of 100 grams *Moringa oleifera* leaf powder.

Nutrients	Mean Value per 100g
Dry matter (%)	90-95
Proteins (g)	20-26
Total ash= total minerals (g)	8-11g
Minerals	
Calcium (Ca) (mg)	1600-2200
Potassium (K) (mg)	800-1800
Magnesium (Mg) (mg)	350-500
Phosphorus (P) (mg)	200-600
Iron (Fe) (mg)	18-28
Manganese (Mn) (mg)	5-9
Zinc (Zn) (mg)	1,5-3
Copper (Cu) (mg)	0.7-1.1
Vitamins	
Vitamin C (mg)	15-100
Vitamin A (as β -carotene) μ g retinol eq.	4000-8000
Vitamin E (as tocopherol) (mg)	60-150

CONCLUSIONS

1. From the REAP medicinal plant seminar, it was understood that *Moringa oleifera* plant parts are loaded with nutritional and medicinal benefits for healthy living.
2. Value added products of dried moringa leaf powder, tea and capsules were developed from perishable fresh moringa by collaborating with Kwara State Chapter of Moringa Development Association farmers and Noble Icon Company.
3. By using standardized harvesting, postharvest processing equipment of commercial solar dryer and procedures, value added dried moringa leave powder, tea and capsules were developed.
4. The nutritional values of protein, calcium, iron, vitamin A and vitamin C obtained in this study were higher than those obtained by Armelle de S. S. and Broin, M. (2010) in a similar study, and met the daily recommended values for NAFDAC certification.
5. The laboratory analysis of the value added moringa products indicated their potential use as nutritional and medicinal supplements. And these are supported by Armelle de S. S. and Broin, M. (2010), Anamed International (2011), and REAP (2013) who indicated that dried moringa leaf powder and tea are useful for treating malaria, strengthening the immune system of AIDS patients, and treating several other diseases such as blood pressure, diabetes, pile, nasal congestion and etc.

RECOMMENDATIONS

1. In this study, only value added products from the moringa leaves and flowers were studied. There is the need for more comprehensive studies to identify other nutritional and medicinal values in other parts of moringa plant.
2. More studies need to be done in order to identify the diversity and level of usage of moringa leaf powder, tea and capsules.
3. More comprehensive clinical evaluation need to be done in order to understand the effectiveness of moringa as nutritional and medicinal supplement for healthy living.

REFERENCES

- Anamed (Action for Natural Medicine), (2011).** Welthungerhilfe Rwanda, Artemisia Training Manual. Schafweide 77; 71364 Winnenden, Germany; www.anamed.net. Dr. Hans-Martin Hirt: anamedhnh@yahoo.de; Dr. Keith Lindsey: anamed@t-online.de
- Armelle de Saint Sauveur and Broin, Mélanie (2010)** Growing and processing moringa leaves. Moringanews / Moringa Association of Ghana.
- Busani, M. et. al. (2011).** Nutritional characterization of Moringa (*Moringaoleifera*Lam.) leaves. African Journal of Biotechnology Vol. 10(60), pp. 12925-12933, 5 October, 2011 Available online at <http://www.academicjournals.org/AJB> DOI: 10.5897/AJB10.1599 ISSN 1684–5315 © 2011 Academic Journals
- Cheryl Laures, C. (2009).** THE MIRACLE TREE PART 2: A Deeper Look. www.natural-lifenews.com Natural Life News & Directory.
- Coppin, J. (2008).** A study of the nutritional and medicinal values of *Moringa oleifera* leaves from Sub-Saharan Africa: Ghana, Rwanda, Senegal, and Zambia. A thesis submitted to the Graduate School-New Brunswick Rutgers, The State University of New Jersey For the degree of Masters of Science Graduate Program in Medicinal Chemistry
- Fahey, J. W. (2005).** *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Trees for Life Journal 2005, 1:5 <http://www.tfljournal.org/article.php/20051201124931586>. Accessed 13/11/2013
- Oliver-Bever, B. (1986).** Medicinal Plants in Tropical West Africa. Cambridge University Press, Cambridge
- Ramachandran, C., Peter, K.V. and Gopalakrishnan, P.K. (1980).** Drumstick (*Moringa oleifera*): A multipurpose Indian Vegetable. Economic Botany, 34 (3) pp276-283.<http://www.naturalnews.com/022272.html#ixzz1FZc4OI0>
- REAP (2013).** Agricultural Medicinal Plant Training Seminar Leaflet
- Watt, J. M. and Breyer – Brandwijk, M.G. (1962).** The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd Ed. E. Livingstone, Edinburgh.

AMINO AND FATTY ACID PROFILES OF *MORINGA OLEIFERA* SEED FLOUR

Abiodun, O.A., Adegbite, J.A., Ojo, A. and Amanyunose, A.A.

Osun State Polytechnic, P.M.B. 301, Iree, Osun State, Nigeria

Corresponding author email: *funmiabiodun2003@yahoo.com*

Phone number: 08030701354

ABSTRACT

Amino acid and fatty acid profiles of Moringa oleifera seeds flour and oil were determined in this study. The seed was dried, milled and packaged in polyethylene bag. Analysis carried out on the flour includes proximate, amino acid and fatty acid profiles. Moringa seed flour contains higher protein (28.08%), fat (45.84%) and crude fibre, content (7.73 %). The anti-oxidant value was 38.08 mgAAEg⁻¹. The result revealed higher amino acid in Moringa seed flour with all the essential amino acid. Amino acid profile showed glutamate (14.83 g/100g) as the predominant amino acid in the seed flour. The major fatty acid in moringa seed oil was oleic acid (72%) which is unsaturated fatty acid. Moringa seed flour and oil are promising products from Moringa Oleifera which could be used to improve the diet of human being.

Keywords: *Defatted, Moringa seed, Flour, Amino acid, Fatty acid*

INTRODUCTION

Moringa oleifera (Syn. M. ptrygosperma Gaertn.) is of the family Moringaceae. It is a small graceful tree with sparse foliage, often planted in compounds or used as hedge in northern Nigeria (Kawo *et al.*, 2009). *Moringa oleifera* is a fast growing, aesthetically pleasing small tree. The specie is characterized by its long, drumstick shaped pods that contain its seeds within the first year of growth (Ajar *et al*, 2013). It is well known for its nutritional and medicinal values by many communities in northern Nigeria (Kawo *et al.*, 2009). Most parts of this plant: root, bark, gum, leaf, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia (Fahey, 2005). It has been used for the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Morimitsu *et al.*, 2000; Siddhuraju and Becker, 2003; Ogbunugafor *et al.*, 2011). The seeds are sometimes removed from mature pods and eaten like peas or roasted like nuts (Ajar *et al*, 2013). *Moringa oleifera* seeds are said to be very good and safe for water treatment (Ogbe and Affiku, 2011/12). *Moringa oleifera* extract was reported to have antibacterial properties and conclusion was made to investigate it as a phytotherapeutic agent to combat infectious agents (Patel, 2011). Due to the importance and benefit of this crop, moringa seed flour and oil had to be studied extensively. Although, the protein and fatty acid composition had been studied in other part of the country (Anwar and Rashid, 2007; Kawo *et al.*, 2009) but generally, the protein and fatty acid could be affected by factors such as location, climatic conditions, species, age and soil type. Therefore, the objective of this work was to deter-

mine the amino acid and fatty acid profile of moringa seed obtained in Osogbo, Osun State, Nigeria.

MATERIALS AND METHODS

Moringa seeds were purchased at Oja-Oba market, Nigeria. The seeds were dried, milled and packaged in polyethylene bag.

Analyses

Proximate composition of the flour was determined using modified methods of AOAC (2006)

Total antioxidant capacity

The total antioxidant capacity was evaluated by phosphomolybdenum method as described by Nabasre and Bratatti (2007). The assay was based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. Extract solution in ethanol (0.3 mL; 100 $\mu\text{g mL}^{-1}$) was mixed with reagent solution (3 mL; 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). A blank composed of 3 mL of reagent solution and methanol was also prepared. All tubes were capped and incubated in boiling water bath at 95 °C for 90 min. Absorbance of samples were read against blank at 695 nm. The antioxidant capacities of samples were expressed as mg Ascorbic Acid Equivalent per gram of sample (mg AAE g⁻¹)

Amino acid determination

The sample (10 g) was weighed into 250ml conical flask and the sample was defatted using soxhlet extraction apparatus. The defatted flour was hydrolyzed with 6M HCl three times for complete hydrolysis to be achieved. The amino acid content of the flour recovered was concentrated and 1ml of the extract was introduced into the gas chromatography (HP 6890 powered with HP ChemStation Rev. A 09.01 [1206] Software). The content was concentrated to 1 μl for gas chromatography analysis before injected into the injection port of GC. Carrier gas was Nitrogen, Inlet temperature was 250°C, column dimension 30m $\times$ 0.25mm $\times$ 0.25 μm . Initial temperature was 60°C, first ramping at 12°C for 2 min, while second ramping at 15°C for 8 min. Detector temperature for the analysis was 320°C.

Fatty acid profile determination

Fatty acid composition was determined using Gas Chromatography (HP 6890 powered with

HP ChemStation Rev. A 09.01 [1206] Software). Oil sample (50mg) was esterified for five minute at 95°C with 3.4 ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl and 3 ml of 14% boron trifluoride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90°C to achieve complete methylation process.

The Fatty Acid Methyl Esters were thrice extracted from the mixture with redistilled nhexane. The content was concentrated to 1 µl for gas chromatography analysis and 1 µl was injected into the injection port of GC. Carrier gas was Nitrogen, Inlet temperature was 250°C, column dimension 30m × 0.25mm × 0.25 µm. Initial temperature is 60°C, first ramping at 12°C for 2 min, while second ramping at 15°C for 8 min. Detector temperature was 320°C (Awolu *et al.*, 2013).

RESULTS

Proximate and antioxidant composition of moringa seed flour are shown in Table 1. Moringa seed flour contains protein (28.08 %), fat (45.84 %) and crude fibre (7.73 %). The anti-oxidant value was 38.12 mgAAEg⁻¹. Amino acid profiles of Moringa seed flour are shown in Table 2. The predominant amino acid in *Moringa oleifera* seed was glutamate with highest value (14.83 g/100g), followed by arginine (8.31 g/100g) and aspartate (7.2 g/100g). Table 3 showed the amino acid score of *Moringa oleifera* flour. The amino acid score showed that moringa seed flour was limiting in lysine (55), leucine (85.91), valine (89.71) and threonine (98.82) for pre-school children. Table 4 showed the fatty acid composition of moringa seed oil. The major fatty acid composition of *Moringa oleifera* seed oil were oleic acid (72.59 mg/100 g), behenic acid (7.33 mg/100g), palmitic acid (6.77 mg/100g), arachidic acid (4.86 mg/100g) and stearic acid (3.29 mg/100g).

DISCUSSION

Moringa oleifera was classified as protein source due to the high protein contents and has potential to improve nutrition, boost food security, foster rural development and support sustainable land care (National Research Council, 2006). Crude protein of soybean was higher than the moringa seed while the oil yield of moringa was higher than that of soybean (USDA, 2007). Proteins are needed in the body for repair and replacement of worn-out tissues, serve as antibodies, primary sources of amino acids and the building block of cellular protein Adeleke and Odedeji (2010). The seed contain appreciable amount of crude fibre which aid in digestion process. It contributes to the bulk density which could help in the bowel movement, lower blood cholesterol and helps prevent cancer of the colon (Hung *et al.*, 2004). Antioxidant value observed was slightly higher than that of 37.94 mgAAEg⁻¹ reported by Ogbunugafor *et al.* (2011). Antioxidant fights against free radicals in the body thereby preventing cancer in human. It intercepts free radical and protects cells from the oxidative damage that leads to aging and disease (Olayinka *et al.*, 2012).

Table 1: Proximate and anti-oxidant composition of Moringa seed flour

Parameter	Value
Protein (%)	28.04±0.86

Fat (%)	45.84±0.42
Crude fibre (%)	7.73±0.10
Total anti-oxidant (mgAAEg ⁻¹)	38.12±0.09

The amount of amino acid in the flour was 77.43 g/100g which was higher than soybean (36.49 g/100g) and Africa pumpkin seed (48.60 g/100g) (USDA, 2007; WHO, 2003). Methionine had lower value (1.04 g/100g) in moringa seed flour. Moringa seed flour contained all the essential amino acid needed for growth and repairs of worn out tissues in the body. This corroborates the report of Olugbemi *et al.*, (2010) that *Moringa oleifera* is a good source of vitamins and amino acids.

Table 2: Amino acid profile of *Moringa oleifera* seeds

Names	Amount g/100g of samples
Glycine	4.95
Alanine	3.03
Serine	4.50
Proline	2.28
Valine	3.14
Threonine	3.36
Isoleucine	4.36
Leucine	5.67
Aspartate	7.15
Lysine	3.19
Methionine	1.04
Glutamate	14.83
Phenylalanine	4.10
Histidine	2.40
Arginine	8.31
Tyrosine	2.66
cysteine	2.23
Total	77.43

The percentage (%) score of the essential amino acids in moringa seed was compared with those of the reference standard amino acids profile for foods established for pre-school children and adults by WHO/FAO/UNU (1985) (Table 3). The results indicated that most of the essential amino acids in moringa seed scored above 100%

of the reference standard value for adults established by WHO/FAO/UNU (1985). This showed that *Moringa oleifera* is a source of essential amino acids for adult. Leucine, lysine, valine and threonine of moringa seed had amino acid scores below 100 % indicating the limiting amino acid in pre-school children. The amino acid score of moringa seed compared well with beef and other protein sources such as peanut flour and soy protein. The chemical scores of beef ranged from 85-94 limiting in valine, aromatic amino acids while soy protein score ranged from 58-96 limiting in valine when compared with the FAO reference pattern (FAO/WHO, 1973, Seligson and Mackey, 1984). Chemical score for peanut flour ranged from 39 to 65 with lysine being the limiting amino acid (Seligson and Mackey, 1984).

Table 3: Amino acid score of *Moringa oleifera* flour

Essential amino acid	WHO Ideal protein		Amino acid concentration (g/100 g)	Percentage Amino acid score	
	Children	Adult		Children	Adult
Isoleucine	2.8	1.3	4.36	155.71	335.38
Leucine	6.6	1.9	5.67	85.91	298.42
Lysine	5.8	1.6	3.19	55.00	199.38
Total sulphur Amino Acid	2.5	1.7	3.27	130.80	192.35
Total aromatic amino acid	6.3	1.9	6.76	107.30	355.79
Threonine	3.4	0.9	2.66	98.82	373.33
Valine	3.5	1.3	3.14	89.71	241.54
Histidine	1.9	1.6	2.40	126.32	150.00

A = WHO/FAO/UNU ideal protein for pre-school children aged 2 to 5 years; B = WHO/FAO/UNU idea protein for adult. Percentage (%) amino acid score = (amino acid in sample/ideal) × 100. Source: WHO/FAO/UNU (1985).

Moringa seed oil contained higher value of unsaturated fatty acid and this compared well with the soybean oil and melon oil (WHO, 2003). The oil has four major unsaturated fatty acid including, oleic, linoleic acid, linolenic acid and Palmitoleic acid which are essential in human diet. Oleic acid was the predominant fatty acid in moringa oil with lower values in the saturated fatty acid composition. Oleic acid

lowers the risk of heart attack and aids in cancer prevention. The oil serves as a good source of fat in human nutrition.

Table 4: Fatty acid profile of *Moringa oleifera* seed oil

Name	Amount mg/100g
Lauric acid	0.06
Myristic acid	0.14
Palmitic acid	6.77
Palmitoleic acid	1.11
Margaric acid	0.16
Stearic acid	3.29
Oleic acid	72.59
Linoleic acid	1.19
Linolenic acid	0.67
Arachidic acid	4.86
Behenic acid	7.33
Erucic acid	0.26
Lignoceric acid	1.56

CONCLUSION

Moringa seeds compared well with soybeans and other legumes in their nutritional value. It serves as good source of essential amino acids for adult and the fatty acid composition were predominantly the unsaturated types which are good for the body. The higher antioxidant in the seed flour helps to fight the free radicals in the body thus preventing formation of cancerous cells.

REFERENCES

- Adeleke R.O., Odedeji J.O. (2010).** Acceptability studies on Bread fortified with Tilapia Fish Flour. *Pakistan Journal of Nutrition* 9(6): 531-534.
- Ajar, P.M., Ibiam, U.A., Uraku, A.J., Orji, O.U., Offor C.E. and Nwali, B.U. (2013).** Comparative Proximate and Mineral Composition of *Moringa oleifera* Leaf and Seed. *Global Advanced Research Journal of Agricultural Science*, 2(5): 137-141
- AOAC, (2006).** Association of Official Analytical Chemists Official methods of Analysis 18th edition, Revision 4. Washington, DC, USA.

- Awolu, O.O., Obafaye, R.O. and Ayodele, B.S. (2013).** Optimization of Solvent Extraction of Oil from Neem (*Azadirachta indica*) and Its Characterizations. *Journal of Scientific Research and Reports* 2(1): 304-314
- Anwar, F. and Rashid, U. (2007).** Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. *Pakistan Journal of Botany*, 39: 1443-1453.
- Fahey, J.W. (2005).** A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. *Trees life J.*, 1.
- FAO/WHO (1973).** Energy and protein requirements: Report of a Joint FAO/WHO Ad Hoc Expert Committee. WHO Tech. Rep. Series No. 522, WHO, Geneva, Switzerland.
- Hung H.C., Joshipura K.J. and Jiang R. (2004).** Fruit and vegetable intake and risk of major chronic disease. *Journal of National Cancer Institute* 96, 1577.
- Kawo, A.H., Abdullahi, B.A., Gaiya, Z.A., Halilu, A., Dabai, M. and Dakare, M.A. (2009).** Preliminary phytochemical screening, proximate and elemental composition of moringa oleifera lam seed powder. *Bayero Journal of Pure and Applied Sciences*, 2(1): 96 - 100
- Morimitsu, Y., K. Hayashi, Y. Nakagama, F. Horio, K. Uchida and T. Osawa, (2000).** Antiplatelet and anticancer isothiocyanates in Japanese horseradish, wasabi. *BioFactors*. 13: 271-276
- Nabasree D. and Bratati D. (2007).** Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chemistry*, 101:471-474.
- National Research Council (2006).** Moringa. Lost crops of Africa; Volume II: Vegetables. National Academies Press. P 247.
- Ogbe, A.O. and Affiku, J.P. (2011/12).** Proximate study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Sciences*, 1 (3) 296-308
- Ogbunugafor, H.A., Eneh, F.U., Ozumba, A.N., Igwo-Ezikpe, M.N., Okpuzor, J., Igwilo, I.O., Adenekan, S.O. and Onyekwelu, O.A. (2011).** Physico-chemical and Antioxidant Properties of *Moringa oleifera* Seed Oil. *Pakistan Journal of Nutrition* 10 (5): 409-414
- Olayinka, O.O., Kareem, A.M., Ariyo, I.B., Omotugba, S.K. and Oyebanji A.O. (2012).** Antioxidant Contents (Vitamin C) of Raw and Blanched Different Fresh Vegetable Samples. *Food and Nutrition Sciences* 3: 18-21
- Olugbemi, T.S, Mutayoba, S.K. and Lekule, F.P. (2010).** Effect of Moringa (*Moringa oleifera*) Inclusion in Cassava based diets to broiler chickens. *International Journal of Poultry Science* 9 (4): 363-367.

- Patel, J.P. (2011).** Antibacterial activity of methanolic and acetone extract of some medicinal plants used in India folklore. *International Journal of Phytomedicine*, vol. 3, p. 261-269.
- Seligson, F.H. and Mackey, L.N. (1984).** Variable predictions of protein quality by chemical score due to amino acid analysis and reference pattern. *Journal of Nutrition*, 682-691
- Siddhuraju, P. and K. Becker, (2003).** Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.). *J. Agric Food Chem.*, 15: 2144-2155.
- USDA (2007).** Full Report (All Nutrients) 16111, Soybeans, mature seeds, dry roasted. National Nutrient Database for Standard Reference Release 26
- WHO/FAO/UNU (1985).** Report: Energy and protein requirements. WHO technical report series No. 724. WHO, Geneva. pp. 220-223.
- WHO (2003).** *Diet, Nutrition and the Prevention of Chronic Diseases*. Geneva, Switzerland: Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series. No. 916: 4-81.

PERFORMANCE OF RABBITS FED *MORINGA OLEIFERA* LAM. BASED DIETS

***Oyedele, O. J. and Odeyinka, S. M.**

**National Biotechnology Development Agency, Bioresources Development Centre,
Ogbomoso, Nigeria*

Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Nigeria

***Corresponding Author's e-mail address:** oolusegunjulius@gmail.com

Phone number: +234 08056113935

ABSTRACT

In a 12-week trial, 70, 4 to 6 weeks old weaner rabbits of both sexes, (average weight of $388 \pm 0.8\text{g}$) comprising crosses of New Zealand White, Chinchilla, and California breeds, were randomly allotted to five treatments of graded levels of

Centrosema pubescens (C) and *Moringa oleifera* (M). The treatments were 100%C, 75%C25%M, 50%C50%M, 25%C75%M and 100%M diets in a completely randomized design. The mean daily dry matter intake (DMI g/day) was higher ($p > 0.05$, $F_{cal} (0.76) < F_{tab} (2.69)$) in rabbits fed 25C75M than those on 75C25M, 50C50M and 100M and lower in rabbits fed 100C. The apparent digestibility coefficient of the dry matter, CP, CF, EE, ash, NFE, organic matter and energy were significantly ($p < 0.05$, $F_{cal} (66.03) > F_{tab} (3.01)$) affected by increasing levels of *Moringa*. The total nitrogen retention (g/day) of rabbits fed 100M diet was significantly ($p < 0.05$, $F_{cal} (38.77) > F_{tab} (3.01)$) higher than those obtained by feeding 75C25M, 25C75M, 50C50M and 100C diets, respectively. It was concluded that the vegetative part of *Moringa oleifera* can effectively replace *Centrosema pubescens* in rabbit's diets.

Keywords: rabbits, *Centrosema pubescens*, *Moringa oleifera*, dry matter, digestibility coefficient

INTRODUCTION

In recent years, there has been rising global awareness on the virtues of rabbit meat production in developing countries as an alternative means of alleviating world food shortages. This basic understanding is largely attributable to the rabbit's high rate of reproduction and early maturity, rapid growth rate, high genetic selection potential, efficient feed and land space utilization, limited competition with humans for similar foods and high quality nutritious meat (Cheeke, 1980). Rabbit practices coprophagy; a process whereby product of microbial synthesis in the digestive tract are not entirely lost. Although rabbits can survive on all-forage diet, optimum performance can only be ensured in a mixed feeding regime involving forage and formulated feeds (Cheeke and Patton, 1987; Arijenwa, *et al.*, 2000). Although rabbits can survive on all forage diet, optimum performance can only be ensured in a mixed feeding regime involving forage and formulated feeds (Cheeke, 1987; Arijenwa, 2000). Bamikole *et al.*, (2005) when investigating the potential of mulberry leaves in rabbit production in Nigeria, concluded that, total dry matter (DM) intake of the concentrate: mulberry diet remained at the level of that of the all-concentrate ration (~38g/d), until mulberry leaves comprised >50% of the ration before it declined significantly.

Studies performed by Jokthan *et al.*, (2003) on the utilization of fig (*Ficus thonningii*) and Mango (*Mangifera indica*) leaves by rabbits, however showed that the average daily intake and weight gain of rabbit fed *Ficus thonningii* leaves was significantly higher than that of *Mangifera indica* leaves. *Centrosema pubescens* is a tropical pasture legume, trailing and twining with stem which root at the node when the stems are close to the ground. It has elevated growing points when climbing up

companion grasses (Humphreys, 1999). *Centrosema* is also implicated with one or more of these deleterious effects (CIEPCA, 2000).

Moringa oleifera Lamarck (synonym: *Moringa pterygosperma* Gaertner) belongs to an onogeneric family of shrubs and tree, also indigenous to Northwest India (Ramachandran *et al.*, 1980). *Moringaceae* is considered to have its origin in Agra and Oudh, in the Northwest region of India, South of the Himalayan Mountains. Although the name "Shigon" for *M. oleifera* is mentioned in the "Shushruta Sanhita" which was written in the beginning of the first century A.D., there is evidence that the cultivation of this tree in India dates back many thousands of years. The Indians knew that the seeds contain edible oil and they used them for medicinal purposes. It is probable that the people also knew of its value as a fodder or vegetable. Most reports indicate that *Moringa* leaves twigs and stems are rich in protein and present an amino acid composition, which is suitable for human and animal nutrition (Makkar and Becker 1996; Freiburger *et al.*, 1998). In a study in Nicaragua, the fresh leaves were found to contain 23% crude protein (CP) in DM, 12.3MJ ME/kg DM and had an in vitro DM digestibility of 79.7% (Becker, 1995), *Moringa* can thus be a valuable supplement for animals.

The experiment conducted by Odeyinka *et al.*, (2008), on reproductive performance of rabbit fed *Moringa oleifera* as a replacement for *Centrosema pubescens* showed that there was a significant difference in average daily weight gain per kid and in milk yield across all the treatments. Milk yield was higher in M0, but not significantly different in M75 and M100 does. They finally concluded that *Moringa oleifera* can be used to replace *Centrosema pubescens* without adverse effect on the reproductive performance of rabbits. Hence, the general objective of this study was to evaluate the performance characteristics of rabbits fed *Moringa oleifera* and *Centrosema pubescens*.

MATERIALS AND METHODS

The experiment was conducted at the Rabbit Unit of the Teaching and Research Farm, Obafemi Awolowo University. Seventy weaner rabbits of both sexes, comprising crosses of New Zealand white, Chinchilla, and Californian breeds, 4 to 6 weeks old ranges between 205 and 534 g were randomly allotted to five treatments in a completely randomized design by weight. Each rabbits each were housed in a cage with a dimension of 76cm x 62cm x 42cm. Adequate feed and water were provided daily with the use of local clay mortal bowl of 12-15cm in diameter.

Freshly harvested *Centrosema pubescens* and *Moringa oleifera* leaves were offered to the animals at the ratio of 100: 0 (100C), 75 : 25 (75C25M), 50 : 50 (50C50M), 25 : 75 (25C75M) and 0 : 100 (100M). *C. pubescens*: *M. oleifera* ratio, respectively. The animals were fed 4% of their body weight, at a ratio of 2% experimental concentrate (Table 1) and 2% *C. pubescens* and or *M. oleifera* on dry matter basis.

The vegetative part of *Centrosema pubescens* and *Moringa oleifera* leaves were collected from established plots at the Teaching and Research Farm and Food Technology, Obafemi Awolowo University; Ile- Ife; at Modakeke; Ipetumodu and Edunabon in Osun State.

The animals were subjected to a 7-day adaptation period. Growth and digestion trials lasted for 12 weeks. Three digestion trials of 7 days each were carried out (6th, 9th and 11th week) during the experimental period. Urine and faeces were collected before the morning feeding (i.e. 0900 hours) during the digestibility periods. Each animal was weighed before the commencement of the experimental period and subsequently weekly throughout the experimental period. Left-over feed were weighed to estimate the feed intake from the total feed offered. The daily urine volume was also measured during the digestibility period. Both the forages and the concentrate diets offered were weighed daily.

Proximate analyses of feed and faeces sample were determined, and urine samples were analyzed for nitrogen (AOAC, 1990).

Data obtained were statistically analyzed with the general linear model of SAS (2008). Significant means were separated using the Duncan option of the Multiple Range Tests of the same package.

Table 1: Composition of experimental concentrate for rabbits

Ingredients (%)	(%)
Groundnut Cake (GMC)	8.00
Wheat offal	19.00
Palm Kernel Cake	50.00
Maize	15.00
Fish	5.00
Bone Meal	2.00
Salt	0.25
Methionine	0.25
Lysine	0.25
Vitamin premix	0.25
Total	100.00

Calculated:

Crude Protein: 17.89

Crude fibre: 13.52

RESULT AND DISCURSION

The experimental concentrate diet used had mean crude protein content 20.61% and mean crude fibre content of 11.56% which agreed with the recommended 18-20% crude protein and 10-12% crude fibre for weaners and growing rabbits (Cheeke, 1987).

The average total dry matter intake g/day, though not significant, ($p>0.05$) showed that 50% supplementation of weaner rabbits diet with different ratio of *Moringa oleifera* and *Centrosema pubescens* increased the total dry matter intake (Table 3). The higher forage intake in 75C25M, 50C50M, 25C75M and 100M than C100 ($p>0.05$) is an index of the better acceptability of the *Moringa oleifera* to rabbits which promoted dry matter intakes. This agreed with the work of Sanchez *et al.*, (2005). The average dry matter intake range from 3.44 to 3.97% of the body weight this compares with the 4% obtained by Odeyinka *et al.*, (2007; 2008).

Animals in all the treatments were in a positive weight balance and the liveweight of the animals increased with the period of experiment (Table 3). Generally, the mean weight gains in this experiment were high when compared with the results in other recent studies. The highest daily weight gain was recorded for weaner rabbits fed the 100M (13.3g/day) which was statistically similar ($p>0.05$) to what was obtained with those on 25C75M, 50C50M, 100C and 75C25M. These daily weight gains can be compared with the result of Odeyinka *et al.*, (2007), who obtained 12.0g per day for weaner rabbits fed diet containing household wastes and Omoikhoje *et al.*, (2006), who obtained 11.06g per day on the response of weaner rabbits fed concentrate supplemented with varying levels of *Syndrella nodiflora* forage. However, weight gain obtained was higher than the value reported by Jokthan *et al.*, (2003) and Bamikole *et al.*, (2005) who obtained 7.62 and 5.72g/day respectively. The presence of deleterious effect and tanins reported to be present in *Moringa oleifera* and *Centrosema pubescens* (Makkar and Becker, 1997; Oliveira *et al.*, 1999 and CIEPCA, 2000) have not remarkably limited its utilization for weight gain in the animals.

The mean result of the two digestibility trials (Table 4) showed that there was significant difference ($p<0.05$) in digestible dry matter intake. Animals on 100M had higher dry matter digestibility (79.9%), this could be attributed to the better digestibility of the nutrients than the other treatments. The result agreed with the findings of Sanchez *et al.*, (2005) who reported that inclusion of *Moringa* as a protein supplement to low quality diets improved DM intake and digestibility of the diet and increased milk production but did not affect milk composition of creole dairy cows.

Results of the coefficient crude protein digestibility ranged from (66.8 to 82.4%). The higher value of 100M suggests that Moringa could serve as a good of replacement for Centrosema in weaner rabbit's diets (Odeyinka *et al.*, 2008). This also agrees with the report of Kakengi *et al.*, (2003) who observed high pepsin and total soluble protein in *Moringa oleifera* leaf meal (MOLM) than other parts of the plant and high pepsin and total soluble protein makes MOLM more suitable to monogastric animals. The crude fibre was poorly digested in 100C (36.4%) but highly digested in 100M (65.2%), ($p < 0.05$). This could be attributed to high acid detergent fibre (ADF) which is the fibrous and the least-digestible portion of roughage. Neutral detergent fibre (NFE) and hemicellulose contents of *Centrosema pubescens* compared to *Moringa oleifera* and rabbits digest cellulose poorly (Fraga, 1990). The ether extract though well digested in all the treatment diets, 67.1 to 83.3% ($p < 0.05$), animals in 100M had the highest digestibility. This is due to high ether extract content in Moringa than Centrosema. The ash and organic matter were well digested in 100M but poorly digested in 100C. This might be due to the attributes of Moringa reported by Delaveau and Boiteau, (1980). They reported that Moringa is an exceptionally good source of provitamin A, vitamins B, and C, minerals (in particular iron), and the sulphur-containing amino acids methionine and cystine. 100M promoted better energy digestibility (77.6%), which was significantly higher than the energy digestibility in 100C (64.5%), ($p < 0.05$). Moringa due to its high digestible energy content may be fed to growing rabbits. The presence of digestible carbohydrate may be responsible for the higher NFE digestibility observed for the weaner rabbits fed Moringa in their diets.

Animals fed 100M, 75C25M, 25C75M and 50C50M diets, retained more nitrogen (g/day) than 100C, this could be attributed to the better quality of nitrogen content in *Moringa oleifera*, (Foidi *et al.*, 2001; Kakengi *et al.*, 2003; Sarwatt, 2004 and Sanchez *et al.*, 2005) and lower amount of nitrogen retained in CI00 might be due to its tannin content. The results agreed with the findings of Onwuka (2007) that browse tannin was capable of affecting N-balance and protein digestibility coefficients.

Table 2: Chemical composition of the diets fed to experimental rabbits

Parameter	Concentrate	Centrosema	Moringa
Dry Matter Fresh (%)		38.84 ± 1.07	28.43 ± 0.21
Dry Matter (%)	91.34 ± 1.15	87.17 ± 0.94	86.83 ± 0.43
Analysis % of DM			
Organic Matter	93.51 ± 0.63	93.54 ± 0.97	91.41 ± 0.17
Crude Protein	20.61 ± 1.15	21.59 ± 1.21	26.18 ± 1.49
Crude fibre	11.56 ± 1.30	28.06 ± 0.62	14.52 ± 1.14

Ether Extracts	18.59 ± 1.79	9.95 ± 0.51	26.10 ± 2.24
Nitrogen free extracts	42.73 ± 0.67	34.59 ± 0.21	24.60 ± 0.53
Ash	6.49 ± 0.64	6.46 ± 0.33	8.59 ± 0.17
Gross Energy (Mcal/g)	3.62 ± 0.07	2.93 ± 0.48	3.06 ± 0.18

Table 3: Performance characteristics of experimental rabbits

Parameter	100C	75C25M	50C50M	25C75M	100M	SEM	PROB
ADFC (g) Concentrate	17.4	17.2	18.3	18.6	18.4	0.44	0.85
Centrosema	14.9 ^a	13.7 ^a	8.8 ^{ab}	4.9 ^c	-	0.28	0.0001
Moringa	-	5.2 ^d	8.6 ^c	13.9 ^b	17.3 ^a	0.25	0.0001
Total	32.3	36.1	35.7	37.4	35.7	1.42	0.55
AILW (g)	387.6	389.1	388.5	388.5	387.6	22.7	1.00
AFLW (g)	1472.5	1460.1	1477.8	1493.6	1505.5	44.6	0.95
AMLW (g)	930.1	924.6	933.2	941.1	946.6	29.4	0.98
TWG (g)	1084.9	1071.0	1089.3	1105.1	1118.0	39.2	0.95
ADG (g)	12.9	12.8	13.0	13.2	13.3	0.46	0.92
Feed conversion	2.50	2.83	2.76	2.84	2.69	0.06	0.59

a, b, c, d : Means within each row with different superscript are significantly different (p< 0.01)

ADFC: Average daily feed consumption; AILW: Average initial live weight;

AFLW: Average final live weight; AMLW: Average mean live weight

TWG: Total weight gain; ADG: Average daily gain

Table 4: Apparent digestibility coefficient of the dry matter and nutrients by rabbits on the experimental diets

Parameter %	100C	75C25M	50C50M	25C75M	100M	SEM	PROB
Dry Matter	56.9 ^d	60.9 ^d	66.2 ^c	70.8 ^b	79.9 ^a	1.52	.0001
Crude Protein	66.8 ^c	74.4 ^b	77.1 ^b	74.6 ^b	82.4 ^a	1.00	.0001
Crude fibre	36.4 ^c	36.4 ^c	40.1 ^{bc}	44.9 ^b	65.2 ^a	2.16	.0001
Ether Extract	67.1 ^d	76.3 ^{bc}	78.7 ^b	75.0 ^c	83.3 ^a	1.04	.0001
Ash	44.2 ^d	43.5 ^d	53.6 ^c	60.8 ^b	67.8 ^a	1.85	.0001
Organic Matter	57.6 ^c	62.0 ^d	66.9 ^c	71.6 ^b	80.8 ^a	1.50	.0001
Energy	64.5 ^{bc}	67.4 ^{bc}	64.3 ^c	69.0 ^b	77.6 ^a	0.03	.0001
Nitrogen free extract	59.8 ^d	61.6 ^d	66.8 ^c	76.7 ^b	83.0 ^a	1.63	.0001
Nitrogen retention, g/day	0.60 ^b	0.76 ^{ab}	0.73 ^{ab}	0.74 ^{ab}	0.92 ^a	0.04	0.04

a, b, c, d, e: Means within each row with different superscript are significantly different (p< 0.01)

CONCLUSION

It was concluded that the vegetative part of *Moringa oleifera* can effectively replace *Centrosema pubescens* in rabbit's diets also serve as a potential inexpensive good protein supplementation to rabbits especially in dry season.

Acknowledgements

The authors gratefully acknowledge funding from Obafemi Awolowo University Research Committee

REFERENCES

- A. O. A. C. (1990)**, Association of Official Analytical Chemist. Official methods of analysis, 15th ed. Washington D. C. USA
- Arijeniwa, A., Otaikhian, S., Omaseun, J.A. (2000)**. Performance of weaner rabbits fed Poultry Grower supplemented with different grass legume ration. Proceedings of 5th Annual Conference of Animal Sci. Ass. Nig. (ASAN) Sept. 19-22, 2000. pg. 103-105
- Bamikole, M. A., Ikhatua, M. I., Ikhatua, U. J and Ezenwa, I. V. (2005)**. Nutritive Value of Mulberry (*Morus Spp.*) Leaves in the Growing Rabbits in Nigeria. *Pakistan Journal of Nutrition* 4 (4): 231-236
- Becker, K. (1995)**. Studies on utilization of *Moringa oleifera* leaves as animal feed. *Institute for Animal Production in the Tropics and Subtropics* vol. 480. University of Hohenheim, Stuttgart, 15pp
- Cheeke, P. R. (1980)** Editorial: The Potential role of the rabbit in meeting world food needs. *Journal of Apply Rabbit Research* 3 (3): 3-5
- Cheeke, P. R. (1987)**. Digestive Physiology: In Rabbit Feeding and Nutrition. Orlando, FL: Academic Press. pp. 20-32
- Cheeke, P. R. and Patton, N. M. (1987)**. Rabbit production and research in the Peoples' Republic of China. *Journal of Applied Rabbit Research* 10:68-75
- CIEPCA, (2000)** Center for Cover Crops Information and Seed Exchange In Afica. Number 5 .08.B.P.0932 Tri Postal,Cotonou
- Delaveau, P. and Boiteau, P. (1980)**. Huiles à interet pharmacologique, cosmetologique et dietique. IV. Huiles de *Moringa oleifera* Lamk. et de *M. Drouhardii* Jumelle. *Plantes medicinales et phytotherapie*. 14: 29-33.
- Foidl, N., Makkar H. P. S. and Becker K. (2001)** The potential of *Moringa oleifera* for agricultural and industrial uses in: "The Miracle Tree –The Multiple Attributes of Moringa" (Ed. Lowell J. Fuglie). CTA, USA

- Fraga M., (1990).** Effect of type of fibre on the rate of passage and on the contribution of soft faeces to nutrient intake of finishing rabbits. *Journal of Animal Science* 69:1566-74.
- Frieberger, C. E., Vanderjagt, D. J., Pastuszyn, A., Glew, R. S., Mounkaila G., Millson, M. and Glew, R. H. (1998).** Nutrient content of the edible leaves of seven wild plants from Niger. *Plant Food Human Nutrition*. 53: 57-69.
- Humphreys, L.R. (1999)** Tropical Pastures and fodder Crops. Second Edition. *Intermediate Tropical Agricultural Series*. 155pp.
- Jokthan, G. E., Afikwu, E. V. and Olugbemi, T. S. (2003).** The Utilization of Fig (*Ficus thonningii*) and Mango (*Mangifera indica*) Leaves by Rabbits. *Pakistan Journal of Nutrition* 2(4): 264-266.
- Kakengi, A. M. V., Shem M. N., Sarwatt S. V., and Fujihara T., (2003).** Can *Moringa oleifera* be used as a protein supplement to ruminants? *Asian-Australasian Journal of Animal Sciences* 18 (1) 42-47.
- Makkar, H. P. S and Becker, K. (1996).** Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science Technology* 63: 211-228.
- Makkar, H. P. S. and Becker, K. (1997).** Nutrients and antinutritional factors in different morphological parts of the *Moringa oleifera* tree. *Journal of Agriculture Science* 128: 311-322.
- Odeyinka, S. M., Olosunde, A. S. and Oyedele, O. J. (2007a).** Utilization of Soyabean Milk Residue, Cowpea Testa and Corn starch residue by weaner rabbits. *Livestock Research for Rural Development* 19 (9): 1-6.
- Odeyinka S.M, Oyedele O.J., Adeleke T. O and Odedire J.A. (2008).** Reproductive Performance of Rabbits fed *Moringa oleifera* as a replacement for *Centrosema pubescens*. *Proceedings of the 9th World Rabbit Congress*, Verona (Italy), June 10-13.
- Oliveira, J.T.A., Silveira, S.B., Vasconcelos, I.M., Cavada, B.S and Moriera, R.A. (1999).** Compositional and nutritional attributes of seeds from the multipurpose tree *Moringa oleifera* Lamarck. *Journal of Sciences Food and Agriculture* 79:815-820.
- Omoikhoje, S. O., Bamgbose, A. M., Aruna, M. B. and Animashahun, R. A. (2006).** Response of weaner rabbits to concentrate supplemented with varying levels of *Syndrella nodiflora* forage. *Pakistan Journal of Nutrition*. 5(6): 577-579.
- Onwuka, C. F. I. (2007).** Browsing goats and sheep: Reaping the wisdom in their foolishness, UNAAB Inaugural Lecture Series No. 21: 1-55.
- Ramachandran, C., Peter, K.V. and Gopalakrishnan, P.K. (1980).** Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. *Economic Botany* 34: 276-283.
- Sanchez, N. R., Sporndly, E. and Ledin, I. (2005).** Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition.

<http://www.moringanews.org/documents/LivestProd.pdf>.

Accessed on 10/02/07.

SAS, (2008). *SAS User's Guide Statistics*, SAS Inc. Cary., North Carolina, 2008 edition.

Sarwatt, S. V., Milang'ha, M. S., Lekule, F. P. and Madalla, N. (2004). *Moringa oleifera* and cotton seed cake as supplements for smallholder dairy cows fed Napier grass. *Livestock Research for Rural Development*, 16: 1-38.

MORINGA GERMPLASM COLLECTION AND SCREENING

Getachew Mulugeta

Southern Agricultural Research Institute

Awassa Agricultural Research Center

P.O. Box 6 Awassa, Ethiopia

Corresponding Author's e-mail address: *getachew1968@yahoo.com*

ABSTRACT

Moringa stenopetala and *Moringa oleifera* germplasm collection & screening trial was conducted in three locations at Konso, Arbaminch and Awassa in SNNPR, Ethiopia. The study was carried out for three consecutive years during, with the following objectives: to assess natural variability of agronomic yield and to generate germplasm pool for subsequent genetic improvement. In the experiment 33 *Moringa* accessions were comprised among which 28 accessions belong to indigenous specie of *Moringa stenopetala* and 5 accessions to exotic *Moringa oleifera* species also included and were compared by using Randomized Complete Block Design with four replications. The results three years after establishment revealed that there was a significant variation in accession Konso Nagayle with mean values of survival rate (98.88 %), height growth (4.53 m), collar diameter (28.56 cm), number of branches (96.99 branches per tree) and dry leaf biomass (47.62 kg per tree) at three tested sites. This could be due to the environmental factors and natural genetic variability of the *Moringa* accessions, which generally expressed in terms of tree growth and yield. Therefore, the native *Moringa* accession Konso Nagayle could be recommended for further genetic pool improvement and leaf production at Konso, Arbaminch, Awassa areas and places with similar agroecologies.

Keywords: *Moringa stenopetala*, *Moringa oleifera*, locations/sites, accession, natural variability, growth, yield

INTRODUCTION

Moringa stenopetala, indigenous to Southern Ethiopia and Northern Kenya, belongs to the single genus family *Moringaceae* (Azene Bekele *et al.*, 1993). The tree is popularly known as *Shifara* or *Alako* in Ethiopia. *Moringa stenopetala* is widely cultivated in Southern Ethiopia especially in Konso, Derashe, Gamo-Gofa and the adjoining districts. A German geographer (Nowackw, 1954) was observed the use of leaves from *M. stenopetala* as cabbage in the Konso highlands, south of Lake Chamo for the first time. *Moringa* naturally growing in riverine forests and *Acacia-commiphora* woodlands on rocky ground (Edward, *et al.*, 2000). Cultivation of the tree in home gardens, on terraces and marketing of the leaves is also practiced by several other tribes of Southern Ethiopia (Kuls, 1958). Even today these leaves are an important vegetable during the dry season, not only for Konso, Gamo, Derashe and Gofa in Ethiopia but also for minorities from the same tribe in the Marsabit area of Northern Kenya (Gottch, *et. al.*, 1984). *Moringa stenopetala* is also known as vegetable tree. The Njemb tribe, living in Kenya, utilizes this tree as medicinal plant (Berger, *et. al.*, 1984). In southern Ethiopia it is used as kale or cabbage for human consumption and animal feed. Over five million people depend on this plant as a vegetable source. In Gamo-Gofa, immature leaves of *M. stenopetala* are part of the staple diet of the population.

Moringa is drought tolerant and is reported to tolerate an annual precipitation of 500 to 1500 mm and annual temperatures from 18.7 to 28.5 ° C. *Moringa* grows in a wide range of soil types (pH of 4.5 to 8.0) except heavy clays and prefers a neutral to slightly acidic soil. The tree grows well in altitudes from 0 to 1800 m.a.s.l. (Duke, 1978; F/FRED, 1992). *Moringa* is a fast-growing tree which also has fast regrowth after pruning (Foidl *et al.*, 2001) and capacity to produce high quantities of fresh biomass per square meter even at high planting densities. The dry matter (DM) yield is high, from 4.2 to 8.3 tons ha⁻¹ when harvested every 40 days and fresh leaves contain between 19.3% and 26.4% crude protein (CP) in DM (Makkar and Becker, 1996; Makkar and Becker, 1997; Foild *et al.*, 1999; Aregheore, 2002). *Moringa* leaves have a negligible content of tannins, a saponin content similar to that of soybean meal and no trypsin and amylase inhibitors or cyanogenic glucosides (Makkar and Becker, 1996; Makkar and Becker, 1997). *Moringa* can grow in a variety of soil conditions, from well drained sandy or loam soils (which the plants prefer) to heavy clay soils. The tree is reported to be tolerant of light frosts and can be established in slightly alkaline soils up to pH 9. Currently, the young leaves and pods are used as vegetables, the oil extracted from kernels for culinary and industrial purposes, the water extract of the kernels as water purifying agent, the seed cake as fertilizer, and various parts of the tree in traditional medicine (Foidl *et al.*, 2001).

Besides its fast growing, drought resistance it offers many advantages to subsistence farmers (John, 1979). *Moringa* grows in dry climate where most of the native trees hardly survive. One tree of *M. stenopetala* is able to support a large family for several years. Apart from being cultivated traditionally as cabbage tree and planted as ornamental tree, unripe and mature seeds of the *Moringaceae* family are used as foodstuff and spice in many Asian and African countries and also consumed in drinks prepared in folk medicine (John, 1979). *Moringa stenopetala* leaves can also be eaten in salads and are important in regions where vegetables are scarce. Seeds of the *Moringaceae* family contain 40 % by weight of high quality oil that is of equal value both cooking oil and as the main ingredient for soap manufacture (Ibrahm, *et. al.*, 1974). *Moringa* leaves are free from antinutrients except for saponins and phenols. The concentration of phenol is much below the toxic threshold levels for animals (Makkar and Becker, 1997).

According to (Fuglie, 1999) the many uses for *Moringa* include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). *Moringa* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products (Terra, 1966). In the West, one of the best known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water (Berger, *et. al.*, 1984; Gassenschmidt, *et. al.*, 1995; Olsen, 1987), but the seeds are also eaten green, roasted, powdered and steamed for tea or used in curries (Gassenschmidt, *et. al.*, 1995). This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called “developing” regions of the world where undernourishment is a major concern. The high true protein content of leaves (23 per cent in DM, Makkar and Becker, 1997), the high proportion of this protein potentially available in the intestine (Makkar and Becker, 1997), the presence of adequate levels of essential amino acids (higher than the levels present in the FAO reference protein), and low levels of antinutrients indicate their high nutritional quality. The high pepsin soluble nitrogen (82 - 91 per cent) and the low acid detergent

insoluble protein (1 - 2 per cent) values for the meal suggest that most of the protein in the meal is available to most animals (Makkar and Becker, 1997).

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular—Trees for Life, Church World Service and Educational Concerns for Hunger Organization—have advocated Moringa as “natural nutrition for the tropics.” Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce. Despite of all these facts the indigenous *Moringa stenopetala* is poorly studied. Hence, screening trial has conducted for the following objectives: to assess the natural variability in agronomic yield and to generate a germplasm pool for subsequent genetic improvement.

MATERIALS AND METHODS

Study sites & climate

Collected Moringa accessions were screened at three locations viz. Konso, semi-arid lower altitude; Arbaminch, semi-arid lower altitude and Awassa, sub-humid lower altitude of Southern Ethiopia. Konso and Arbaminch are the well known traditional Moringa stenopetala growing belts and Awassa was non-traditional area of this species.

Seed collection

Moringa stenopetala seeds have been collected from 29 sites in Konso, Arbaminch and Kucha areas in January 2002 whereas the seeds of *Moringa oleifera* were obtained from ECHO-seed bank. From each site of *Moringa stenopetala*, more than 1000 seeds were collected from a random sample of up to 20 trees. The location of each tree accession was recorded with the help of GPS -Global Position Standard (Appendix 1).

Farmers' Participation

The farmers have participated from the beginning to the end of research process. Seed collection carried out based on the farmers' indigenous knowledge and their preferences for reduced leaf bitterness which was identified by less unpleasant/ more pleasant smell of the fresh leaf stalk sap that exudes after crushing.

Experimental Design

All collected accessions were screened at Konso; Arbaminch and Awassa. At each location, a single row plot with seven trees per accession was replicated 4-times in a RCBD.

Survival rate

Survival rate refers to number of established seedlings divided by total number of seedlings planted times hundred and expressed in terms of per centage. In forestry the survival rate of the seedlings is recorded after one year of planting,

Collar diameter

The collar diameter of the tree is measured with the help of caliper at ground level of stem and then expressed in terms of centimeters (cm). This parameter is recorded at the end of growing/vegetative season at a given period of time to quantify radial increment of the tree.

Height growth

Height growth of tree is measured with the scientific instrument known as Hypsometer. Time of recording is the end of vegetative growth and expressed in terms of meter.

Number of branches

Number of branches per tree has been obtained by counting the total number of branches. The right time of counting the tree branches is just before collection of leaves for recording of dry leaf biomass at the end of growing season.

Dry leaf biomass

The collected leaf sample is dried in the oven for 48 hours at 65 °C of temperature to constant weight and then weighed with the help of weighing balance and expressed in terms of kilo gram per tree.

RESULTS AND DISCUSSIONS

Survival Rate

The survival rate per cent of the tree species is an indicator of site suitability or the adaptation capability of species to the particular site conditions. Among all screening sites the best survival rate or establishment performance of entire Moringa accessions were recorded at Arbaminch (table.1). Accession Konso Nagayle revealed the highest survival rate percentage with the mean value of 98.88 % at Arbaminch site. On the other hand the minimum value of survival rate was observed on accession no. 41 (Baringo seed source) with mean value of 69.66 %. Awassa was not a traditional Moringa growing area even though it survived better than its native home of Konso. This study suggests that the Arbaminch area is a best site to under take huge commercial or industrial plantation of

Moringa species since the mean survival percentage was much greater than 80 %.

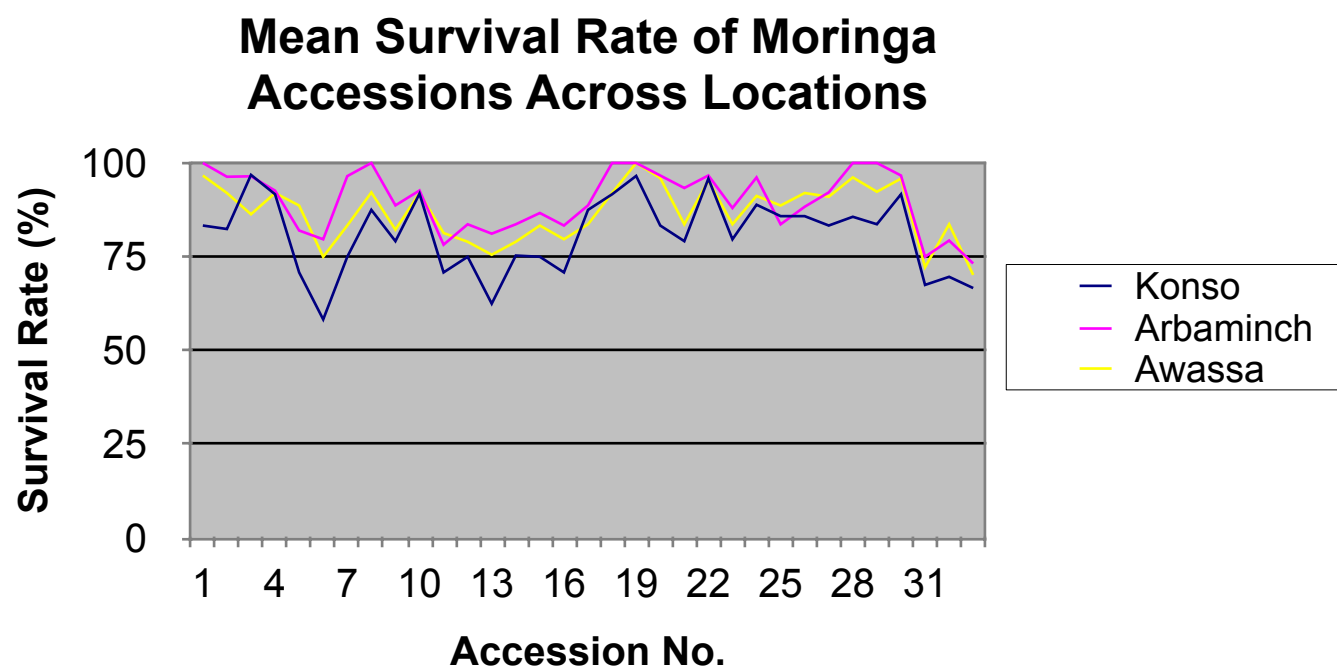


Fig. 1: Mean Survival Rate of Moringa Germplasm Collection Across the Sites

Collar diameter

The mean value of collar diameter was significantly influenced by natural genetic variability of Moringa accessions across locations (Table 1). The maximum collar diameter was observed in accession Lasho (28.56 cm), which was followed by accession Konso Nagayle (28.37cm). The minimum collar diameter was recorded for accession Boringo (20.62 cm). All accessions of *Moringa stenopetala* have shown better collar diameter increment than *Moringa oleifera* accessions in all testing sites.

Table 1: Mean Collar diameter (cm) of Moringa accessions across locations

Acc. No.	Konso	A/Minch	Awassa	Mean
1	27.33	29.8	28.56	28.56
2	24.82	27.6	25.39	25.93
3	26.47	28.51	27.63	27.53
4	25.31	26.93	25.80	26.01
5	23.18	25.22	24.67	24.35
6	17.50	20.38	18.40	18.76
7	25.66	28.40	27.53	27.19
8	23.29	25.86	23.90	24.35
9	24.70	27.00	26.35	26.01
10	22.45	26.58	24.37	24.46
11	23.34	26.61	25.89	25.28
12	25.32	26.14	25.92	25.96
13	23.59	25.33	23.85	24.25
14	24.76	27.55	26.61	26.30
15	23.64	26.47	25.50	25.20
16	24.95	25.40	24.76	25.03
17	25.81	27.62	26.19	26.54
18	24.47	26.30	24.95	25.24
19	25.22	30.25	28.41	28.37
20	26.53	29.57	26.30	27.03
21	22.70	24.46	24.25	23.80
22	27.69	26.80	25.74	25.41
23	22.44	25.53	22.92	23.63
24	23.87	26.36	25.54	25.25
25	24.51	25.53	24.87	24.97
26	25.39	27.78	25.94	26.36
27	24.27	26.40	25.38	25.35
28	23.62	25.91	24.70	24.74
29	25.40	27.15	25.66	26.07

30	22.79	24.33	23.58	23.56
31	20.58	21.24	20.96	20.92
32	20.90	22.59	21.35	21.61
33	20.41	20.85	20.60	29.62
Loc.Mean	26.35			
CV.	2.97%			

Height Growth

The height growth of three years old Moringa accession was significant across locations which in turn implies the natural variability among accessions and used as one of the characteristics to screen well performing germplasm (Figure 2). The plant growth is the cumulative effect of genetic potential and environment of the tree species. Moringa accessions were exhibited the maximum height growth at Aribaminch site. In contrary the minimum height growth was observed in Konso due to harsh environmental conditions of the site. Height growth of exotic *Moringa oleifera* accessions was better than indigenous *Moringa stenopetala* across all tested locations. Konso Nagayle was the only indigenous Moringa accession that has shown significant height growth out of the natives. The tree height may not be a good trait to screen tree species for leaf yield. Height growth of exotic accessions was double of Moringa stenopetala but the diameter/radial growth of Moringa oleifera was significantly lesser. The natural height increment variability among accessions is a good distinguishing trait of the Moringa species.

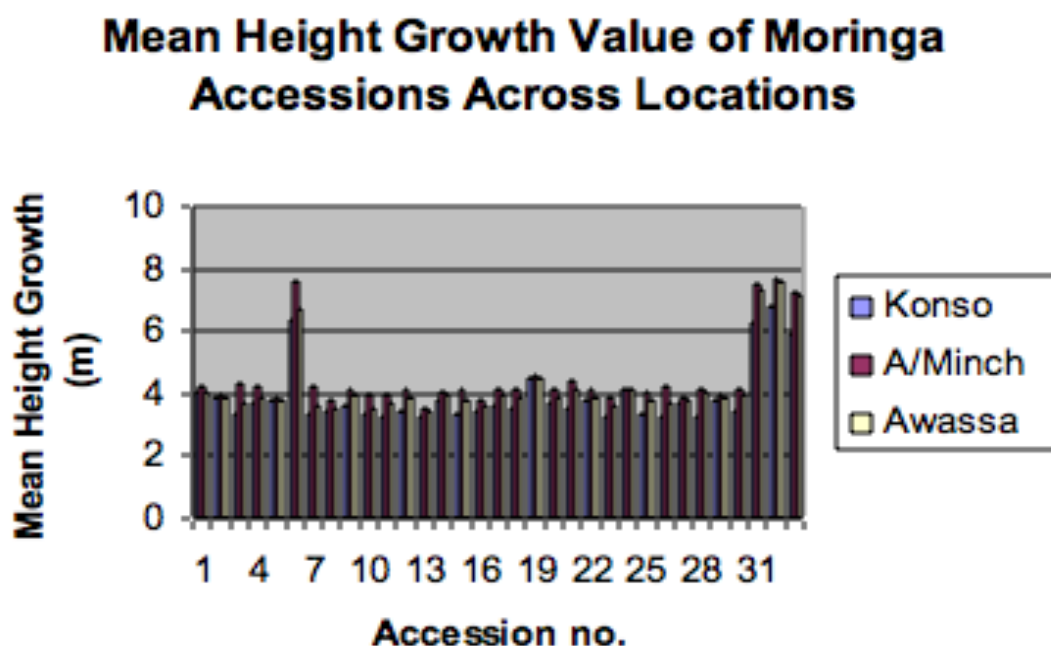


Figure 2: Mean height growth value of Moringa germplasm across locations

Number of Branches

Most of the time number of branches has direct correlation with leaf yield of the tree species. The mean number of branches per tree were the highest in Konso Negayle (96.99 branches per tree) followed by Arbaminch Kolashara (82.09 branches per tree).

Number of branches was lower in exotic accessions as compared to indigenous Moringa accessions. The trend of number of branches across sites was shown identical genetic trait for all accessions irrespective of the seed origin. But the number of branches was revealed natural variability among accessions (Figure 3). Branching habit of indigenous accessions was erect and horizontal for exotic. *Moringa stenopetala* has strong and long branches than *Moringa oleifera* accessions. Figure 3 shows the separate trend of mean number of branches per tree across locations, which is similar among the accessions but the variability in number of branches was due to site factor or environment.

Trend of Mean Branch Number of Moringa Across Locations

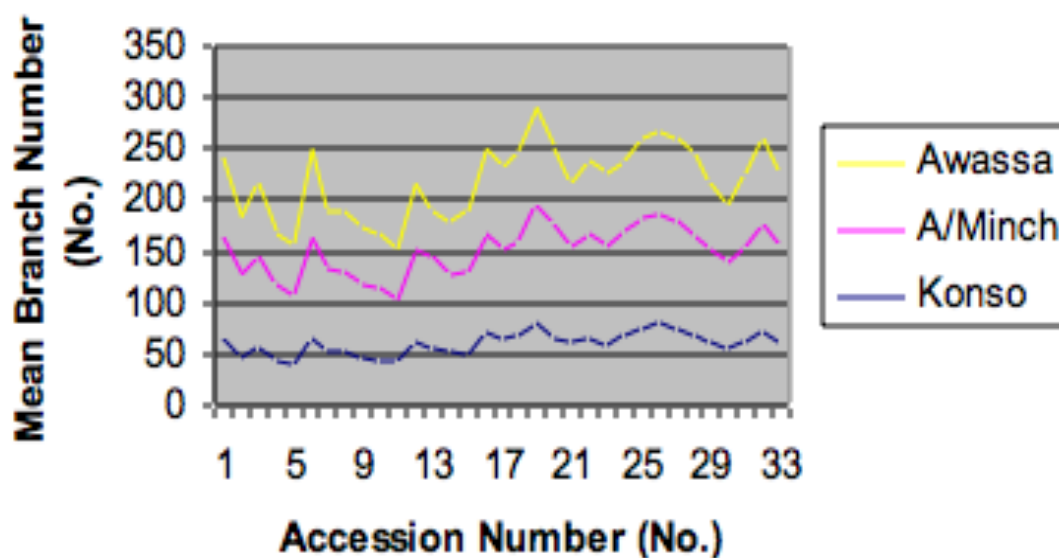


Figure 3: Trend of mean branch number of Moringa accessions across locations

Dry Leaf Biomass

Dry leaf biomass per tree was the highest in Konso Negayle (47.62 kg/tree) and the lowest in exotic accession Nicaragua-O (22.22 kg/tree) in table 2 and figure 4. The dry leaf biomass is very important selection criteria for Moringa accessions since the leaves are food for human beings and feed for animals. The exotic accessions gave the lowest dry leaf biomass than indigenous Moringa accessions, which implies a better leaf yield potential of the native germplasm. Currently *Moringa stenopetala* is primarily grown for home consumption of the leaf and the surplus for sale in the markets to generate cash income to the family

Mean Dry Leaf Biomass Value of Moringa Accession Across Locations

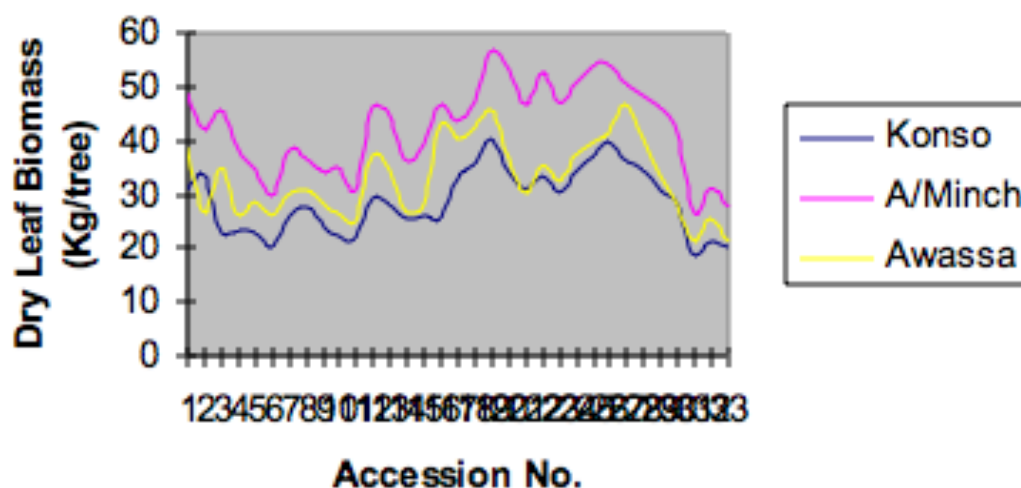


Figure 4: Mean dry leaf biomass value of Moringa accessions across locations
The reasons for the reduced dry leaf biomass per tree of *Moringa oleifera* was due to smaller leaf size and lesser number of branches compared to *Moringa stenopetala*. The lowest dry leaf biomass was obtained at Konso sites (Figure 4). Indigenous accessions have shown genetical superiority over the exotic *Moringa oleifera*, which implies that it is very advisable to improve the native germplasm rather than introducing the exotic accessions.

CONCLUSION AND RECOMMENDATION

The result of the trial on Moringa germplasm collection and screening indicated that there was significant difference between accessions for yield. Among the tested Moringa accessions, Konso Negayle is thus recommended for leaf production at Konso, Arbaminch, Awassa and in places with similar agro ecologies. Therefore, this accession is also recommended for further tree improvement research.

REFERENCES

- Aregheore, E.M. (2002).** Intake and digestibility of *Moringa oleifera* batiki grass mixture for growing goats. *Small Rumin. Res.* 46: 23-28.
- Berger, M.R., Habs, M., Jahn, S.A.A. and Schmahl, D., (1984).** Toxicological assessment of seeds from *Moringa oleifera* and *Moringa stenopetala*. Two highly efficient primary coagulants for domestic water treatment of tropical raw water. *East. Afr. Med. J.* 61:712-71.
- Duke, J. A. (1978).** The quest for tolerant germplasm. In: G. Young (ed). *Crop tolerance to suboptimal land conditions.* Amer. Soc. Agron. Spec. Symp. 32. Madison, Wis. pp. 1-61.

- Edward, S., Mesfin Tadesse, Sebsebe Demissew and Hedberg, I. (2000).** Flora of Ethiopia and Eritrea. Vo. 2, part 1: Magnoliaceae to Flacourtiaceae, Addis Ababa, Eth.
- F/FRED. (1992).** Forestry/Fuelwood Research and Development Project. Growing Multipurpose Trees on Small Farms. Bangkok, Thailand: Winrock International.
- Foidl, N., Mayorga, L. and Vásquez, W. (1999).** Utilización del Marango (*Moringa oleifera*) como forraje fresco para el ganado. Conf. Electrónica de la FAO sobre agrofor. para la Prod. Anim. en América Latina. www.fao.org/livestock/agap/frg/agrofor1/foidl16.htm
- Foidl, N., Makkar, H.P.S. and Becker, K. (2001).** The potential of *Moringa oleifera* for agricultural and industrial uses. In: Proceedings of International Workshop What development potential for *Moringa* products? October 29th to November 2nd 2001. Dar Es Salaam, Tanzania
- Fuglie L. J. (1999)** The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar. 68 pp.; revised in 2001 and published as The Tree: The Multiple Attributes of *Moringa*, 172 pp.
- Gassenschmidt U., Jany, K.D., Tauscher, B. and Niebergall, H., (1995)** Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. *Biochimica Biophysica Acta* 1243: 477-481.
- Gottsch, E., Engles, J. and Demisse. (1984).** Crop diversity in Konso agriculture. PGRC/E- ILCA Germplasm Newsletter. 1984 7: 18-26.
- Ibrahim, S.S., Ismail, M., Samuel, G. and Kamel El Azhari, T. (1974).** Bean seeds: A potential oil source. *Agric. Res. Rev.*; 52:47.
- Jahn, S.A.A. (1979).** African plants for the improvement of drinking water. *Curare*. 2:183-199.
- Kuls. W. (1958).** Beitrage zur Kulturgeographie der Sued Aethiopischen Seenregion *Frankfurter Geogr. Hefte*. 32: 106-107.
- Makkar, H.P.S and Becker, K. (1996).** Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Anim. Feed Sci. Techn.* 63: 211-228.
- Makkar, H.P.S. and Becker, K. (1997).** Nutrients and antinutritional factors in different morphological parts of the *Moringa oleifera* tree. *J. Agric. Sci. Camb.* 128:311-332.
- Nowacke, (1959).** Land und der Konso (Sub Athiopien). *Bonner Geogr. Abh.* 14: 35pp.
- Olsen, A. (1987).** Low technology water purification by bentonite clay and *Moringa oleifera* seed flocculation as performed in Sudanese villages. Effects on *schistosoma mansoni* cercariae. *Water Research* 21(5): 517-522.
- Terra, G.J.A. (1966).** Tropical vegetables, vegetable growing in the tropics and subtropics especially of indigenous vegetables. Communications No. 54e of the Department of Agricultural Research; Publication of the Royal Tropical Institute, Amsterdam, The Netherlands

Appendix 1. GPS data of Moringa accessions										
Ser. No.	Accessions	Lat	Deg	Min	Sec	Lon	Deg	Min	Sec	Altitude in (m)
1	Lasho	N	6	35	21	E	37	50	13	1273.1
2	Wajiffo	N	6	27	50	E	37	44	54	1224.4
3	Yayke	N	6	23	49	E	37	44	52	1266.4
4	Kerber-O	N	6	17	43	E	37	46	3	1244.5
5	Lante	N	6	8	12	E	37	39	7	1222.7
6	Arbaminch Univ.-O	N	6	3	55	E	37	33	41	1267.1
7	Arbaminch Univ.	N	6	3	55	E	37	33	41	1267.1
8	AM. Shekela	N	6	1	56	E	37	33	26	1285.9
9	Konso Dokatu	N	5	19	56	E	37	25	15	1629.8
10	Konso Dokatu	N	5	20	20	E	37	25	34	1538.0
11	Gofa(Sawla)	N	6	-	-	E	37	-	-	-
12	Konso Gamolle	N	5	20	10	E	37	24	31	1609.4
13	Konso Gamolle	N	5	20	10	E	37	24	31	1609.1
14	Konso Karate	N	5	20	14	E	37	26	21	1495.2
15	Konso Kube	N	5	20	10	E	37	27	38	1295.5
16	Konso Dera	N	5	19	55	E	37	26	41	1415.9
17	Konso Gerssale	N	5	21	17	E	37	27	4	1285.2
18	Konso Gerssale	N	5	21	17	E	37	27	4	1285.2
19	Konso Negayle	N	5	22	34	E	37	26	34	1287.8
20	Derahse Gatto	N	5	33	21	E	37	24	51	1259.4
21	Derashe Dnota Minder	N	5	38	32	E	37	26	46	1171.5
22	Arbaminch Wosseka	N	5	43	32	E	37	25	49	1186.6
23	Shele	N	5	51	55	E	37	28	41	1129.2
24	Shele	N	5	51	55	E	37	28	41	1129.2
25	Secha	N	6	0	36	E	37	32	15	1414.7
26	Arbaminch Kolashara	N	6	5	26	E	37	33	56	1245.7
27	Arbaminch Kolashara	N	6	5	26	E	37	33	56	1245.7
28	Molle	M	6	16	3	E	37	46	20	1232.8
29	Wacha	N	6	19	43	E	37	18	17	1244.5
30	Selambire	N	6	28	20	E	37	27	30	1334.7

31	Nicaragua-O	N	-	-	-	E	-	-	-	-
32	Ex. Mbolo-O	N	-	-	-	E	-	-	-	-
33	Ex. Baringo-O	N	-	-	-	E	-	-	-	-

Legend: O = *Moringa oleira*; AM = Arbaminch; Ex = Exotic; Lat = Latitude, Lon = Longitude Deg= Degree, Min= Minute, Sec = Second, E=East, N=North

EVALUATION OF THE EFFECT OF *MORINGA OLEIFERA* LEAF POWDER ON DIGESTION OF SOME NUTRIENTS IN WEANLING ALBINO RATS

***Oladiji, A.T., Omole, O. M., Oloyede, H. O. B., Yakubu, M. T.**

*Department of Biochemistry, Faculty of Life Sciences, University of Ilorin,
P. M. B. 1515, Ilorin, Nigeria*

Corresponding author's e-mail address: temidayooladiji@yahoo.com

ABSTRACT

The effect of Moringa oleifera leaf powder (MOLP) on digestion in weanling albino rats was investigated. Four-week-old weanling albino rats (5 per group) were maintained for 28 days on experimental diets. Group 1 served as the control and was fed on a Moringa oleifera free diet. Groups 2, 3, 4 and 5 were fed on diets prepared by replacing soymeal with MOLP at levels of 25, 50, 75, and 100% respectively. The digestion of nutrient was measured via the balanced method (percentage of differences between nutrient intake and faecal nutrient) and an evaluation of the activities of selected digestive enzymes. Average daily feed intake, weight gain and apparent daily water intake was not significantly different ($p>0.05$) in the treatment groups when compared to the control, whereas Feed Efficiency Ratio was significantly lower ($p<0.05$) in the 100% Moringa oleifera supplemented diet (MD) group. Serum ferritin concentration, gastric pH, pepsin and lipase activity were also not significantly different ($p>0.05$) in the treatment groups, when compared to the control. Amylase activity was significantly higher in rats placed on 100% MD, when compared to the control. The % apparent digestibility of protein, carbohydrate, fat, Mg, K, Zn and Fe in the test groups were significantly lower ($p<0.05$) when compared to the control. However, the serum mineral levels revealed an appreciation for some minerals with the inclusion of Moringa oleifera in diet. The results of the enzymatic study has showed that Moringa oleifera leaf powder may not affect the digestibility of nutrients since similarities and stimulatory effects were observed in the activity of digestive enzymes and other biochemical parameters evaluated when compared to the control. The balanced study however revealed a decline in the digestibility of macronutrients and some minerals evaluated: this may be due to endogenous losses.

Keywords: Nutrient, digestibility, digestive enzymes, minerals, Moringa oleifera.

INTRODUCTION

Digestion is a vital biological process which involves the breakdown of food into products that can be absorbed and utilized in the body. The survival of cells and various metabolic functions is dependent on the availability of nutrients. Thus a defect in the digestive process will impair absorption and assimilation of nutrients and hence alter the body function.

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Stevens *et al.*, 2013). According to Fahey (2005), it is also known as the horseradish tree, drumstick tree, benzolive tree (french), kelor (bali), marungo (portuguese), marango (spanish), mlonge (swahili), mulangay (philippines), nébéday (serere), sahijna (hindi) or Ben oil tree. It is locally known as 'Zogale-gandi' in Hausa, 'Eweigbale' in Yoruba and 'Okweoyibo' in Igbo (Dalziel, 1956).

In developing tropical countries, Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers (Fahey, 2005). Three non-governmental organizations in particular - Trees for Life, Church World Service and Educational Concerns for Hunger Organization - advocate Moringa as “natural nutrition for the tropics” (Fuglie, 1999).

Research findings confirms that the leaves are a powerhouse of nutritional value: they have been reported to contain seven times the vitamin C in oranges, four times the calcium in milk, four times the vitamin A in carrots, two times the protein in milk and three times the potassium in bananas (Fahey, 2005). Though the health benefits, as well as the nutritional properties of *Moringa oleifera* are now so well known, they have been reported to contain antinutrients such as; tannins, phytates, trypsin inhibitors, saponins, oxalates and low levels of cyanide (Ogbeet *et al.*, 2011), which may affect digestion and absorption processes and thus interfere with normal feed utilization (Nuhu, 2010).

Previous studies have shown that *Moringa oleifera* have the following properties: antioxidant (Sreelatha and Padma, 2009), anti-hypertensive (Dangiet *et al.*, 2002), anti-ulcer (Pal *et al.*, 1995), cholesterol lowering (Okwari *et al.*, 2013), anti-diabetic (Giridhari *et al.*, 2011), anti-bacterial (Rahman *et al.*, 2009), anti-hyperglycemic (Luangpiom *et al.*, 2013), and anti-fungal (Chuang *et al.*, 2007). Little is however documented on its effect on the digestion of food nutrients (Nuhu, 2010; Yuangsoi and Masumoto, 2012). Nuhu (2010) reported a significant increase in the apparent digestibility of dry matter (DM) and crude protein (CP) in weanling rabbit placed on *Moringa oleifera* supplemented diets, when compared to the control, but no significant effect was observed for ether extract (EE). On the contrary, the results obtained by Yuangsoi and Masumoto (2012) in a report on replacing *Moringa oleifera* partially by protein replacement in soybean meal of fancy carp (*Cyprinus carpio*) showed no difference ($p > 0.05$) in the in vitro protein digestibility assay and pepsin digestibility study. Adeniji and Lawal (2012) also reported that *Moringa oleifera* incorporated diets had no significant effect ($P > 0.05$) on the nitrogen digestibility of the grower rabbits. With the exception of Yuangsoi and Masumoto (2012), the inferences drawn from the prior mentioned experiments in terms of nutrient digestion were majorly based on the balanced study (differences in the input and output of nutrients), with a minimal emphasis on biochemical occurrences that underlie the results obtained. In view of this, the effect of *Moringa oleifera* leaf powder on the digestive process was investigated using both the balance method and evaluation of various biochemical parameters.

MATERIALS AND METHODS

MATERIALS

Plant Materials and Authentication: The leaves of *Moringa oleifera* were obtained From Tanke region, Ilorin, Nigeria and were authenticated with a voucher number UIH 1011, at the Herbarium unit, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Experimental animals: Twenty five (25) weanling rats (*Rattus norvegicus*) of both sexes with average weight of 34.23 ± 5.14 g were obtained from Department of Biochemistry Animal Holding Unit, University of Ilorin, Ilorin, Nigeria and were housed under standard conditions in metabolic cages to prevent coprophagia and to facilitate the collection of spilled food, faeces and urine. All animal management and experimental procedures were performed in strict accordance with the specified requirements of the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 1985).

Assay kits and reagents: Bicinchoninic acid protein assay kit was a product of Sigma-Aldrich, 3050 Spruce Street, St. Louis, USA. Ferritin Elisa microwell kit was a product of Monobind Inc., Lake Forest, USA and Lipase kit was a product of Chemelex, Barcelona. All other reagents used were also of analytical standard.

Other materials: Soymeal and wheat offal (source of cellulose) were obtained from Gbemisola Feeds, Offa Garrage, Ilorin, Kwara State, Nigeria. Corn starch and Methionine were obtained from Oja Oba and Rofat Feeds respectively. Soya oil was obtained from Yoruba Road, Ilorin, Nigeria and was a product of UAC. Vitamin mix was a product of Globe Veterinary Pharmaceuticals, Bristol, England. U. K.

PROCEDURES

Processing of *Moringa oleifera* leaves: The Moringa leaves were dried in oven at 45°C until they were crispy to touch, while retaining their greenish colour. They were then milled with a blender and sieved to obtain the Moringa leaf powder (MOLP) which was stored in properly sealed cylindrical glass bottles until needed.

Experimental diets: The test diets were prepared by replacing soymeal with *Moringa oleifera* leaf powder at levels of 0, 25, 50, 75, and 100%. The composition of the diets (each of specific quantity as shown in Table 1) were thoroughly mixed, made into pellets to ensure easy picking during feeding, and oven-dried at 45°C. The feeds were packed into air-tight polythene bags and stored in the freezer to prevent microbial infestation.

Table 1: Composition of the experimental diets

Ingredients	CSD	25% MD	50% MD	75% MD	CMD
Corn starch	476	476	476	476	476
Soymeal	250	187.5	125	62.5	-
MOLP	-	62.5	125	187.5	250
Sucrose	100	100	100	100	100
Methionine	4	4	4	4	4

*Vitamin/**Mineral mix	50	50	50	50	50
Cellulose	40	40	40	40	40
Soya oil	80	80	80	80	80

**Mineral mix (g/kg): CoCl₂·6H₂O (0.001), CuSO₄·5H₂O (0.079), MnSO₄·7H₂O (0.178), KI (0.032), NaCl (3.573), ZnCO₃ (1.6), CaSO₄ (11.61), MgSO₄·7H₂O (2.292), KH₂PO₄ (10.559), FeSO₄·7H₂O (1.078).

*Vitamin mix: Vitamin A (100,000 IU), Vitamin D₃ (10,000 IU), Vitamin E (100mg), Vitamin B₁ (20 mg), Vitamin B₂ (40 mg), d-Calcium Pantothenate (100 mg), Vitamin B₆ (15 mg), Vitamin C: 250 mg, Vitamin K₃: 15 mg, Folic acid: 5000 mcg, Nicotinic acid: 200 mg, Biotin: 150 mcg, Inositol: 80 mg.

Legends; MOLP: *Moringa oleifera* leaf powder, CSD= Complete Soymeal based diet, MD = *Moringa oleifera* meal based diet, CMD= Complete *Moringa oleifera* meal based

Research Design and Animal grouping: The animals were randomly assigned into 5 groups of 5 animals each using a Complete Randomized Design. Group 1 served as the control and was fed on a *Moringa oleifera* free diet. Groups 2, 3, 4 and 5 were fed on diets prepared by replacing Soymeal with MOLP in fractions of 25, 50, 75, and 100% respectively. The rats were acclimatized for 1 week on commercial pellets and tap water and were thereafter fasted for 18 hours, prior to commencing feeding with experimental diets. Feed intake was recorded during the last 3 days of each week, body weights were recorded on a weekly basis and apparent water intake was measured throughout the experiment. Faeces and urine for the determination of proximate and mineral nutrients were collected during the last 7 days of the study. The faecal samples were dried for analysis, while the urine samples were kept frozen at -20°C.

Proximate analysis of samples: The amount of protein, fat, fibre and carbohydrates in the feed and faecal samples was determined in accordance to AOAC (1990). While proximate ash and minerals was determined as recommended by AOAC (2005). Food energy contents were calculated as reported by Edem *et al.* (2003), by applying the conversion factor of 16.74kJ/g for the energy content of protein and carbohydrate and 37.67kJ/g, for fat.

Determination of Feed intake: Feed intake (FI) was derived as the difference between the quantity of feed given (QFG) and the sum of the quantity of feed left (QFL) and spilled (QFS) on dry weight basis. $FI = QFG - (QFL + QFS)$

Determination of water intake: Apparent water intake (AWI) was determined as the difference between the quantity of water given (QWG) and the quantity of water left (QWL), throughout the experiment.

$$AWI = QWG - QWL$$

Determination of body weight gain: The weight gain per rat per day for each week was obtained as follows:

Determination of Feed efficiency ratio (FER) was calculated as shown below:

Determination of Nutrient digestibility: The percentage of each nutrient digested was calculated in accordance to Edem *et al.* (2003) on dry matter basis as shown below:

Where, I = Nutrient intake and F = amount of nutrient excreted in faeces

Collection of Serum: The rats were subjected under ether anaesthesia in a glass jar container. The neck region was quickly cleared of fur and skin to expose the jugular veins. The jugular veins were slightly displaced from the neck region to prevent contamination with interstitial fluid and then cut with a sharp sterile blade. The rats were held downwards and allowed to bleed into the clean dry plain sample bottles (Akanji and Ngaha, 1989). The collected blood samples were allowed to clot and centrifuged at 3000 rpm for 15 minutes and the serum decanted into plain bottles and kept frozen for analysis.

Collection of Gastric Juice and determination of Gastric pH: The rat stomachs were excised out under ether anaesthesia and the gastric contents were collected. The stomachs were washed with 2 ml of luke-warm sterile water. Both the washings and gastric contents were centrifuged together at 4000 rpm for 10 minutes. The supernatants were collected in plain bottles stored at -20°C until needed for analysis. The pH of all supernatants was measured using a digital pH meter.

Preparation of Intestinal Homogenate: The preparation of intestinal homogenates was done by a modification of the methods of Forman and Schneeman (1980) and Percival and Schneeman (1979). The small intestine was removed and each half of the gut was flushed with 2.0 ml of cold physiological saline (0.9%). The intestinal segment was opened lengthwise and was scraped with a glass slide to remove the mucosa plus any chyme remaining. The whole intestine, gut washes and mucosal scrapings were homogenized in cold physiological saline (0.9%). They were thereafter centrifuged at 4000 rpm for 20 minutes and the supernatant decanted and stored frozen at -20°C until required for further analysis.

Determination of Pepsin activity: Pepsin activity was determined by the modified Anson method (Anson, 1938) in the centrifuged gastric juice using bovine albumin as a substrate (Hirohashi *et al.*, 1993; Xio-Bo S *et al.*, 1992; Yoshida *et al.*, 1993).

For each sample, 1 ml of gastric secretion supernatant and 5 ml of buffer solution (comprising of 0.2 N HCl and 0.2 N Sodium citrate solutions in the ratio 4:1) was mixed in a test tube and incubated at 37°C for 30 minutes. 2 ml (5 mg/ ml) of bovine serum albumin was added and the test tubes re-incubated at 37°C for 30 minutes. The unreacted protein of bovine serum albumin was detected with the addition of 1.0 ml of biuret reagent and the absorbance read at 546 nm exactly 30 minutes against reagent blank (which consisted of a mixture of 8 ml of distilled water and 1 ml of Biuret reagent) using a spectrophotometer. The pepsin activities were determined from the standard turbidity curve.

Where, V_t = Total reaction volume
 V_e = Volume of enzyme used
 $[\text{Reacted albumin}] = [\text{Albumin used}] - [\text{unreacted Albumin}]$ in mg/ml

Determination of Pancreatic Amylase activity: The saccharolytic activity of pancreatic amylase was measured with the 3,5-Dinitrosalicylic Acid (DNS) method (Bernfeld, 1955).

Determination of Pancreatic Lipase activity: This was measured using commercial Kits (Labkit, Chemelex, SA, Barcelona). Pancreatic Lipase in the presence of colipase, deoxycholate and calcium ions, hydrolysed the substrate, DGGMR, (1, 2-o-dilauryl-rac-glycero-3-glutaric acid- (6'- methylresorufin) ester) at 580 nm. The rate of formation product (methylresorufin) was measured photometrically.

Determination of Serum Ferritin concentration: This was done using commercial kit (Ferritin Elisa microwell kit, monobind Inc., Lake Forest, USA). In this method serum samples of the control and groups placed on Moringa supplemented diets were each added to a streptavidin coated well. A biotinylated monoclonal antibody (specific for ferritin) was then added and the reactants mixed and incubated for 30 minutes at room temperature. Reactions between the biotinylated ferritin antibody and native ferritin resulted in the formation of an immune complex deposited on the streptavidin coated well. The excess serum protein was washed away via a wash step and another ferritin specific antibody, labeled with an enzyme was added to the wells. Further incubation was carried out at room temperature for 30 minutes. Another wash step was carried out to remove excess enzyme and a substrate (containing tetramethylbenzidine and hydrogen peroxide in buffer) was added. The addition of substrate resulted in the generation of colour of which intensity is proportional to the concentration of ferritin in the sample. The absorbance, read at 450 nm using a

microplate reader was compared with a dose response curve (generated by utilizing different serum references of known antigen concentration) to obtain the Ferritin concentration in (ng/ml).

Statistical analysis: All data are expressed as the mean of five replicates $\pm$ standard error of mean (S.E.M.). Statistical evaluation of data was performed using analysis of variance (ANOVA), followed by Duncan's posthoc test for multiple comparison. Values were considered statistically significant at $P < 0.05$ (Confidence level= 95%).

RESULTS

Proximate and mineral analysis of *Moringa oleifera* leaf powder: Table 2 shows the proximate and mineral composition of *Moringa oleifera* leaf powder on dry weight basis. The dried Moringa leaf powder was found to be rich in crude protein (23.84 ± 0.00 %), ash (11.25 ± 0.01 %), crude fibre (15.51 ± 0.02 %), potassium (1.10 ± 0.00 %), calcium (1.22 ± 0.01), and Iron (22 mg/100 g).

Table 2: Proximate and Mineral Composition of *Moringa oleifera* leaf powder

CONSTITUENT	Quantity (%)
Dry matter	84.50 ± 0.00
Moisture	15.50 ± 0.00
Crude protein	23.84 ± 0.00
Ether extract	5.00 ± 0.58
Crude fibre	15.51 ± 0.02
Ash	11.25 ± 0.01
Carbohydrate	28.90 ± 0.00
Energy (kj)	1071.27 ± 11.65
Mineral composition (mg/100g)	
Sodium (Na)	621 ± 1.45
Calcium (Ca)	1220 ± 5.77
Potassium (K)	1095 ± 8.66
Magnesium (Mg)	425 ± 0.88
Zinc (Zn)	17 ± 0.57
Iron (Fe)	22 ± 0.57

Values are a means of 3 replicates $\pm$ SEM.

Proximate and mineral composition of diets: Table 3 shows the proximate and mineral composition of the experimental diets. The inclusion of varying amount of *Moringa oleifera* leaf powder in the experimental diets resulted in significant increase ($p<0.05$) in crude fibre, ether extract and ash and a significant reduction in crude protein, carbohydrate and food energy content of the experimental diets, when compared to the control. The levels of minerals evaluated (Na, K, Ca, Mg, Zn, and Fe) were also significant higher ($p<0.05$) in the test diets, when compared to the control.

Nutritional performance of rats: The nutritional performance of rats in terms of the average daily feed intake and weight gain, apparent daily water intake and feed efficiency is shown in Table 4. There was no significant change ($p>0.05$) in the average daily feed intake, average daily weight gain, and apparent daily water intake of rats fed on *M. oleifera* leaf supplemented diets, when compared to the control. However, the average daily faecal output (g/rat/day) was significant higher ($p<0.05$) in rats placed on *M. oleifera* supplemented diets when compared to the control. Feed efficiency ratio was significantly lower in rats placed on the complete *M. oleifera* supplemented diet, when compared to the control.

Table 3: Proximate and Mineral Composition of Experimental Diets

Proximate composition (%)	CSD	25%MD	50%MD	75%MD	CMD
Dry matter	87.00±0.58 ^a	87.00±0.00 ^a	81.50±0.00 ^b	86.25±0.14 ^a	88.25±0.14 ^c
Moisture	13.00±0.58 ^a	13.00±0.00 ^a	18.50±0.00 ^b	13.75±0.14 ^a	11.75±0.14 ^c
Crude protein	15.98±0.01 ^a	14.88±0.01 ^b	14.22±0.01 ^c	13.56±0.00 ^d	12.02±0.01 ^e
Ether extract	8.50±0.29 ^a	10.00±0.00 ^{b,c}	9.50±0.29 ^b	10.50±0.29 ^{c,d}	11.00±0.00 ^d
Crude fibre	2.82±0.01 ^a	4.10±0.01 ^b	5.16±0.01 ^c	5.30±0.01 ^d	6.65±0.01 ^e
Ash	3.88±0.01 ^a	5.65±0.01 ^b	5.70±0.30 ^b	6.27±0.01 ^c	7.31±0.02 ^d
Carbohydrate	55.83±0.82 ^a	52.37±0.02 ^b	46.93±0.39 ^c	50.62±0.14 ^d	51.27±0.14 ^{b,d}
Energy (kJ/g)	1522.24±3.24 ^a	1502.53±0.34 ^b	1381.46±8.2 ^c	1469.91±8.55 ^d	1473.84±2.32 ^d
Mineral composition (mg/100g)					
Na (%)	117±0.58 ^a	323±0.88 ^b	317±0.58 ^c	340±0.58 ^d	353±0.88 ^e
Ca (%)	325±0.88 ^a	715±0.58 ^b	697±0.88 ^c	725±0.88 ^d	742±0.58 ^e
K (%)	677±0.88 ^a	735±0.58 ^b	730±0.58 ^c	757±0.58 ^d	770±0.58 ^e
Mg (%)	217±0.88 ^a	294±0.58 ^b	286±0.58 ^c	303±0.58 ^d	315±0.88 ^e
Zn (%)	026±0.58 ^a	040±0.58 ^b	037±0088 ^c	047±0.58 ^d	062±0.58 ^e
Fe (%)	053±0.58 ^a	077±0.58 ^b	073±0.88 ^c	085±0.58 ^d	094±0.88 ^e

Values are a mean of 3 replicates $\pm$ SEM. Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD= *Moringa oleifera* meal based diet, CMD= Complete *Moringa oleifera* meal based diet

Table 4: Nutritional performance of rats fed on experimental diets

Parameters	Diets				
	CSD	25%MD	50%MD	75%MD	CMD
Average feed intake (g/rat/day)	8.51 $\pm$ 2.9 ^a	8.94 $\pm$ 2.8 ^a	9.38 $\pm$ 1.78 ^a	9.72 $\pm$ 1.83 ^a	9.79 $\pm$ 1.94 ^a
Average weight gain (g/rat/day)	2.9 $\pm$ 0.94 ^a	2.51 $\pm$ 0.73 ^a	1.95 $\pm$ 0.31 ^a	1.93 $\pm$ 0.32 ^a	1.41 $\pm$ 0.01 ^a
Apparent water intake (ml/rat/day)	18.17 $\pm$ 3.37 ^a	17.36 $\pm$ 3.53 ^a	15.04 $\pm$ 1.47 ^a	16.66 $\pm$ 2.28 ^a	16.67 $\pm$ 2.33 ^a
Average faecal output (g/rat/day)	1.40 $\pm$ 0.5 ^a	1.90 $\pm$ 0.86 ^b	1.70 $\pm$ 0.70 ^b	2.20 $\pm$ 0.58 ^c	2.30 $\pm$ 0.91 ^c
Feed efficiency ratio (FER)	0.33 $\pm$ 0.06 ^a	0.30 $\pm$ 0.02 ^a	0.22 $\pm$ 0.03 ^{a, b}	0.20 $\pm$ 0.02 ^{a, b}	0.17 $\pm$ 0.04 ^b

Values are a mean $\pm$ SEM (5 rats per group). Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD= *M. oleifera* meal based diet, CMD= Complete *M. oleifera* meal based diet. * Results are based of 28 days evaluation

Consumption and excretion of Nutrients: Table 5 shows the consumption and faecal output of protein, fat, and carbohydrate, while Table 6 shows the consumption and excretion of minerals during the 7-day collection period in rats fed on experimental diets. When compared to the control, there was significant decrease ($p < 0.05$) in the protein and carbohydrate intake of rats fed on 50, 75 and 100% *M. oleifera* supplemented diets. With the exception of the 50 % MD group which compared favourably with the control, fat intake was significantly higher ($p < 0.05$) in the test groups when compared to the control. Na, Ca, Mg, Zn and Fe intake were also significant higher ($p < 0.05$) in the test groups when compared to the control, but K intake was significantly higher ($p < 0.05$) only in rats placed on 25 %MD, 75 %MD and CMD and significantly lower ($p < 0.05$) in those placed on 50 % MD.

The faecal output of all the nutrients was significantly increased ($p < 0.05$) in the test groups, when compared to the control. For protein, carbohydrate, fat, Mg, Zn and Fe, the increase was directly proportional to the amount of *Moringa oleifera* in diet. The urinary output of Mg and Na was significantly lower ($p < 0.05$) in the test groups when compared to the control. For Ca, a significant decrease was observed only in

rats placed on 25 %MD, 50 %MD and CMD. Urinary output of Zn was significantly lower in the 50 %MD and CMD groups, but was significantly higher in rats placed on 75 % MD. No significant change ($p>0.05$) was observed in the urinary output of K in rats placed on 25 % and 75 %MD, but a significant decreased was observed in those placed on 50 %MD and CMD, when compared to the control. Urinary output of Fe was not significantly different in rats placed on 25 %MD, 50 %MD and CMD, but was significantly higher ($p<0.05$) in rats placed on 75 %MD when compared to the control.

Table 5: Consumption and faecal output of protein, fat and carbohydrate of rats fed on experimental diets

Parameters	Diets				
	CSD	25%MD	50%MD	75%MD	CMD
Intake (g/rat/day)					
Protein	6.64±0.04 ^a	6.63±0.00 ^a	5.21±0.00 ^b	5.16±0.01 ^b	4.87±0.01 ^c
Fat	3.53±0.10 ^a	4.46±0.00 ^b	3.48±0.11 ^a	3.99±0.02 ^c	4.45±0.01 ^b
Carbohydrate	23.22±0.50 ^a	23.33±0.01 ^a	17.18±0.14 ^b	19.25±0.02 ^c	20.76±0.0 ^d
Faecal output (g/rat/day)					
Protein	0.96±0.00 ^a	1.10±0.01 ^b	1.13±0.01 ^b	1.25±0.01 ^c	1.06±0.02 ^d
Fat	0.31±0.00 ^a	0.53±0.05 ^b	0.48±0.00 ^b	0.76±0.02 ^c	0.87±0.01 ^d
Carbohydrate	1.46±0.02 ^a	1.89±0.01 ^b	1.73±0.04 ^b	2.12±0.05 ^c	1.79±0.11 ^b

Values are a mean ± SEM (5 rats per group). Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD= *M. oleifera* meal based diet, CMD= Complete *M. oleifera* meal based diet, *Results were obtained during the 7-day collection period

Table 6: Consumption and faecal output of selected minerals in rats fed on experimental diets

Parameters	Diets				
	CSD	25%MD	50%MD	75%MD	CMD
Intake (mg/day)					
Na	48.64±0.24 ^a	143.75±0.39 ^b	116.06±0.2 ^c	129.27±0.22 ^d	142.77±0.36 ^e
K	281.33±0.37 ^a	327.45±0.26 ^b	267.27±0.21 ^c	287.82±0.22 ^d	311.71±0.23 ^e
Mg	90.08±0.37 ^a	130.98±0.26 ^b	104.71±0.21 ^c	115.20±0.22 ^d	127.38±0.36 ^e
Ca	134.99±0.37 ^a	318.54±0.26 ^b	255.06±0.32 ^c	275.53±0.34 ^d	300.38±0.23 ^e

Zn	10.81±0.24 ^a	17.82±0.26 ^b	13.42±0.32 ^c	17.87±0.22 ^b	25.10±0.23 ^d
Fe	22.03±0.24 ^a	34.30±0.26 ^b	26.97±0.32 ^c	32.33±0.22 ^d	37.92±0.36 ^e
Faecal output (mg/day)					
Na	2.23±0.03 ^a	3.96±0.15 ^b	3.98±0.03 ^b	5.71±0.06 ^c	5.67±0.04 ^c
K	2.28±0.04 ^a	4.11±0.13 ^b	4.11±0.05 ^b	5.82±0.04 ^c	5.77±0.05 ^c
Mg	1.36±0.04 ^a	3.17±0.08 ^b	3.19±0.05 ^b	4.05±0.04 ^c	5.15±0.04 ^d
Ca	1.6±0.04 ^a	3.48±0.06 ^b	3.42±0.03 ^b	5.34±0.04 ^c	5.47±0.04 ^c
Zn	0.15±0.03 ^a	0.42±0.02 ^b	0.47±0.03 ^b	0.84±0.06 ^c	0.99±0.04 ^d
Fe	0.18±0.03 ^a	0.47±0.03 ^b	0.59±0.05 ^b	0.84±0.04 ^c	1.14±0.05 ^d
Urinary output (mg/day)					
Na	0.52±0.02 ^a	0.34±0.01 ^b	0.28±0.01 ^c	0.38±0.01 ^d	0.18±0.00 ^e
K	0.32±0.02 ^a	0.31±0.01 ^a	0.19±0.01 ^b	0.33±0.01 ^a	0.18±0.00 ^b
Mg	0.73±0.01 ^a	0.50±0.01 ^b	0.30±0.01 ^c	0.47±0.01 ^d	0.13±0.00 ^e
Ca	0.28±0.02 ^a	0.25±0.01 ^b	0.21±0.01 ^c	0.37±0.01 ^d	0.20±0.00 ^c
Zn	0.14±0.01 ^a	0.14±0.01 ^a	0.09±0.01 ^b	0.18±0.01 ^c	0.11±0.00 ^b
Fe	0.11±0.01 ^a	0.11±0.01 ^a	0.10±0.01 ^a	0.17±0.01 ^b	0.10±0.00 ^a

Values are a mean ± SEM (5 rats per group). Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD = *Moringa oleifera* meal based diet, CMD = Complete *Moringa oleifera* meal based diet. *Results were obtained during the 7-day collection period

Nutrient Digestibility: Table 7 shows the results obtained for the digestion of nutrients, in each of the experimental diets, calculated from the nutrient intake and faecal output of the last 7 days of the experiment (balanced method). The percentage apparent digestibility of protein, carbohydrate, fat, Mg, K, Zn and Fe in the rats fed on the *Moringa oleifera* supplemented diets was significantly reduced ($p < 0.05$) when compared to the control. The reduction in fat digestibility was directly proportional to the amount of *Moringa oleifera* leaf powder in diet. Percentage apparent digestibility of Ca was also significantly reduced, but only in rats placed on 75 %MD and CMD group. Na was however significantly increased in the treatment groups, when compared to the control.

Gastric pH and digestive enzyme activities: When compared to the control, there was no significant change ($p > 0.05$) in the gastric pH, pepsin activity and pancreatic lipase activity of rats fed on *Moringa oleifera* supplemented diets. Pancreatic Amylase activity was however, significantly higher but only in the rats fed on complete Moringa diet, when compared to the control (Table 8).

Serum mineral composition and Ferritin concentration: Represented in Table 9 is the mineral composition and ferritin concentration of the serum of rats fed on ex-

perimental diets. There was no significant change ($p>0.05$) in serum ferritin concentration and K levels of rats fed on *Moringa oleifera* supplemented diets, when compared to the control. % Serum Na also compared favourably but only in rats fed 25, 50, and 75 % MD. Those placed on complete *Moringa oleifera* diet had a significantly increased serum Na level. When compared to the control, serum Mg and Zn levels were significantly increased ($p<0.05$) in all the test groups. Ca level was significantly increased but only in rats placed on 25 %, 50 %, and CMD. The increase in serum Zn was directly proportional to the quantity of *Moringa oleifera* in diet.

Table 7: Percentage digestibility of Protein, Fat, Carbohydrate and Minerals in rats fed on experimental diets

Apparent digestibility (%)	CSD	25%MD	50%MD	75%MD	CMD
Protein	85.56±0.08 ^a	83.34±0.15 ^b	78.27±0.15 ^c	78.83±0.19 ^d	78.14±0.31 ^e
Carbohydrate	93.71±0.12 ^a	91.90±0.04 ^b	89.95±0.20 ^c	88.98±0.24 ^d	91.40±0.49 ^b
Fat	91.21±0.26 ^a	88.02±1.19 ^b	86.17±0.50 ^b	80.85±1.10 ^c	80.40±0.28 ^e
Na	92.08±0.01 ^a	96.12±0.09 ^b	95.18±0.02 ^c	94.33±0.04 ^d	94.89±0.01 ^e
Mg	97.56±0.03 ^a	96.93±0.07 ^b	96.15±0.05 ^c	95.29±0.02 ^d	95.30±0.04 ^d
Ca	98.12±0.03 ^a	98.61±0.02 ^b	98.29±0.01 ^c	97.72±0.01 ^d	97.86±0.01 ^e
K	98.48±0.01 ^a	98.13±0.04 ^b	97.71±0.02 ^c	97.28±0.01 ^d	97.51±0.04 ^e
Zn	97.96±0.19 ^a	97.27±0.14 ^b	96.03±0.14 ^c	94.94±0.27 ^d	95.78±0.10 ^c
Fe	98.80±0.10 ^a	98.36±0.11 ^b	97.50±0.15 ^c	97.13±0.10 ^d	96.77±0.12 ^e

Values are a mean ± SEM (5 rats per group). Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD= *M. oleifera* meal based diet, CMD= Complete *M. oleifera* meal based

Table 8: Activity of selected digestive enzymes in rats fed on experimental diets

Enzyme	CSD	25%MD	50%MD	75%MD	CMD
Pepsin (μmol/min)	0.44±0.12 ^a	0.69±0.03 ^a	0.50±0.01 ^a	0.47±0.13 ^a	0.63±0.00 ^a
Pancreatic Amylase IH (μmol/mg protein/min)	37.67±3.78 ^a	46.50±1.75 ^a	40.30±5.86 ^a	48.63±0.17 ^a	146.10±14.24 ^b
Pancreatic Lipase IH (U/mg protein)	1.39±0.26 ^a	1.25±0.31 ^a	3.59±1.60 ^a	3.77±1.17 ^a	2.20±1.16 ^a

Values are a mean ± SEM (5 rats per group). Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD= *M. oleifera* meal based diet, CMD= Complete *M. oleifera* meal based diet. IH= Intestinal homogenate

Table 9: Concentration of Ferritin and selected minerals in serum of rats fed on experimental diets

Parameters	CSD	25%MD	50%MD	75%MD	CMD
Mineral composition ($\times 10^{-3}$ %)					
Na	8.30 $\pm$ 0.26 ^a	9.40 $\pm$ 0.17 ^a	7.10 $\pm$ 0.09 ^a	7.30 $\pm$ 0.12 ^a	17.10 $\pm$ 2.31 ^b
Ca	2.50 $\pm$ 0.20 ^a	3.40 $\pm$ 0.17 ^b	4.00 $\pm$ 0.23 ^c	1.80 $\pm$ 0.06 ^d	4.60 $\pm$ 0.12 ^e
K	6.10 $\pm$ 0.16 ^a	6.70 $\pm$ 0.12 ^a	7.60 $\pm$ 0.18 ^a	6.40 $\pm$ 1.01 ^a	6.50 $\pm$ 0.09 ^a
Mg	1.80 $\pm$ 0.17 ^a	4.50 $\pm$ 0.20 ^b	2.80 $\pm$ 0.12 ^c	3.20 $\pm$ 0.09 ^d	5.20 $\pm$ 0.10 ^e
Zn	1.40 $\pm$ 0.15 ^a	2.50 $\pm$ 0.20 ^b	2.90 $\pm$ 0.17 ^b	4.00 $\pm$ 0.06 ^c	5.50 $\pm$ 0.09 ^d
Ferritin (ng/ml)	22.67 $\pm$ 2.41 ^a	32.53 $\pm$ 5.77 ^a	27.13 $\pm$ 3.56 ^a	24.93 $\pm$ 1.65 ^a	30.60 $\pm$ 2.89 ^a

Values are a mean $\pm$ SEM (5 rats per group). Values along the same row with different superscripts are significantly different ($P < 0.05$). Legends; CSD= Complete Soymeal based diet, MD= *M. oleifera* meal based diet, CMD= Complete *M. oleifera* meal based diet.

DISCUSSION

In this study, attempts have been made to explore the effect of *Moringa oleifera* leaf powder on the digestive process in weanling albino rats, using both the balance method and evaluation of various biochemical parameters.

The nutritional composition of dried *Moringa oleifera* leaf powder was investigated and the values obtained were within the range of values found in literature. The crude protein value (23.83 ± 0.00 %) obtained in this study was higher than the value (17.01 ± 0.10 %) reported by Ogbe *et al.* (2011) and lower than 27.51, 29.25, 27.44% and 30.65%, reported by Oduro *et al.* (2008), Nuhu (2010), Olugbemi *et al.* (2010) and Mutayoba *et al.* (2011) respectively. Nuhu (2010) and Mutayoba *et al.* (2011) reported higher values (19.25% and 14.28%, respectively) for crude fibre and (41.98 % and 36.24 %, respectively) for carbohydrate, but a lower ether extract/fat of 2.23% and ash of 7.13% was reported by Nuhu (2010). These variations may be as a result of differences in geographical locations. The low fat, high protein and carbohydrate content of *Moringa oleifera* leaf is an indication that this plant is health benefitting and may be used to combat malnutrition as earlier reported (Fahey, 2005), provided the nutrients are bioavailable. In addition, the low fat content of *Moringa oleifera* leaf may be responsible for the claimed benefit of *Moringa oleifera* on cardiovascular diseases.

Moringa oleifera leaf powder used in this study contained an appreciable amount of minerals, which also compared well with the values obtained in previous reports. The value obtained for Na and Zn (0.621 and 0.017%) were higher than (199.95 ± 4.48 ppm and 60.06 ± 0.30 ppm) and (0.05 % and 21.79 ppm), reported by Ogbe *et al.* (2011) and Mutayoba *et al.* (2011) respectively. K, Mg, and Fe levels were higher than 0.97 ± 0.01 , 0.38 ± 0.01 and 107.48 ± 8.81 ppm respectively reported by Mutayoba *et al.* (2011). Ca level was lower than the value obtained in both studies. These variations may also be due to the differences in geographical locations. The presence of these minerals in *Moringa oleifera* is beneficial as minerals, though required in trace amounts, play significant roles in living cells. They partake in physiological functions such as fluid regulation, energy production, bone formation, muscle movement and nerve functioning (Oladiji, 2012).

The inclusion of varying amounts of *Moringa oleifera* leaf powder in diet caused a significant change ($p < 0.05$) in the nutritional content of feeds. The significantly higher mineral, ash and fibre content and the lower protein content of the *Moringa oleifera* supplemented diets, when compared to the control is an indication of nutritional differences between the soymeal and *Moringa oleifera* leaf powder used, since all other components of the diets were constant except the protein source

(Soymeal and *Moringa oleifera* leaf powder). Fapohunda (2012) reported 40.96% as the crude protein content of roasted soybean, which is higher than the value of protein (23.84 %) obtained for *Moringa oleifera* leaf powder in this study: crude fibre and ash values (4.62 and 4.21 % respectively) reported in the afore mentioned study were lower than those obtained in this study.

Growth response of rats fed on experimental diets showed that there was a dose-dependent decrease in weight gain of rats fed on *Moringa oleifera* supplemented diets, which was not significant ($p>0.05$). This is in agreement with the result of Yuangsoi and Masumoto (2012). Fashakin and Unikiwedu (1993) reported that the amount and quality of food consumed by experimental animals has a great effect on the growth response. The similarity in the weight gain of rats may be due to the similarity in feed intake of the test groups, when compared to the control. The pattern of weight decline indicates that an excessive intake of *Moringa oleifera* at the expense of other sources of protein may result in a loss of weight, thus affecting growth.

It has been reported that feeds with a relatively high moisture content decreases the quantity of drinking water required (Ward, 2007). In this study, there was a direct dose effect relationship between the moisture content of feed and the apparent water intake of rats. This however did not result in significant effects, when compared to the control.

The Feed Efficiency Ratio of the 25, 50 and 75 % *Moringa oleifera* supplemented diets compared favourably with the control. This was in agreement with the report of Adeniji and Lawal (2012) in which replacement of groundnut cake with 20 % and 40% *Moringa oleifera* leaf meal compared favourably with the control, but higher doses were significantly different ($p<0.05$). It however contradicts the report of Adeniji and Omonijo (2004), in which no significant difference ($p>0.05$) was shown for all the doses. The significant ($p<0.05$) decrease in Feed Efficiency Ratio in rats placed on complete *Moringa oleifera* supplemented diet may be due to the significantly reduced protein and carbohydrate intake of this group.

In the balanced digestibility study, the significantly higher ($p<0.05$) faecal mineral output and reduced percentage apparent digestibility of Mg, K, Zn and Fe in all the treatment groups, as well as Ca in the 75% MD and CMD group, when compared to the control suggests poor absorption. This may be as a result of antinutrients, nutrient interactions, or the high faecal output of minerals. Ogbe *et al.* (2011) reported the presence of tannins, phytates, trypsin inhibitors, saponins, oxalates and cyanide in *Moringa oleifera* leaves harvested from Lafia, Nigeria. Phytates, tannins, and oxalates are known to chelate minerals, especially the divalent ions of iron, calcium, and zinc (Nwokolo and Bragg, 1977; Reed, 1995; Webb, 1999), resulting in the formation complexes which may aggravate competitive effects at the site of intestinal

absorption (Hardwick *et al.*, 1990; Spencer *et al.*, 1994). In addition, the high fibre content of *Moringa oleifera* might have influenced the significant increase in faecal mineral excretion, thus resulting in the reduction in apparent mineral digestibility.

The result of the balanced macronutrient digestibility study showed a significant reduction ($p < 0.05$) in the digestibility of protein, carbohydrate and fat in the test groups, when compared to the control. This contradicts the report of Nuhu (2010), in which a dose dependent increase in apparent digestibility of nutrients was observed. It also negates the report of Fahey *et al.* (2001), that *Moringa* is an outstanding indigenous source of highly digestible protein.

The results obtained from the enzymatic study suggest efficient digestion process, in contrast to the balanced study. This may be due to the fact that endogenous losses were not determined in this study. The similarity in pepsin and pancreatic lipase activities of the test groups, when compared to the control is an indication that *Moringa oleifera* may not interfere with protein and fat digestion process, since these enzymes are major digestive enzymes in mammals. Furthermore, the significantly higher pancreatic amylase (an α -Amylase) activity in the intestine of rats fed on complete *Moringa oleifera* supplemented diet, when compared to the control, may be due to the high calcium content of the diet. Calcium is an essential cofactor for α -amylase (Morris, 2011). It has been proposed to stabilize this enzyme via interaction of its cation with some negatively charged amino acid residues, thus maintaining the 3D structure of the protein (Muralikrishna and Nirmala, 2005).

The gastric pH of rats fed on experimental diets in this study was within the range of 3.4 and 4.95. This was higher than 3.2 and 3.9 reported by McConnell *et al.* (2008) as gastric pH of rats in the fed and fasted state. These variations may be as a result of differences in experimental condition and nature of feed administered. In the gastric pH of the test groups and control (no significant change ($p > 0.05$)) was observed. This is reflected in the pepsin activity of rats, which also showed no significant change ($p > 0.05$). Pepsin is more active at an acidic pH (Nelson and Cox, 2004). Thus the similarity between the gastric pH of the test groups, when compared to the control, as well as the acidic state of the gastric juice further suggests that *Moringa oleifera* may not alter protein digestion.

The levels of mineral in the serum, was also investigated. One of the major factors affecting the concentration of minerals in blood is nutrition (Boguslawska-Tryket *et al.*, 2012). The significantly higher ($p < 0.05$) Mg, Ca, and Zn serum levels in the test groups and the significantly higher ($p < 0.05$) Na level in rats placed on complete *Moringa oleifera* supplemented diet can be attributed to the high level of the minerals in the diets. This observation suggests either an efficient absorption of minerals (in contradiction to the result obtained in the balance study) or defects in the regulatory mechanism required for the maintenance of electrolyte balance. Serum elec-

trolyte level can be used as a marker for assessing the functional capacities of the kidney. Thus the significant increase ($p<0.05$) in the levels of these minerals suggests impairment in the normal functioning of this organ. Absorptive hypercalcemia readily develops in children and patients with chronic kidney disease (CKD) when they receive amounts of dietary calcium that exceed the ability of their kidneys to filter and excrete the calcium load (Pieper, 2006). The significantly increased ($p<0.05$) serum Mg in the test groups and the significantly increased serum ($p<0.05$) Na in rats placed on CMD suggests that glomerular filtration might have been altered, thereby decreasing the excretion of these minerals in the urine (Ogunka-Nnoka *et al.*, 2012), as observed in this study. In addition, the significantly lower ($p<0.05$) serum Ca concentration in rats placed on 75% MD ($p<0.05$) was coupled with a significant increase in urinary Ca output, further attesting to the previous claim that alterations in glomerular filtration might have occurred.

Serum ferritin levels were not significantly different ($p>0.05$) in the test groups and when compared to the control. The similarity in the ferritin concentration of the control and the test groups in this study, indicates that the iron stores of the test groups were not depleted. This implies that although, the absorption of minerals seems to be affected, sufficient amount of iron was still absorbed, probably due to the higher intake in the test groups when compared to the control. High Fe intake may result in the saturation of iron storage (Ferritin) and transporting (transferrin) proteins, leading to a loss of Fe (Murray *et al.*, 2003) as shown in the dose dependent increase ($p<0.05$) in faecal output of Fe when compared with control.

In conclusion, the evidence from this study has given more insight on the possible effect of *Moringa oleifera* on the digestive process in weanling albino rats. *Moringa oleifera* was found to be a rich source of nutrients, but did not stimulate the feed intake and growth response in weanling rats. It rather aided macronutrient digestion, through the activity of the digestive enzymes and other biochemical markers evaluated. The higher calcium content of *Moringa Oleifera* leaf powder may be responsible for the significantly increased amylase activity observed in the rats placed on complete *Moringa Oleifera* supplemented diet, while the high fibre content of *Moringa Oleifera* might be responsible for the reduced apparent mineral absorption observed.

ACKNOWLEDGEMENT

Appreciation is expressed to Dr. A. Igunnu and Mrs R. A. Oyegoke in the Department of Biochemistry, University of Ilorin and to Dr. S. O. Biliaminu, Department of Chemical Pathology and Immunology, College of Medicine, University of Ilorin, Nigeria for the support rendered.

REFERENCES

- Adeniji A. A., Omonijo O. A. (2004).** The replacement value of palm kernel cake for groundnut cake in the diets of weaner rabbits. *Livestock Production Science*, 85: 287-291.
- Adeniji, A. A., Lawal, M. (2012).** Effects of Replacing Groundnut Cake with *Moringa oleifera* Leaf Meal in the Diets of Grower Rabbits. *International Journal of Molecular Veterinary Research*, Vol.2, No.3 8-13.
- Akanji, M. A., Ngaha, E.O. (1989).** Effect of repeated administration of berenil on urinary excretion with corresponding tissue pattern in rats. *Pharmol. Toxicol.* 81: 173-179.
- Anson, M. J. (1938).** The estimation of pepsin with Hemoglobin. *Gen Physiol*, 22: 79.
- Association of Analytical Chemists. (1990).** *In official methods of Analysis*. 15th edition Washinton D. C.
- Association of Analytical Chemists. (2005).** *Official Methods of Analysis*. 18th Edition. AOAC international, Maryland, USA.
- Bernfeld, P. (1955).** Amylase α and β . *Methods in Enzymology*, 1, 149-151.
- Boguslawska-Tryk, M., Szymeczko, R., Piotrowska, A. (2012)** The level of major proteins and minerals in the blood serum of chickens fed diets with pure cellulose. *Folia biologica (Kraków)* 60 (1-2): 65-70.
- Chuang, P., Lee, C., Chou, J., Murugan, M., Shieh, B., Chen, H. (2007)** Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Bioresource Technology* 98(1):232-236.
- Dalziel, J.M. (1956).** Useful plants of West Tropical Africa. *Crown Agents for Oversea Government and Administration*, London, 23pp.
- Dangi, S. Y., Jolly, C. I., Narayanan, S. (2002)** Antihypertensive Activity of the Total Alkaloids from the Leaves of *Moringa oleifera*. 40 (2), 144-148.
- Edem, D. O., Eka, O. U., Umoh, I. B., Udoh, A. P., Akpan, E. J. (2003).** Effect of Red Palm Oil and Refined Palm Olein on Nutrient Digestion in the Rat. *Pakistan Journal of Nutrition*, 2 (5): 271-278.
- Fahey, J.W. (2005).** *Moringaoleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. *Trees for Life Journal*, 1: 5.
- Fahey, J. W., Zalcmann, A. T. and Talalay, P. (2001).** The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 56 (1): 5-51.
- Fapohunda, O.O. (2012).** Evaluation of Processed Soybean Meal in the Feeding of ClariasGariepinus Fingerlings *Journal of Animal Science Advances*, 2(2): 244-249.

- Fashakin, J. B., Unokiwedi, C. C. (1993).** Nutritional evaluation of “warankasi” and “waragusi” prepared from cow milk partially substituted with melon milk. *Nigeria Food Journal*, 11: 128-134.
- Forman, L. P., Schneeman, B. O. (1980).** Effects of Dietary Pectin and Fat on the Small Intestinal Contents and Exocrine Pancreas of Rats. *Journal of Nutrition*, 110: 1992-1999.
- Giridhari, V. A., Malathi, D., Geetha, K. (2011)** Anti Diabetic Property of Drumstick (*Moringa oleifera*) Leaf Tablets *International Journal of Health and Nutrition* 2: 1
- Hardwick, L. L., Jones, M. R., Buddington, R. K., Clemens, R. A., Lee, D. B (1990).** Comparison of calcium and magnesium absorption: in vivo and in vitro studies. *American Journal of Physiology*, 259, G720-726.
- Hirohashi, M., Takasuna, K., Kasi, Y., Usui, C., Tamura, K., Kojima, H. (1993).** General pharmacological profile of the new anti-ulcer drug 3-[[[2-(3, 4-Dimethoxyphenyl) ethyl] carbamoyl] amino- N-methylbenzamide. *ArzneimittelForsch - Drug Research*, 43(1): 569-577.
- Luangpiom, A., Kourjampa, W., Junaimaung, T. (2013)** Anti-hyperglycemic Properties of *Moringa oleifera* Lam. Aqueous Leaf Extract in Normal and Mildly Diabetic Mice. *British Journal of Pharmacology and Toxicology* 4(3): 106-109.
- McConnell. E. L., Basit, A. W., Murdan, S. (2008).** Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *Journal of Pharmacology and Pharmacotherapeutics*, 60(1): 63-70.
- Morris, C. (2011).** Impact of calcium on salivary α -amylase activity, starch paste apparent viscosity and thickness perception. *Chemosensory Perception*, 3, 112-116.
- Muralikrishna, G., Nirmala, M. (2005).** Cereal Alpha-amylases - an overview. *Carbohydrate Polymers*, 322 60(2), 163-173.
- Murray, R. K., Granner, D. K., Mayes, P. A., Rodwell, V. W. (2003)** Harper’s Illustrated Biochemistry. Lange Medical Books/McGraw-Hill, 26th Edition. Chapter 44 page 478.
- Mutayoba, S. K., Dierenfeld, E., Mercedes, V. A., Frances, Y., Knight, C. D. (2011).** Determination of chemical composition and anti-nutritive components for Tanzanian locally available poultry feed ingredients. *International Journal of Poultry Science*, 10(5), 350-357.
- Nelson, D.L. & Cox, M.M. (2004).** *Lehninger Principles of Biochemistry*, 4th ed. W.H. Freeman page 212.

- NRC (1985).** National Research Council. Guide for the care and use of laboratory animals, Publication No. 85-23 (rev). National Institutes of Health, Bethesda, MD.
- Nuhu F. (2010).** Effect of Moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits, Thesis for M.S., Animal Science Department, Faculty of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Accessed 17th June, 2013. <http://hdl.handle.net/123456789/337>.
- Nwokolo, E. N., Bragg, D. B. (1977).** Influence of Phytic acid and crude fibre on the availability of Minerals from four protein supplements in growing chicks. *Canadian Journal of Animal Science*, 57: 475-477.
- Oduro, I., Ellis, W. O. Owusu, D. (2008).** Nutritional potential of two leafy vegetables: *MoringaOleifera* and *Impomeabatatas* leaves. *Scientific research and essay*, 3(2): 57-60.
- Ogbe, A. O., Affiku, J. P. (2011).** Proximate study, mineral and anti-nutrient composition of *Moringaoleifera* leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Sciences*, 1 (3) 296-308.
- Ogunka-Nnoka, C. U., Uwakwe, A. A., Nnabuike, C. J. (2012)** Effects of ethanolic /potash extract of sorghum bicolor leaf sheath on serum electrolytes, liver and kidney indicative on albino rats. *Journal of Natural Sciences Research* Vol.2, No.4.
- Okwari, O.O., Dasofunjo, K., Asuk, A. A., Alagwu, E. A., Mokwe, C. M. (2013)** Anti-Hypercholesterolemic and Hepatoprotective effect of Aqueous Leaf Extract of *Moringa oleifera* in Rats fed with Thermoxidized Palm Oil Diet. *Journal of Pharmacy and Biological Sciences* Volume 8(2), 57-62.
- Oladiji, A. T. (2012).** The little giants in foods. 102nd inaugural lecture of the University of Ilorin. 22nd March, 2012.
- Olugbemi, T. S., Mutayoba, S. K., Lekule, F. P. (2010).** Effect of Moringa (*Moringaoleifera*) Inclusion in Cassava based diets to broiler chickens. *International Journal of Poultry Science*, 9(4), 363-367.
- Pal, S. K., Mukherjee, P. K., Saha, B. P. (1995)** Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytotherapy Research* 9 (6), 463–465.
- Percival, S. S., Schneeman, B. O. (1979).** Long term pancreatic response to feeding heat damaged casein in rats. *Journal of Nutrition*, 109, 1609-1614.
- Reed, J. D. (1995).** Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science*, 73: 1516-1528.

- Spencer, H., Norris, C., Williams, D. (1994).** Inhibitory effects of zinc on magnesium balance and Magnesium absorption in man. *Journal of American College of Nutrition*, 13, 479-484.
- Sreelatha, S., Padma, P. R. (2009)** Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods Hum Nutr* 64(4): 303-311.
- Stevens, G. C, Baiyeri, K. P., Akinnnagbe, O. (2013)** Ethno-medicinal and culinary uses of *Moringa oleifera* Lam. in Nigeria. *Journal of Medicinal Plants Research*. 7 (13), 799-804.
- Ward, D. (2007).** *Water Requirements of Livestock*. Fact sheet ISSN 1198-712X. Queen's printer. Accessed 28th January, 2013.
- Webb, M. A. (1999).** Cell-Mediated Crystallization of calcium oxalate in plants. *Plant cell*, 11: 751-761.
- Xio-Bo, S., Tsukasa, M., Haruki, Y. (1992).** Anti-ulcer activity and mode of action of the polysaccharide fraction from the leaves of *Panax ginseng*. *PlantaMedica*, 58:432-435.
- Yoshida, N., Omoya, H., Kato, S., Ito, T. (1993).** Pharmacological effects of the new gastroprokinetic agent Mosapride citrate and its metabolites in experimental animals. *ArzneimittelForsch - Drug Research*, 43(II) Nr. 10: 1078-1083.
- Yuangsoi, B., Masumoto, T. (2012).** Replacing moringa leaf (*Moringaoleifera*) partially by protein replacement in soybean meal of fancy carp (*Cyprinus carpio*). *Song klanakarin Journal of Science and Technology*, 34 (5), 479-485.

βETA-LACTAMASE PRODUCTION AND PLASMID PROFILING OF PENICILLINS RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FOOD HANDLERS

¹Kayode, R.M.O.*, ¹Adedeji, B.S., ²Oladoye, C.O., ³Laba, S.A., and ¹Salami, K.O.

¹*Division of Food Preservation, Microbial Biotechnology and Toxicology, Department of Home Economics and Food Science,
University of Ilorin, P.M.B 1515, Ilorin, Nigeria*

²*Department of Science Laboratory Technology, Federal Polytechnic, Offa,
Kwara State, Nigeria*

³*Department of Microbiology, Faculty of Life Sciences,
University of Ilorin, P.M.B 1515, Ilorin, Kwara State, Nigeria*

Corresponding author's e-mail address: kayodermosnr@gmail.com

Phone no: +2348035850545.

ABSTRACT

*This study investigated the prevalence and plasmid profiles of β -lactamase positive *Staphylococcus aureus* isolated from the nasal and hand swabs of some healthy food handlers in Tanke area of Ilorin, Nigeria. Sensitivity test of the isolated strains of *Staphylococcus aureus* was carried out with six different types of penicillin based antibiotics using standard procedures. The plasmid profile was carried out using Agarose gel electrophoresis and the result shown in Polaroid gel instant camera through an orange filter. The photograph was developed and observed for bands. There was 100 % occurrence of *S. aureus* from both nasal and hand swab samples. The result reveals 31.57% β -lactamase positive *Staphylococcus aureus* (β PSA) from the nasal swabs, while it was 15.78% from the hand swabs. The strains of *Staphylococcus aureus* isolated from the nose and hand swabs were resistant to most of the selected penicillin based antibiotics in the following decreasing order: Penicillin (100 %), Ampicillin (86.84 %), Amoxicillin (73.68 %), Ampicloxacin (68.42 %), Gentamycin (34.02%) and Erythromycin (15.78%).*

*The results of plasmid profiles of the β -lactamase positive *Staphylococcus aureus* (β PSA) strains showed that 15.7% had distinct plasmid bands. Although, less than half of the total isolated *Staphylococcus aureus* strains were β PSA (23.6%) and 66.6% of the total β PSA carried distinct plasmid bands; which is an indication of a potential microbiological hazard in foods prepared by the food handlers especially, if proper hygienic condition is not maintained during food preparations.*

Key words: *β -lactamase, penicillins, plasmid profile, food handlers, *Staphylococcus aureus**

INTRODUCTION

Staphylococcus aureus, is a spherical aerobic gram positive, catalase positive, oxidase positive, non-motile, spore-forming coccus. It is an opportunistic pathogen in human and animal, and is one of the most frequent sources of hospital and community-acquired infections. Generally, *S. aureus* is responsible for superficial infections and toxic epidermal necrolysis, systemic infections such as endocarditis inflammation of bone or bone marrow, pneumonia and toxinoses such as food poisoning or toxic shock syndrome. However, among gram-positive cocci, only β -lactamase of major clinical significance is *Staphylococcal* β -lactamase positive strains which rapidly hydrolyses benzylpenicillin, ampicillin, cephalosporins, and related antimicrobials (Foster, 1996; Francis *et al.*, 1997; Brumfit and Hamilton, 1989; Sampathkumar 2007; Daini and Akano, 2009; Hotu *et al.*, 2007). Methicillin-resistant *S. aureus* (MRSA) is a special strain of *S. aureus* that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class. MRSA have acquired genes encoding antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum β -lactamase-resistant penicillin antibiotics (O'Brien *et al.*, 1999; Maltezou and Giamarellou, 2006; Boyce, 1994; Chambers, 2001; Maltezou and Giamarellou, 2006).

Staphylococcus aureus is a bacterium found on the skin and in the nasal passages of up to 25% of healthy people and animals. *S. aureus* is bacterial specie that can contaminate food by direct bodily contact, through skin fragments, or through respiratory droplets produced when people cough and sneeze. Most *Staphylococcus aureus* food borne illness results from food contamination by food handlers, meat grinders, knives, storage containers, and cutting blocks. Food-borne diseases are of major concern worldwide. To date, around 250 different food-borne diseases have been described, and bacteria are the causative agents of two thirds of food-borne disease outbreaks. Among the predominant bacteria involved in these diseases is *Staphylococcus aureus*. The organism is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food. Strains of *Staphylococcus aureus* that are resistant to methicillin (MRSA) is a major concern to public health workers throughout the world because of its ability to grow in the presence of antibiotics of the penicillin group such as methicillin, oxacillin and dicloxacillin which is made possible by its ability to produce enzyme beta-lactamase (pennicillinase) which breaks the beta-lactam ring contained in the antibiotic thereby causing infections that are difficult to treat such as otitis, bacteraemia and osteomyelitis. Hence, the study is designed to assess the mechanism of resistance of this strain to selected chemotherapeutic agents.

MATERIALS AND METHODS

Swab Sample Collection

Thirty-eight hands and nasal swaps were collected from nineteen (19) food service centers in Tanke area, Ilorin. In each of the food service centers, two swap sticks were aseptically collected from the food handlers as follows: One hand swap and one nasal swap were collected from the personnel working for each of the food service center.

Isolation of *Staphylococcus aureus*

The Baird Parker Agar was prepared according to the manufacturer's specification. The agar surface was smooth and moist, but without excessive moisture. Medium was inoculated as soon as the specimen arrives at the laboratory. The culture was made by using the specimen from the swab, medium was inoculated by rolling the swab over a third portion of the agar surface, and the remainder of the plate was streak to obtain isolated colonies. A total of thirty-eight (38) plates were used for all the swaps.

Screening of Bacterial Isolates for Beta-Lactamase Production

All the bacteria species isolated from the various hand swaps and nasal swaps of the food handlers were tested for the production of β -lactamase.

Penicillin Starch Paper Strips Preparation

Penicillin starch paper strip was prepared according to the method used in a study on pediatric gonorrhea (Odugbemi and Onile, 1988) and the β -lactamase production of bacterial isolates from smoked bush meats (Kayode and Kolawole, 2008). An ample of benzyl penicillin containing 1,000,000 iu of antibiotic was dissolved in 10ml of

phosphate buffered saline (PBS) at pH 7.3 such that each ml of the solution contain 100,000 iu of benzyl penicillin. Two percent (2%) starch solution was prepared by the addition of 2g of starch powder to 98ml of phosphate buffered saline; the starch was warmed until dissolved completely. A whatman no 3 filter paper was cut into strips of size 7cm by 4cm to fit the bottom of the petri-dish. 10ml of the benzyl penicillin solution was added to 90ml of the 2% starch solution and mixed properly. The paper strips were then soaked in the mixture for 5 min, and removed and spread in a hood to dry and stored in the refrigerator at 4°C before use.

Detection of β -Lactamase

A strip of the starch paper was soaked in benzyl penicillin for 10min and spread smoothly in a neat petri-dish. Two millimeter sterile bacteriological loop was used to collect bacterial culture from the surface of the culture medium and transferred to the test paper in a petri-dish; this was spread smoothly over an area for 2-3mm, inoculants was placed at least 1.5cm apart. These was incubated at 37 °C for 30 min after which the paper was then flooded with iodine solution (which was drained immediately). This causes the starch to turn uniformly blue black; a negative region also turn blue black, white positive result in a region where the bacteria produces β -lactamase becomes decolorized immediately.

Antibiotics Resistance Test

A total of six (6) antibiotics namely: Gentamycin (Lek pharm, Slovenia), Ampicillin (Broad spectrum, Nigeria). Amoxicillin, CSPC Zhongnuo pharm, China). Erythromycin (Mekophar Chemical Pharmaceutical, GMP, Vietnam). Penicillin (Sishui xier kang, China) Ampicloxacillin (Nichben pharm Nigeria). were purchased from the whole sale / retail store of Tuyil Pharmaceutical Company in Ilorin town, Kwara State Nigeria. The antibiotics were prepared into disc form according to the performance standard for antimicrobial disc susceptibility testing given by CLSI. Thus, the antibiotic discs were used with their concentration as follows: Ampicloxacillin (5 μ g), Ampicillin (10 μ g), Erythromycin (15 μ g), Gentamycin (10 μ g), Penicillin (30 μ g), Amoxicillin (25 μ g).

Sensitivity Test

Nutrient agar and antibiotic sensitivity multi-discs containing penicillin or its derivative antibiotics were used for the work. The inoculated plates were incubated at 37°C for 24 hrs before checking for the formation of zone of inhibition around the antibiotic discs; an indication that the organism is sensitive to the antibiotic studied.

Plasmid Extraction Purification and Digestion

Plasmid was extracted by the modification of the alkaline lysis method of Bimboim and Doly (1979). First, the MRSA isolates was grown overnight on Mueller-Hinton agar at 35°C, harvested and washed once with 0.1ml of the buffer in eppendorf tubes, vortexed and centrifuged. The supernatant was discarded while, the cells were suspended in 0.2ml Tris buffer (Te buffer) containing 40 μ g of lysostaphin per ml, vortexed once to properly mix and then incubated in a water bath at 37°C for 30mins. To these, 0.4ml of 0.2M NaOH-1% (w/v) Sodium Duodecyl Sulphate (SDS) was added and tubes placed on ice for 10 mins., Exactly 0.3ml of sodium ac-

etate was then added and the lysate was cooled on ice for 10 mins. The mixture was centrifuged at 13,000 rpm for 10 mins on ice. The supernatant was extracted once with buffered-saturated phenol and once with ether. The preparation was then incubated with 2µl of heat treated pancreatic RNASE for 15 mins at 37°C in a water bath. The DNA was extracted with 1.0ml of phenol chloroform (1:1) and then precipitated with ethanol. The precipitate was collected by centrifugation and washed with cold 70% ethanol, dessicated inside vacuum oven and dissolved in 40µl of TE buffer.

Plasmid Digestion and Electrophoresis

Exactly 10µl of plasmid preparation from each MRSA isolate was added to 1µl of TE buffer (SURE/CUT Buffer 11) and digested with 10 units of EcoRI restriction enzyme for 3hrs in a water bath at 37°C. The electrophoresis was carried out in a horizontal agarose electrophoresis gel unit (Model HE 33 Hoeffer scientific instruments, San Francisco). Then volume (15µl) of plasmid preparation and digested DNA sample from each isolate with added 0.2µl bromophenol blue dye (tracking dye) were applied to the head of the horizontal wells containing 50ml of 0.8% (w/v) agarose into which two drops of ethidium bromide dye has been added. This was followed with the addition of exactly 15µl of marker DNA molecule (Roche Diagnostic Germany 2027-23130 kbp) was applied to the middle well as the standard. The agarose slab was placed in an electrophoresis performed at 75v for 1hr 30mins at 37°C. The gel was dried and observed under a 302 nm wavelength trans-illuminator and then photographed onto Polaroid gel cam instant camera through an orange filter (peca product inc. USA). The photographs were develop and observed for bands.

RESULTS

Frequency of Isolation of *Staphylococcus aureus*

A total of seventy-six swab samples were collected for this research, out of which thirty-eight were hand swabs and the remaining thirty-eight were nasal swabs, there was *S. aureus* growth in all the swab samples based on the selective medium used. The prevalence rate of *S. aureus* from hand to nose were 38 (100%) and 38 (100%) respectively as shown in Table 1.

β-Lactamase Production

Table 2 shows the level of β-Lactamase production by the isolates from the hand and the nose of healthy food canteen workers. The percentage β-lactamase positive *S. aureus* obtained from the nasal swabs was 31.57%. While, 15.78% was obtained for *S. aureus* isolated from the hand swabs. Off the 76 cultures of *S. aureus* isolated from two body parts of the food handlers, not less than 23.6% of the isolates were producers of the enzyme.

Antibiotic Resistance Pattern of *Staphylococcus aureus*

The result of the antibiotic susceptibility pattern of the isolates to the six antibiotics used is shown in Table 5. The result shows total resistance to penicillin (100%) and high resistance to ampicillin (86.84%), amoxicillin (73.68%), ampicloxacillin

(68.42%), moderate resistance to gentamycin (34.2%) and low resistance to erythromycin (15.78%).

Percentage Prevalence of Multi-drug Resistance among *Staphylococcus aureus*

High level of multiple drug resistance was observed in MRSA isolated from healthy food handlers in the study. 97.3 % of the *S. aureus* isolated were resistant to at least two antibiotics tested and only 2.63% of the *S. aureus* isolated were resistant to one of the antibiotics tested as indicated in Table 3.

Plasmid content of the β -lactamase positive *Staphylococcus aureus*

Table 5 shows the plasmid profiling of the β -lactamase positive *Staphylococcus aureus* isolated from the food handlers. Not less than 75.0% (9 isolates) of the 31.57% (12 isolates) of the total β -lactamase positive *Staphylococcus aureus* (38 isolates) strains from the nose and 50.0% (3 isolates) of the 15.78% (6 isolates) of the total β -lactamase positive *Staphylococcus aureus* (38 isolates) strains from the hand of the food handlers contained plasmid. This result reveals that the probability of the occurrence of plasmid in β -lactamase Positive *Staphylococcus aureus* is high; an indication that the gene causing resistance to the antibiotics is situated in the plasmid.

DISCUSSION

This study was carried out to investigate the prevalence of β PSA on food handlers. Of the 76 isolates collected from the food handlers tested, only 23.6% were β lactamase positive and are thereby methicillin-resistance *Staphylococcus aureus*. The value obtained seems to be close to 22.4% β lactamase positive *Staphylococcus aureus* strains isolated from food handlers in Botswana (Klaske *et al.*, 2007).

This value (23.6%) β PSA obtained in this study is observed lower than the results obtained in a study conducted in Jos (Ikeh, 2003) and Ilorin (Taiwo *et al.* 2004) in Nigeria; which had prevalence rates of 43.0 and 34.7% respectively. In another study comprising eight large hospitals in Africa and Malta, a prevalence rate of 16% was observed. MRSA was reported to be relatively high in Nigeria, Kenya and Cameroon with values ranging from 21-30% (Boyce, 1994). Multidrug resistance was defined in this study as resistance to two or more of the antibiotics tested. Thus 74 (97.37 %) of the isolates showed multidrug resistance to the antibiotics tested. None of the MRSA was fully susceptible to all the antibiotics but 2 (2.63%) was resistant to all the six antibiotics used. This observation confirms the postulation that healthy members of the community including food handlers are the highest reservoir of antimicrobial resistant bacteria (Laminkanra *et al.*, 1996).

There was 100% resistance to penicillin, which agrees with Esan *et al.* (2009) who also reported 100% resistance to penicillin antibiotic in a study carried out on some bacterial species. The β PSA isolated in this study showed high resistance to Ampicillin (86.8 %) and Amoxycillin (73.68 %), which supports the findings that MRSA strains are equally resistant to all β -lactam antibiotics (Gross- Schulman *et al.*, 1998). The high level of resistance observed in ampicillin is also in agreement with the reports by Onanuga *et al.* (2005) who observed a resistivity of 91.7 % and 100% to ampicillin respectively in their two different findings. The β PSA isolated also

showed a high resistance to ampicloxacillin (68.4%) but had low resistance to Erythromycin (15.7 %). The resistance observed toward ampicloxacillin may be attributed to the capability of the organism to exhibit remarkable versatility in its behavior towards antibiotics and that most strains of *S. aureus* are resistant to commonly prescribed antibiotics (Grassi, 1998; Olayinka *et al.*, 1995).

The low resistance observed by the β PSA isolated in this study towards Erythromycin is also similar to 38% as reported by Hantash *et al.* (2008). The differences observed in the resistivity of the organisms to the antibiotic tested may be due to the source of isolation, genetic variability and environmental conditions that may cause microbial cell mutation. The high resistance to ampicillin and low resistance to erythromycin also agree with previous report by oyetunji *et al.* (2012). The high sensitivity to Gentamycin (65.7 %) supports some previous reports (Ehinmidu, 2003; Fridkin *et al.*, 2005; Ikeh, 2003; Onanuga *et al.*, 2005; Nordmann and Nass, 2005; Olayinka *et al.*, 2004; Uba and Umar, (2004). In 2001, Polyzou *et al.*, reported that high susceptibility to Gentamycin may be as a result of conferring genes being absent in these MRSA strains. Also, gentamycin, an aminoglycoside though very cheap, its high susceptibility may be due to the complexity of the aminoglycoside and the route of administration. While the fluroquinolones are newer drugs with mode of action centre on inhibition of the DNA replication which stops the multiplication of bacterial cells and relatively expensive therefore are more likely not available for abuse (Onanuga *et al.*, 2005). In addition, susceptibility of the MRSA isolated to Gentamycin supports the findings of Onanuga *et al.* (2005) that these non- β -lactam antibiotics may provide an opportunity for the recommendation of these drugs for the treatment of CA-MRSA infections. Different studies have shown that the beta-lactamase enzyme (penicillinase) destroys the penicillin antibiotic by hydrolyzing the beta-lactam ring and this decreases the usefulness of the penicillin derivative antibiotics such as methicillin, oxacillin amoxicillin among others.

High level of multiple drug resistance was observed in MRSA isolated from healthy food handlers in the study. About 97.3 % of the *S. aureus* isolated were resistant to at least two antibiotics tested and only 2.63% of the *S. aureus* strains were resistant to one of the antibiotics tested. Twelve (66.6%) of the 18 *Staphylococcus aureus* strains that are β -lactamase positive had plasmid; an indication that the probability of the occurrence of plasmid or gene causing resistance situated in the plasmid in β PSA is high.

This study observed high prevalence of *Staphylococcus aureus* among the food handlers and that the strains of *Staphylococcus aureus* that are β -lactamase positive are very low when compared with similar reports carried out in the hospital environment. The number of *Staphylococcus aureus* isolated from the food handlers carrying plasmid was observed to be low; this may be one of the reasons why infection due to β -lactamase positive *Staphylococcus aureus* is not common in this part of the world. However the high prevalence of *Staphylococcus aureus* on food handler's body parts should be curtailed by high standard of hygiene practices.

In conclusion, effective and scrupulous hygienic practices should always be carried out by food service providers to reduce contamination, for instance during food preparation or serving, Food handlers should be discouraged from smoking, chewing gum or eating food. Food handlers should minimize bare-hand contact of ready-

to-eat food. Food handlers should always put on clean clothes, hat and caps and should wear hand gloves when necessary. Workers should not put on fingernail polish or artificial fingernail food handlers should always wash their hands after using the bathroom, after coughing, sneezing, smoking, eating, or drinking.

Thorough disinfection should be carried out regularly by food service providers, disinfectants such as sodium hypochlorite, quaternary ammonium compounds, and iodophors can be used to scrub the floor of the canteen and to wash the eating tables every weekend to retard contamination.

Food service providers should always serve foods when hot, because the temperature of the hot food would have an inhibitory effect on *S. aureus* that might be present in such food.

Proper prescription of antibiotics and implementation of an agreed control of infection policies are needed to curtail abuse and irrational use of antibiotics which may lead to development of drug resistance.

Contact between livestock such as poultry, pigs etc and their processor should be adequately controlled, this may aid transmission of resistant strains from animals or livestock to humans and further complicating the epidemiology of this organism.

Table 1: Frequency of isolation of *Staphylococcus aureus* from the hand and nose of healthy canteen workers

Source of Isolation	Total Number of Samples Analyzed	Occurrence of <i>S. aureus</i> in Food Handlers (%)
Hand	38	100%
Nose	38	100%
Total	76	100%

Table 2: Beta-lactamase production by *Staphylococcus aureus* isolated from nasal and hand swabs of healthy food canteen workers

Source of Isolation	Total Number of Samples Analyzed	β-Lactamase Production	
		Positive (%)	Negative (%)
Nose	38	31.57	68.42
Hand	38	15.78	84.21
Total	76	23.6	76.3

Table 3: Antibiotics resistance pattern of *Staphylococcus aureus* isolated from the nasal and hand swabs of healthy food canteen workers.

Antibiotics	Resistance (%)	Susceptible (%)
Ampicillin	92.10	7.89
Amoxicillin	84.21	15.78
Erythromycin	15.78	84.21
Penicillin	100	0.00
Gentamycin	28.94	71.05
Ampicloxacillin	65.78	34.21

Table 4: Percentage prevalence of multi-drug resistance among *Staphylococcus aureus* isolated from nasal and hand swabs of healthy food canteen workers

Parameter/ Number of Antibiotic Tested	Number of isolates tested	Number of isolates resistant to antibiotics	Prevalence (%)
Sensitive to all the antibiotics used	76	0	0.00
Resistance to only one of the antibiotic	76	2	2.63
Resistance to two of the antibiotics	76	6	7.89
Resistance to three of the antibiotics	76	14	18.42
Resistance to four of the antibiotics	76	32	42.10
Resistance to five of the antibiotics	76	18	23.68
Resistance to all the antibiotics tested	76	4	5.26

Table 5: β -lactamase positive *Staphylococcus aureus* containing plasmids isolated from nasal and hand swabs of healthy food canteen workers.

Source of Isolation	β -Lactamase Production		
	Total Isolates Tested	β -Lactamase positive strains (%)	Plasmid positive strains (%)
Nose	38	31.57 (12)	75 (9)
Hand	38	15.78 (6)	50 (3)

REFERENCES

- Boyce, J.M. (1994).** Methicillin-resistant *Staphylococcus aureus*: a continuing infection control challenge. *European Journal of Clinical Microbiology Infection Disease*, 13: 45-49.

- Brumfit, W., Hamilton-Miller, J. (1989).** Methicillin-resistant *Staphylococcus aureus* *New England Journal of Medicine*, 320: 1188-1196.
- Chambers, H.F. (2005).** Community-associated MRSA-resistance and virulence converge. *New England Journal of Medicine*, vol. 352: 1486-1487.
- Daini, O.A. Akano, S.A. (2009).** Plasmid-mediated antibiotic resistance in *Staphylococcus aureus* from patients and non patients. *Sci. Res. Essay*, 4(4): 346-350.
- Ehinmidu, J.O. (2003).** Antibiotic susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. In *Tropical Journal of Pharmaceutical Research*, vol. 2: 223-228.
- Esan, C.O. Oladiran, F., Johnson, L. and Adebayo, O.S. (2009)** Characterization of *Staphylococcus aureus* isolates obtained from health care institutions in Ekiti and Ondo States, South-Western Nigeria. *African Journal of Microbiology Research* Vol. 3(12): 962-968.
- Foster, T. (1996).** *Staphylococcus*, Barron's Medical Microbiology (Barron, S. *et al.*, eds.). 4th edition; University of Texas Medical Branch. Francis O, Harold, L.P., Roger, F.G., David, G. (1997). Antibiotic and Chemother. 7th ed., Churchill Livingstone, U.K., P. 26.
- Francis, O., Harold, L.P., Roger, F.G., David, G. (1997).** Antibiotic and Chemotherapy. 7th edition Churchill Livingstone, U.K., p. 26.
- Fridkin, S.K. (2001).** Vancomycin-intermediate and resistant *Staphylococcus aureus*: What the infectious disease specialist need to know. In *Clinical Infect. Disease*, vol. 32: 108-115.
- Grassi, G.G. (1998).** Infections by Gram-positive bacteria: An overview. *International Journal of Antimicrobials and Chemotherapy*, vol. 21: 1-7.
- Gross-Schulman, S., Dassey, D., Mascola, L., Anaya, C. (1998).** CA-MRSA disease in three communities. In *New England Journal of Medicine*, vol. 352: 1436 - 1444.
- Hantash, A.E., Muna, J., Abdul, J.T. (2008).** Nasal carriage of methicillin-resistant *Staphylococcus aureus* in individuals exposed and not exposed to hospital environments. In *European Journal of Scientific Research*, vol. 22 (4): 570-574. *Chemotherapy*, vol. 40: 135-136.
- Hotu, B., Ellenbogen, C., Hayden, M.K., Aroutcheva, A., Rice, T.W., Weinstein, R.A. (2007).** Community-associated methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections at a public hospital: Do public housing and incarceration amplify transmission? *Archeological International Medicine* 167: 1026-1033.
- Ikeh, E.I. (2003).** Methicillin-resistant *Staphylococcus aureus* (MRSA) at the Jos University Teaching Hospital. In *Afric Jour of Clinic and Experimental Microbiol.*, vol.4: 52-62.
- Kayode, R.M.O. and Kolawole, O.M. (2008).** Studies of the β - lactamase production of bacterial isolates from smoked bush meats correlated with bacterial resistance to three β - lactam antibiotics. *Journal of Applied Sci. and Environmental Management*. 12(2):89-92 (ISSN: 1119-8362). <http://www.bioline.org.br/ja>

- Klaske, V .H. (2007)** Methicillin-resistant *Staphylococcus aureus* isolated from food handlers in Botswana. *Journal of Food Protection*, Vol. 70 (12) 2674-2768.
- Lamikanra, A., Ako-nai, A.K., Ogunniyi, D.A. (1996).** Transferable antibiotic resistance in *Escherichia coli* isolated from healthy Nigerian school children. In *International Journal of Antimicrobial Agents*, vol. 7: 59-64.
- Maltezou, H.C., Giamarellou, H. (2006).** Community-acquired methicillin-resistant *Staphylococcus aureus* infections. *International Journal of Antimicrobial Agents*, 27: 87-96.
- Nordmann, P., Nass, T. (2005).** Transmission of methicillin-resistant *Staphylococcus aureus* to a microbiologist. In *New England Journal of Medicine*, vol. 352: 1489-1490.
- O'brien, F.G. Pearman, J.W. Gracy, M. Ritley, T.V. Grubb, W. B.(1999).** Community stain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. In: *Journal of Clinical Microbiology*, vol. 37: 2858-2862.
- Odungbemi, T. and Onile, B. A. (1988).** Peadiatric gonorrhoeae: Is it receiving adequate attention? *America Journal. Reproductive Immunology. Microbiology* 18: 32.
- Olayinka, B. O., Olayinka, A. T., Onaolapo, J. A., Olurinola, P. F. (2005).** Pattern of resistance to vancomycin and other antimicrobial agents in a University Teaching Hospital. In *African Journ. of Clinical and Experimental Microbiology*, vol. 6: 46- 52.
- Onanuga, A., Oyi, A. R., Onaolapo, A. J. (2005).** Prevalence and susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. In *African Journal of Biotechnology*, vol. 4 (11) 1321- 1324.
- Oyetunji, I.A., Ikenna, O. O., Olumide, A., Odeyemi, A.E., Okwori, J. (2012).** Prevalence of methicillin-resistant *Staphylococcus aureus* from healthy community individuals volunteers in Jos South, Nigeria. *Journal of Microbiology, Biotechnology and Food Sciences*, 1 (6) 1389-1405.
- Polyzou, A., Slavakis, A., Pournaras, S. (2001).** Predominance of a MRSA clone susceptible to erythromycin and several other non-beta lactam antibiotics in a Greek hospital. In: *Journal of Antimicrobial agents and Chemotherapy*, vol. 48: 231-234.
- Sampathukumar, P. (2007).** Methicillin-resistant *Staphylococcus aureus*: The latest Health Scare. *Moyo Clinical. Protection*, 82: 1403-1467.
- Taiwo, S.S., Bamidele, M., Akinside, K.A., Omonigbehin, E.A., Smith, S.I., Onile, B.A., Olowe, O.A. (2005).** Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Ilorin, Nigeria. *West Afrianc Journal of Medicine*. Vol 24: 2.
- Umar, U., Uba, A. (2004).** Incidence and antibiotic susceptibility patterns of *Staphylococcus aureus* from clinical specimens in Bauchi, Nigeria. Book of abstracts, in 26th Annual Conference of *Nigeria Society of Microbiology*. University of Uyo, Akwa-Ibom State, Nigeria.

THE ECONOMICS OF *MORINGA OLEIFERA* USE BY PEOPLE LIVING WITH HIV/AIDS (PLWHA) IN KWARA STATE, NIGERIA

^{1*} Animashaun, J.O., ²Williams, F.E., ³Ibrahim, H. and ⁴Toye, A.A.

¹Department of Agricultural Economics and Farm Management, P.M.B. 1515, University of Ilorin, Ilorin, Nigeria.

Department of Clinical Pharmacy and Pharmacy Practice, P.M.B. 1515, University of Ilorin, Ilorin, Nigeria.

³Department of Pharmacognosy and Drug Development, P.M.B. 1515, University of Ilorin, Ilorin, Nigeria.

⁴Department of Animal Production, P.M.B. 1515, University of Ilorin, Ilorin, Nigeria.

Corresponding author's e-mail address: Animashaun.jo@unilorin.edu.ng; real-s4u@yahoo.com

Phone no: +2348038550618

ABSTRACT

Despite widespread use and belief in the efficacy and affordability of Moringa plant among users, and anecdotes of its utility in treating HIV in Africa, little is known of the actual economics and attitudes to the use of Moringa oleifera for therapeutic benefits among People Living With HIV/AIDS (PLWHA) in Kwara State, Nigeria. To fill this knowledge gap, the

present study randomly sampled 167 respondents comprised exclusively of PLWHA who use the Moringa plant for therapeutic purpose in Kwara State, Nigeria. A questionnaire was designed and administered on the respondents in the study area. Findings revealed that respondents show a positive attitude to the use of the moringa plant, and the part consumed most by users is the leaf (82%). Majority of the respondents (72%) reported not paying money to have access to the plant as it is in most cases obtained free as a gift from friends and grown in the backyard. Among those who incurred cost in accessing the plant, the average amount paid was N1,163.00 (USD 7) per 200g. Respondents with higher monthly income are more likely to pay higher for the plant ($p=0.07$). The study concludes by recommending that clinical trials be intensified to examine the claims of the plant's therapeutic value in HIV management which underpin the relatively positive attitude towards its use by PLWHA. Furthermore, purified extract(s) of moringa phytochemicals needs to be isolated and examined for efficacy and affordability as drugs for managing HIV related pathology in PLWHA.

Keywords: *Moringa, HIV/AIDS, Affordability, Nigeria.*

INTRODUCTION

Ever since Nigeria first reported the first HIV/AIDS case in 1986, the country had taken several steps to limit the spread of the disease. In contrast to other African nations like South Africa and Zambia, the rate of prevalence of the disease is low in Nigeria (UNAIDS, 2010; UNDP, 2011). Nevertheless, HIV/AIDS related death and disease has had a significantly negative impact on the national economy; In 2009 alone, AIDS claimed the lives of approximately 220,000 people in Nigeria (UNAIDS, 2010). Furthermore, at an infection rate of 3.6 percent (UNDP, 2011; UN-GASS, 2010), an estimated 6 million people lived with the virus in Nigeria as at the end of 2013. With AIDS claiming so many lives and the possibility of claiming many more lives, there may be a need to consider alternative and adjunctive therapies in adopting new strategies for treatment and/or management of HIV/AIDS.

Meanwhile, certain plants have been recognized to possess the ability to synthesize a wide variety of phytochemical compounds that are used to perform important biological and defensive functions. Since time immemorial, these phytochemicals have been utilized by humans in the treatment of disease condition, and, according to the World Health Organization (WHO), 80 percent of the populations in the Asian and African countries make use of herbal medicine (ethno-medicine) for some aspect of primary health care (Edgar *et al.*, 2002). Advocates of herbal medicines posit that they possess reduced risk of side effects, are effective with chronic conditions, relatively affordable, easily available, culturally acceptable and are known to be relevant and vital in many areas in which there is an absence or inadequate provision of

modern medical services (Animashaun *et al.*, 2013; Williams *et al.*, 2013; Edgar *et al.*, 2002; Kudi and Myint, 1999).

Specifically there are reported cases of the use and benefits of herbal medicines in the context of treating HIV/AIDS related symptoms in Africa (Babb *et al.*, 2004; Peters *et al.*, 2004). Studies conducted in Malawi document the relative lower cost of use of Moringa for vitamin and mineral sources than any other plant sources (Babu and Chale, 1994). Though there is no clear scientific evidence of its nutrient and phytochemical availability or its effectiveness as a phytomedicine in the context of treating HIV/AIDS conditions for which it is routinely used (Sebit *et al.*, 2000), several related studies have, confirmed the use of Moringa for several health benefits and perception among users that it is effective in relieving symptoms of ill health (Williams *et al.*, 2013; Animashaun *et al.*, 2013). Equally, the same studies showed that such users were willing to pay more than current prices for moringa products in order to enjoy the health benefit they believe it gives.

However, as with other herbal therapies, the use of moringa for therapeutic purposes may not be without some potential adverse effect (Talalay and Talalay, 2001) and cost implications. In relation to this unclear therapeutic and cost effectiveness benefits associated with the use of the moringa plant, specifically by PLWHA, this study is designed to examine the cost implication and the attitudinal disposition to Moringa use by PLWHA. This is purposively done to ascertain the economics of use and perception of efficacy of the plant in its context of usage (HIV/AIDS management). Outcomes of the study will furnish requisite background knowledge for experimental studies on the therapeutic benefits of Moringa in people living with the virus.

This study therefore broadly examines the economics and perceived attitudes of users to the therapeutic benefits of Moringa among People Living With HIV/AIDS (PLWHA) in Kwara State, Nigeria, and specifically;

1. identifies the various forms of medications used by respondents,
2. identifies the Moringa parts used by respondents,
3. examines respondents' attitude and knowledge to the use of Moringa plants, and,
4. estimates the relative cost of Moringa use by respondents.

METHODOLOGY

The study was carried out in Kwara State, Nigeria. Kwara State is located in the North-Central geographical zone of Nigeria within latitudes 7° 45' N and 9° 30' N and longitudes 2° 30' E and 6° 25' E. The State comprises a mixture of urban and rural settlements. The study was descriptive and cross-sectional, and applied a pilot-tested questionnaire. The study population comprised people diagnosed with HIV/AIDS who are registered and receiving treatments across the HIV/AIDS Treatment Centers

in the study area. Specifically, a list of registered PLWHA patients was collected from identified HIV/AIDS treatment clinics in the study area, and out of the identified 8 HIV/AIDS clinic, 7 were used for this study and one clinic was used for pre-testing the questionnaire. In the first stage, 1000 respondents which comprised of PLWHA were randomly sampled across the 7 Treatment centers and were administered with a questionnaire that asked whether they consume or use the Moringa plant in addition to their regular medications for the treatment of HIV/AIDS. Because the study targets respondents living with HIV/AIDS that also use Moringa for treating HIV/AIDS, a second sampling stage involved the purposive selection of respondents that use Moringa plant. At the end of the sampling exercises, a total of 170 Moringa user respondents were selected of which 167 provided valid responses.

Data was collected between April and May 2013 by trained enumerators. The survey instrument assessed demographic details including sex, age, ethnicity, income, education, livelihood activity, therapies used for treating HIV/AIDS; Moringa use, perceived effectiveness); quantity and frequency of Moringa purchase per month, parts of Moringa plants used, cost per unit of Moringa used, other therapies used (conventional/herbal medicines)/, and the respondent's attitude toward Moringa use.

The survey instrument/questionnaire was designed in English and translated into native languages of the respondents who did not understand English language. Attitudes of respondents to the use of moringa plant were assessed using a 5-point Likert type item questions. Responses generated from the questionnaire were coded and subsequently analysed by use of descriptive and inferential statistical methods.

RESULTS AND DISCUSSION

Socio-Demographic Distribution of Respondents

The result of the demographic characteristics of the respondents is presented in Table 1

Table 1: Socio-demographic Distribution of Respondents

Variables		Frequency	Percentage
Ethnicity	Yoruba	99	61.1
	Hausa	24	14.8
	Igbo	18	11.1
	Other (tribe)	26	16
Sex	Male	43	23.9

	Female	124	76.1
Age	18-38	51	30.5
	39-59	101	60.1
	60>	15	9
Mean Age	43		
Marital status	Single	19	11.4
	Married	122	73.1
	Divorced	24	14.4
	Widowed/Widower	2	1.2
Education	No formal	27	16.2
	Adult	2	1.2
	Primary	24	14.4
	Secondary	46	27.5
	Arabic	23	13.8
	Tertiary	45	27.2
Livelihood	Artisan	67	40.1
	Agriculture	16	9.6
	Private salaried job	17	10.2
	Public service	36	21.6
	Student (tertiary level)	5	3
	No response	26	15.6
M o n t h l y income (Naira)	< 18,000	51	31
	18,000-36,000	64	38
	36,100-54,000	42	25
	54,100-72,000	4	2.4
	72,100-90,000	3	1.8
	91,000-108,000	1	0.6
	>108,100	2	1.2

Source: Field Survey, 2013

As revealed in Table 1, the ethnic composition of the respondents indicates that majority are Yoruba (61%), followed by Hausa/Fulani (14%) and Igbo (11%). The composition of other ethnicities is 16%. The apparent larger representation of the Yoruba ethnicity may be related to the demographic composition of the population characteristics of the study area. In addition, female sex appears to be more represented (76%) than male (23.9%). With regard to the age distribution of the respondents, the age range of 39-59 years (60%) occupied the modal age whilst the average age was 43 years. Majority of the respondents were married (73%) and had one form of formal education (84%). About 38% of the respondents reported a monthly income in the range of N18,000-N36,000..

Frequency of Respondents' Use of Prescribed Medical Practices for the Treatment HIV/AIDS

The result of the distribution of respondents' frequency of use of prescribed medical practices for the treatment of HIV/AIDS is presented in Figure 1.

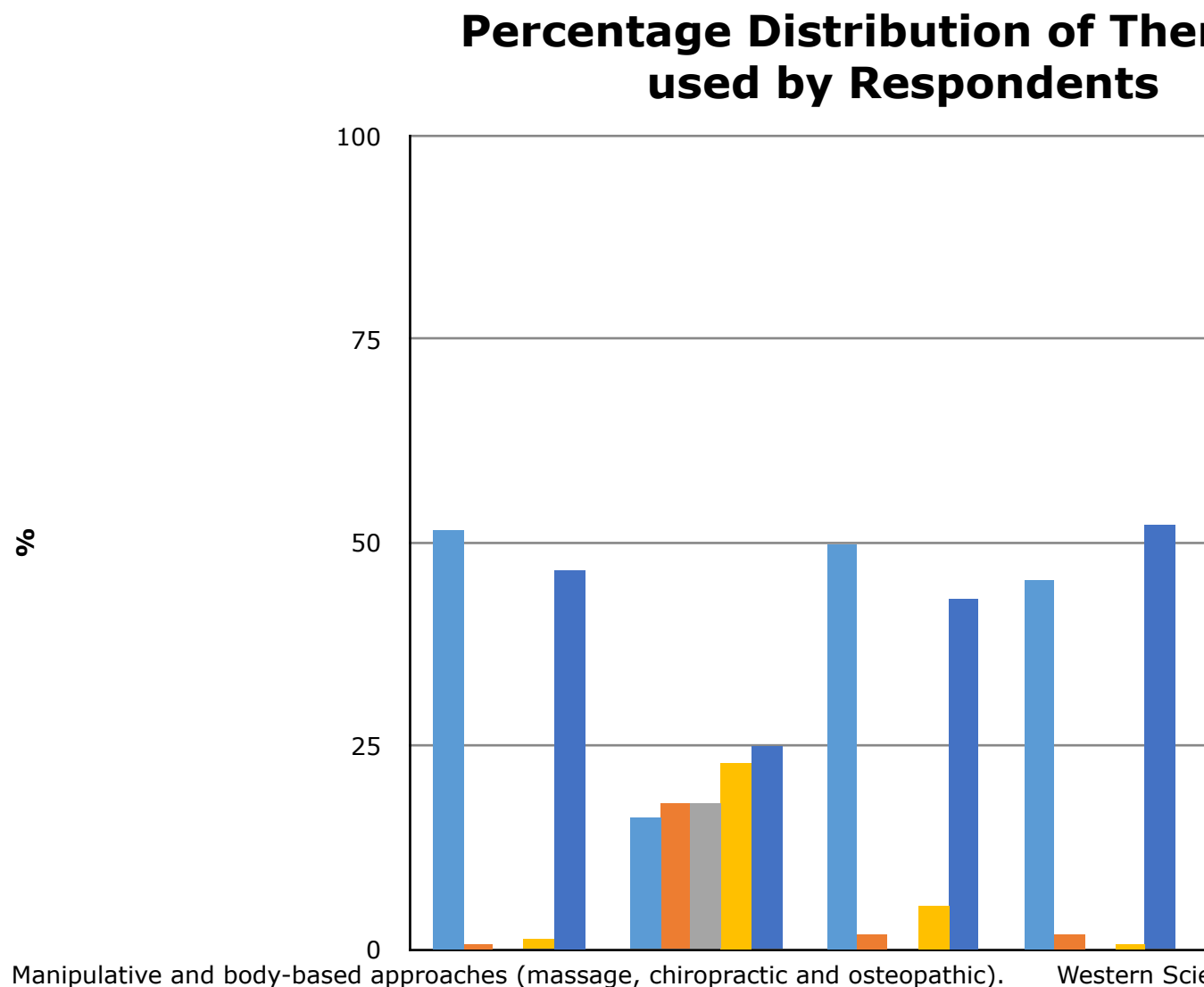


Figure 1: Distribution of Respondents' Use of Prescribed Medical Practices

As shown in the Figure, all of the respondents (PLWHA that also use Moringa) indicate the use of western prescribed medical therapy in the treatment of HIV/AIDS. This is however not unexpected given that the sampling of the respondents was carried out in approved medical HIV/AIDS clinics. The study also showed that 22% of PLWHA that use Moringa and Western prescribed medical therapy did use mind-body spiritual and meditative medicine also.

Unlike observed for Western medicine and Mind-body Spiritual therapy, use of Manipulative therapy, Acupuncture, and Energy Medicine was less frequently represented in the respondents. (Figure 1). This may be due to the relative unavailability of these methods in the study area.

3.3 Moringa Parts Used by Respondents

The distribution of the moringa plant parts used by respondents is presented in Figure 2.

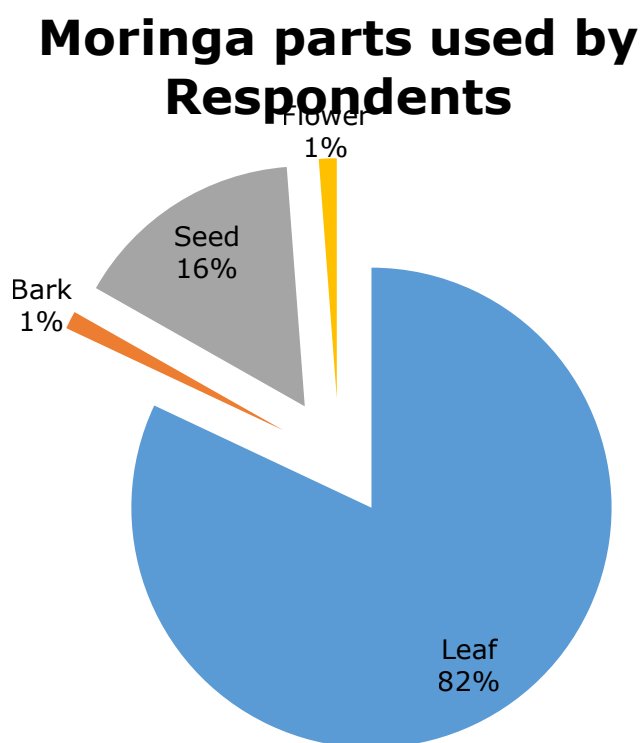


Figure 2: Distribution of the Moringa Plants Parts Used by Respondents

According to Figure 2, 82% of the respondents report the use of Moringa leaf and 16% report the use of the seed. The apparent inclination of the use of these parts of the plant may signify the perceived benefits in terms of the phytonutrients contained in these parts than the others, though it may simply reflect relative availability and convenience of use. Indeed, as reported in other studies, moringa consumers are more likely to consume the moringa leaf than any other part of the plant (Williams et al, 2013). Studies have also investigated and reported that the moringa leaf have the

potentials of being a nutraceutical; an immunity booster, fertility and reproductive health booster and can also serve as nutritional supplement (Animashaun *et al.*, 2013; Adejumo *et al.*, 2012; Anwar *et al.*, 2007; Fahey, 2005).

Attitude of Respondents to the Use of Moringa

The distribution of respondents response to item questions measuring attitudes to the use of moringa is presented in Figure 3.

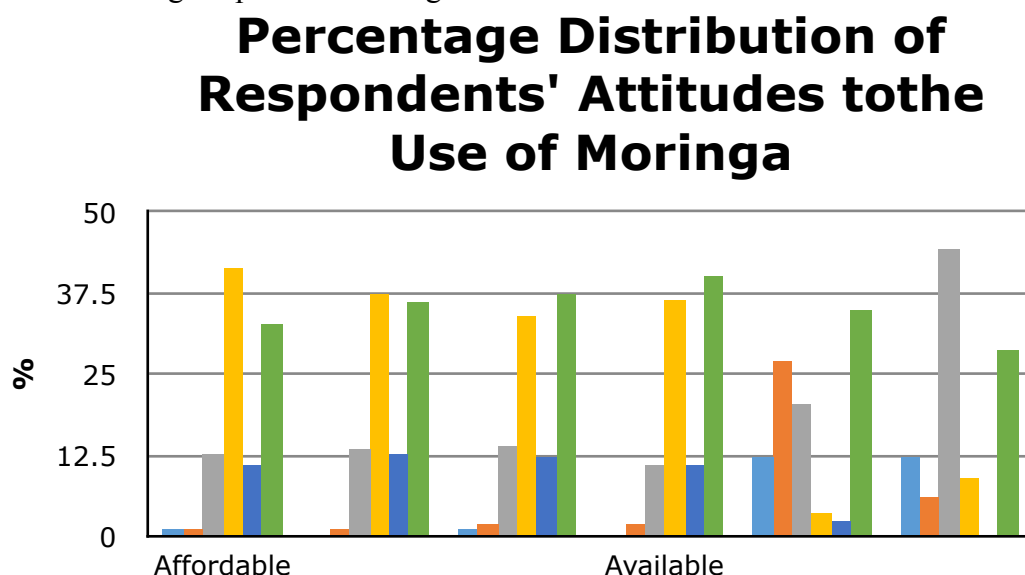


Figure 3: The Distribution of Respondents' Response to item questions Measuring Attitudes to the use of Moringa in the study area.

As indicated in Figure 3, more than half of the valid responses received from respondents for the perception of the affordability, safety, and effectiveness and on availability of the moringa herbal remedy are in the Agree and Strongly Agree categories. Interestingly, however, 44.3% of the valid 77% responses recorded for lack of knowledge of the exact dosage to use suggest that a higher proportion of moringa users are not really sure whether not knowing the exact dosage of moringa to use is a deterrent to the use of the Moringa plant. And more than half of the valid response disagreed that Moringa interferes with the biological functioning of the other prescribed drugs.

The distribution of respondents' attitude to the use of moringa suggests a favorable disposition towards the plant by PLWHA. However, since this study is merely descriptive in nature with no test of significance of the responses, the implication suggested by these results may have to be taken with caution.

Relative Cost Implication of use of Moringa among PLWHA

The ranges of price paid by respondents in accessing a 200g of Moringa powder is presented in Table 2.

Table 2: Distribution of Price Paid by Respondents per 200 g of Moringa

Ranges of Price paid per 200g of Moringa powder (Naira)	Frequency	Percent
Free (Gift, Grown in the backyard garden)	121.0	72.5
less than 500	11.0	6.6
510-1000	9.0	5.4
1001-1500	5.0	3.0
1501-2000	6.0	3.6
above 2000	2.0	1.2
Average Cost= N1,163.36		

Source: Field Survey, 2013

As shown in Table 2, the average price reported paid by respondent in accessing 200 g of Moringa is N1,263.00. Furthermore, about 6% of the 27% of respondents who paid for the moringa used less than N500 to access the moringa plant and 5% paid between N500 to N1,000. However, about 72% of the respondent reported not having to pay to have access to the plant as it is usually grown as a backyard garden or received as a gift from friends and form neighbors.

Furthermore, the study examined the relationship between respondents' monthly income earnings and amount spent on Moringa. The result is presented in Table 3.

Table 3: Correlation Between Respondents' Expenditure on Moringa and Monthly Income

Monthly Income	Total Cost spent on Moringa purchased
Monthly Income	0.176*

*Significance (2-tailed) level at 10% p value

As revealed in the Table, there is a significant positive linear relationship between respondents monthly income and amount expended on the purchase of moringa ($p=0.07$). The implication of the relationship between these two variables is that respondents who earn higher income are more likely to pay more to have access to moringa plant.

CONCLUSION AND RECOMMENDATION

This study examined the attitudes and the relative cost implication of the use of Moringa by PLWHA who also use Moringa in Kwara State, Nigeria. The study collected information from Moringa users with the use of a structurally designed questionnaire. Results showed that the three major ethnicities in the country are well represented in the study, with a majority of them being Yoruba tribe found majorly in the South-West region of the country. Furthermore, majority of the respondents are married, possess a formal form of education and earn at least N18,000 per month. All of the respondents reported using western medical therapies and this is followed by the mind-body spiritual and meditative medicine. However, the use of manipulative therapy, acupuncture, and energy medicine appear relatively rare among respondents.

More respondents reported using moringa leaf than other parts of the plant, and this is followed by use of seeds. More than half of the responses on perception of the affordability, safety, effectiveness and availability of moringa herbal therapy are in the Agree and Strongly Agree categories. And more than half of the responses disagreed that Moringa interferes with the biological functioning of prescribed drugs (Western medicine).

The average cost of 200 g of Moringa was N1,163.00, and about 72% of the respondents reported not having to pay to have access to the plant as it is usually grown as a backyard garden plant or received as a gift from friends and from neighbors. Finally, the study found that a positive correlation exists between amount earned by respondents and expenditure paid for Moringa.

In view of these findings, this study recommends that clinical trials be intensified to examine the claims of the plant's therapeutic value in HIV management which underpin the relatively positive attitude towards its use by PLWHA. Furthermore, purified extract(s) of moringa phytochemicals needs to be isolated and examined for efficacy and affordability as drugs for managing HIV related pathology in PLWHA.

REFERENCES

- Adejumo, O. E., Kolapo, A. L. and Folarin, A. O. (2012).** Moringa oleifera Lam. (Moringaceae) grown in Nigeria: In vitro antisickling activity on deoxygenated erythrocyte cells. *J Pharm Bioallied Sci.* Apr; 4(2): 118-122.
- Animashaun J.O Williams F.E. and Teye A.A. (2013).** Towards Validating Moringa's Nutraceutical Benefits: Examining Consumers' Perspectives vis-à-vis Health Satisfaction and Willingness to Pay. *Journal Agri on-line Papers in Economics and Informatics Vol V (2)* pp 11-21
- Anwar F., Latif S., Ashraf, M. and Gilani, A. H. (2007).** Moringa oleifera: a food plant with multiple medicinal uses. *Phytother Res.* Jan: 21(1): 17-25

- Babb, D.A., Pemba, L., Seatlanyane, P., Charalambous, S., Curchyard, G., and Grant, A.D. (2004).** Use of traditional medicine in the era of antiretroviral therapy: experience from South Africa. *eJIAS, MedGenMed*, **6**:B10640
- Babu SC., and Chale, B. (1994).** Indigenous vegetables for rural nutrition: An evaluation of Moringa in Salima. BUNDA/IFPRI Working Paper No. 6. Lilongwe: BundaCollege of Agriculture.
- Edgar J. D., Elias, B., and Adnan, B., (2020).** Biotechnology and the developing world. *Electronic Journal of Biotechnology* **5** (1).
- Fahey W. J. (2005).** Moringa oleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic and Prophylactic Properties. Part 1. Trees for Life Journal. 1:5.
- Kudi, A.C., and Myint, S.J., (1999).** Antiviral activity of some Nigerian medicinal plant extracts. *Journal of Ethnopharmacology*, **68**:289-294
- Peters, E.J., Immananagha, K.K., Essien, O.E., and Ekott, J.U. (2004).** Traditional healers' practices and the spread of HIV/AIDS in south eastern Nigeria. *Trop Doct* 2004, **34**:79-82.
- Sebit, M.B., Chandiwana, S.K., Latif, A.S., Gomo, E., Acuda, S.W. MAKoni, F. and Vushe, J. (2000).** Quality of Life evaluation in patients with HIV-I infection: the impact of traditional medicine in Zimbabwe. *Cent Afr J Med.*, **46**(8): 208-13
- Talalay, P and Talalay, P. (2001).** The importance of using scientific principles in the development of medicinal agents from plants. *Academic Medicine* **76** (3): 238–47.
- UNAIDS, (2010).** UNAIDS report on the global AIDS epidemic. Accessed on 24/3/2014 from http://www.unaids.org/globalreport/Global_report.htm
- UNDP, (2011).** Human Development Report 2011. Accessed on 24/3/2014 from http://www.beta.undp.org/undp/en/home/librarypage/hdr/human_developmentreport2011.html
- Williams, F.E., Animashaun, J.O., Toye, A.A and Ibrahim, H., (2013).** Use and Determinants of Adoption of Moringa Products for Nutraceutical Benefits in Ilorin-South Local Government Area of Kwara State, Nigeria. *Journal of Agricultural Research and Development*. Vol.13 no 1

