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Nitrogen Maintenance and Blood Constituents of Rats Fed Rat Chow Supplemented with 2 Levels of Dry Moringa Oleifera Leaf Extract

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ABSTRACT

This study evaluated the nitrogen maintenance and blood constituents of rats who received 2 levels of dry Moringa leaf extract. The proximate, mineral, vitamin and phytochemical composition of the extract were determined. Twelve albino rats were used for the study. Each group consisted of four rats and they were allotted on the basis of body weight so that the difference between two groups will not be more than 5g. The first group (A) was fed dry Moringa oleifera leaf extract (10% protein level) and a commercial animal feed whose iron, protein and vitamin A composition was known. Group B was fed dry Moringa oleifera leaf extract (at 5% protein level) and the commercial animal feed while group C was fed the commercial animal feed and water only and this group served as the control. Means, Standard deviation and One Way Analysis of Variance were used to analyse the data. The results showed that the ash, crude fibre, fat and moisture contents of dry Moringa oleifera were 0.04, 0.00, 0.001 and 96.68%, respectively. The protein and carbohydrate contents were 0.66 and 2.63%, each. Iron and calcium were 2.07 and 33.35mg, each. Vitamin C and beta-carotene contents were 6.26mg and 223RE, each, while flavonoids and alkaloids contents were 0.20 and 0.07%, respectively. Dry Moringa oleifera leaf extract maintained positive nitrogen balance in rats. Except in the nitrogen balance, all the parameters tested (serum iron, protein and vitamin A) proved superior in the test groups and differed significantly from the control groups ($p < 0.05$).

KEYWORDS: Leafy vegetables, bioavailability, leaf extract and Moringa oleifera

INTRODUCTION

Vegetables are an essential part of the human diet. It is also a major source of biologically active substances such as vitamins, dietary fiber, antioxidants, and phytochemicals. It is known that eating a diet rich in an abundance of fruits and vegetables was associated with the prevention and/or treatment of at least four of the leading causes of death in the developing countries. Vegetables are excellent sources of complex carbohydrates, dietary fibre, and several vitamins and minerals. Fruits and vegetables contain phytochemicals which have a unique protective effect on health (Lydia, 2006).

Moringa oleifera is the best known of the thirteen species of the genus *Moringacae*. It is native to India but has been planted around the world and naturalised in many locales (Martin, 2007). In Nigeria, it is mostly grown in the northern part and locally known as *Zogeli* among the Hausa speaking people. The Yoruba of south-west Nigeria calls it 'ewe ile' or 'igi iyaanu' because of its medicinal uses (Saalu et al., 2011).

Moringa oleifera has in the past two decades been advocated as an excellent source of essential nutrients (protein, iron, calcium, vitamins, carotenoids and other phytochemicals) (Fahey, 2005). One tablespoon of leaf powder provides 14% protein, 40% calcium, 23% iron and most of the Vitamin A needs of a child aged 1-3 (Martin, 2007). An aspect of the plant *Moringa oleifera* which has not been fully explored is the extent to which these nutrients contained in the plant are bioavailable. An understanding of bioavailability is important because consumers change their dietary patterns for reasons of health and economics. Hence, knowledge of bioavailability may affect their choices (Mike, 2011).

In recent decades, malnutrition was reported to be the leading cause of deaths especially in under-five children in developing and under-developed countries. Among the various forms of malnutrition, micronutrient deficiency (iron, iodine, vitamin A and zinc deficiencies) and Protein energy malnutrition (PEM) is of public health concern.
Studies in Nigeria revealed that 20.5% of children had PEM according to World Health Organisation/National Centre for Health (WHO/NCHS) standards (Odunaya, 2006). It is also estimated that about 1.62 billion people are iron deficient which corresponds to 24% of the population (Francis, 2010). Similarly, 24.9% of children in Nigeria suffer from vitamin A deficiency (Francis, 2010). The plant, *Moringa oleifera* has been used to combat malnutrition, especially among infants and nursing mothers (Fahey, 2005). This work therefore aims to:

I. determine the proximate composition of dry Moringa oleifera leaf extract.
II. determine the mineral (iron, calcium), and Vitamins (Vit A and C) composition of dry Moringa oleifera leaf extract.
III. determine the phytochemical (flavonoids and alkaloids) composition of dry Moringa oleifera leaf extract.
IV. assess nitrogen maintenance and blood (iron, protein and vitamin A) constituents of rats fed 2 levels of dry Moringa oleifera leaf extract.

MATERIALS AND METHODS

Plant materials: The leaves of *Moringa oleifera* were collected from home garden in the University of Nigeria, Nsukka campus, Nigeria.

Sample preparation: The leaves of *Moringa oleifera.* were shade-dried for three (3) days and were subsequently ground to powder using household blender.

Sample extraction: Two grammes of dry *Moringa oleifera.* leaf powder was soaked in 200ml of boiling water for 30 minutes after which the solution was drained using a 1-mm mesh. The extract was used to determine its chemical composition.

Chemical analysis: Total nitrogen (N) was determined using the micro-kjeldahl method (Harbone, 1973). The crude protein was obtained by multiplying N by the conversion factor of 6.25 (%p = TN x 6.25). The fat content of the sample was determined using the method of AOAC (1995). The ash content of the sample was determined using AOAC (1995) method. Moisture content of the sample was determined using the method described by Pearson (1976) and total carbohydrate content was determined by difference as follows: 100 – (% ash + protein + fat + moisture). The iron (Fe) content was determined using the Phenanthroline method AOAC (1995). The calcium (Ca) and ascorbate contents of the sample was determined using the AOAC (1995) method. Beta-carotene was determined according to Pearson (1976) method. Flavonoids and alkaloids contents were determined using Bohm and Kocipai-Abyazan and Harborne (1973) methods.

Balance study (bioavailability study)
Twelve rats were allotted to three groups and used in this study. Each

group had four rats on basis of body weight alloted in individual metabolic cages. The rats were alloted such that the difference in body weight between the groups were not more than 5g. These cages are equipped with steel bottom to separate urine and faeces. The rats were fed a commercial diet for rodents for 4 days to acclimatize them to both the diet and environment. Subsequently, the rats were fed their respective diet and fluid ad-libitum for 9 days. The daily food intakes were recorded to calculate their food intake.

Rat chow was the main source of nutrient for a 12-day balance study. The first group was fed rat chow and water ad libitum and served as the control. The second group was fed rat chow and dry *Moringa oleifera* leaf extract to supply 5% protein. The third group was also fed rat chow as well as dry *Moringa oleifera* leaf extract to supply 10% protein.

Urine samples were collected daily for seven days. Drops of 0.01N HCL were added to each urine sample daily to prevent loss of ammonia. The urine samples were stored in refrigerator until analysed for urinary nitrogen (N). Faeces of individual rats were pooled and dried for 12 hours, weighed prior to grinding into fine powder and stored for faecal N determination. At the end of the balance study, blood samples of the rats were collected from the ocular vein using blood collecting tubes for serum protein, iron and vitamin A determination.

Statistical analysis

Means, standard deviation and standard error of the mean were calculated for two determinations. One way analysis of variance (ANOVA) and Duncan Multiple Range Test was used to separate and compare means (Steel & Torrie, 1980) ($p < 0.05$).

RESULTS

Table 1: Proximate, micronutrient and phytochemical composition of dry *Moringa oleifera* leaf extract (% per 100ml)

Moringa oleifera	
Moisture (%)	96.68± 0.028
Protein (%)	0.66±0.003
Fat (%)	0.001±0.000
Ash (%)	0.04± 0.001
Crude fibre (%)	0.00±0.000
Carbohydrate (%)	2.63± 0.021
Iron (mg)	2.07± 0.049
Calcium (mg)	33.35 ±0.919
Beta-carotene (RE)	223 ±5.657
Vitamin C (mg)	6.26 ± 0.028
Flavonoids (%)	0.20 ± 0.035
Alkaloids (%)	0.07 ± 0.00

Mean ± SD of two determinations

Table 1 presents the proximate, micronutrient and phytochemical composition of dry *Moringa oleifera* leaf extract (% per 100ml). The moisture content of dry *Moringa oleifera* leaf extract was high (96.68 %) and protein was 0.66%. Fat, ash, carbohydrate and crude fibre values for the leaf extract were 0.001, 0.04, 2.63 and 0.000%, respectively.

Micronutrients and phytochemical values for the leaf extract were 2.07mg iron, calcium 33.35mg, beta-carotene 223RE, and vitamin C 6.26mg. Flavonoids content and alkaloids values were 0.20% and 0.07%, each (Table 1).

Table 2: Mean weight gain, food, fluid and nitrogen intake, faecal and urinary nitrogen and nitrogen retention and biochemical content

	Commercial feed Group B and M. oleifera at and (10% protein level)	Commercial feed Group C (M. oleifera at commercial 5% protein level feed + water)	
Weight gain (g)	18.25±12.28	26 ± 4.76	16±10.23
Food intake (g)	62.5±5.05	55±8.67	63.38±7.35
Fluid intake (ml)	92.75±8.45	94.88±9.35	110.75±13.06
Nitrogen intake (g)	2.02±0.50	1.76±0.27	1.72±0.20
Faecal nitrogen (g)	0.05±0.038	0.05±0.05	0.07±0.057
Urinary nitrogen (g)	0.06±0.088	0.01±0.003	0.07±0.003
Nitrogen balance (g)	1.92±0.45	1.70±0.264	1.58±0.557
Serum protein(g)	3.66±0.116	3.66±0.003	2.56±0.018
Serum iron (mg)	0.66±0.000	0.77±0.004	0.49±0.002
Serum vitamin A (mg)	1.32±0.004	1.33±0.003	0.92±0.002

Mean ± SD of four determinations

Table 2 shows that there was difference in mean weight gain among the groups of rats and the control. Rats in group B that had 5% leaf extract had the highest weight gain (26.00g). The group of rats (A) that had 10% supplement had 18.25g and rats in group C had 16.00g weight gain. Food intake of rats fed rat chow and 5% extract had the least intake (55g). The control and the group A with 10% supplement had comparable values 63.38 and 62.50g, each which were higher than the B group. Weight gain for control and group A rats were comparable and lower ($p < 0.05$) relative to those in group B (16.0 and 18.25 vs. 26.00g). The mean fluid intake of rats in groups A, B and C were 92.75, 94.88 and 110.75ml, each. Rats in groups A and B had comparable ($p > 0.05$) values (92.75 and 94.88ml). On the other hand, rats in group C (control) had much more fluid intake ($p < 0.05$) relative to the other two groups (110.75ml vs. 92.75 and 94.88ml). Nitrogen intake of rats in group A was the highest (2.02g), group B had 1.76g and group C had 1.92g. The three groups of rats had positive nitrogen balance of 1.92, 1.57g and 1.51g, each. This was because their faecal and urinary N outputs were low 90.05 and 0.079). There was slight difference among the nitrogen balance of the control and the test groups. The differences were not significant ($p > 0.05$). The mean serum iron for rats in groups A, B and C were 0.66, 0.77 and 0.49mg, each. Control group had the lowest (0.49mg) relative to 0.66 and 0.77mg. The serum iron level for rats in group A differed from those in group B ($P < 0.05$) (0.66 vs. 0.77mg). The serum iron for rats in group C differed from the test groups ($P < 0.05$). The serum protein level for the control group was the lowest (2.56g). Group B had 3.66g and group A had 3.66g, each. The serum protein levels for rats in group C differed significantly from the test groups ($p < 0.005$). The rats in groups A and B had similar serum protein levels ($p > 0.05$) (3.66mg). The serum vitamin A level for rats in groups A and B were 1-32mg and 1.33mg. Group C had 0.92mg. The serum vitamin A level for rats in group C differed from those of the test groups ($p < 0.005$).

Discussion

The low dry matter (protein, fat, ash, fibre and carbohydrate) was not a surprise. It is a common phenomenon that foods that have high moisture would have low dry matter Johnson and Pace, 2010). The slightly higher weight gain, fluid intake, serum iron and vitamin A intake indicated that 5% supplementation had an edge over 10% as well as the control (Table 2). On the other hand, the lower food and N intakes, N retention and iron and beta-carotene intake meant that 5% Moringa oleifera supplementation did not have mutual supplementation effect with rat chow. The lower values except for food and fluid intakes for control rats (table 2) meant that the test diets were superior to the control diet. On the other hand, the higher food, N intakes as well as N retention or balance and serum iron and beta-carotene values for rats fed chow with 10% supplementation might be associated with palatability. It is shown that adequate dietary protein increases palatability. Rats consume much more foods when they are palatable.

Rats in group B had the highest nitrogen balance because 96% of nitrogen ingested was retained irrespective of its reduced food intake. Rats in group A retained 95% of its nitrogen intake while rats in group C had the lowest nitrogen balance. Nitrogen loss was lowest in rats in group

B and was the highest in rats in the control group. This work confirms the report of Foidl et al. (2001), who reported that *Moringa oleifera* has high quality protein which is easily digested and that is influenced by the quality of its amino acids. *Moringa oleifera* leaves contain all the eight essential amino acids and 19 of the 20 prominent amino acids (Fahey, 2005).

The serum protein levels of rats in groups A and B were comparable (3.66g), irrespective of the lower food intake of rats in group B. Rats in group B competed favourably with those in group A because it consumed more of the extract of *Moringa oleifera* containing high quality protein. The serum protein level of rats in the control was the lowest and differed significantly from the test groups ($p < 0.001$). This is surprising because commercial poultry feeds have been reported to contain animal proteins such as meat and bone meal, blood meal and poultry by-product meal (FAO, 2002). Supplementation of these commercial poultry feeds with dry *Moringa oleifera* improved the serum protein concentration of the test groups more than the control. This is similar to the report by the Trees for Life organisation, that the protein quality of *Moringa oleifera* leaves rivals that of milk and eggs (Fahey, 2005).

The serum iron level of all three groups of rats was low compared to their intakes. However, the serum iron level of rats in the control was the lowest and differed significantly from that of the test groups ($p < 0.015$). Similarly, the serum iron level of rats in group A differed significantly from that of the rats in group B ($p < 0.03$). The low serum iron levels in the three groups of rats may be due to poor absorption of dietary iron. This confirms the reports of the study carried out by Idohou et al., (2011) who found out that daily consumption of *Moringa oleifera* dried leaf powder did not improve iron status in anaemic lactating women but prevented significant weight loss. The results of this work showed that the bioavailability of iron in dry *Moringa oleifera* leaf extract ranged from 4-5%. This is higher than the 2.2% bioavailability reported by (Ndong et al., 2001). It is known that increased calcium, fibre and polyphenols intake reduces iron bioavailability and these substances are found in *Moringa oleifera* leaves in high quantities. However, the improved bioavailability of iron in dry *Moringa oleifera* leaf extract may be attributed to the low fibre and reduced calcium content of the extract relative to the dried leaf powder.

The serum vitamin A levels of the three groups of rats were high although that of the control group was the lowest and differed from the test group significantly ($p < 0.001$). The serum vitamin A levels of rats in groups A and B were comparable and there was no significant difference between them ($p > 0.05$). Similarly, a study carried out by (Nambiar & Seshadri, 2001) using a rat model reported that rats receiving *Moringa oleifera* increased their food intake and weight gain and had significantly higher serum and liver vitamin A levels compared to rats given either synthetic vitamin A or vitamin A adequate diets.

Conclusion

This study has shown that supplementation of foods using dry *Moringa oleifera* leaf extract result in weight gain and improved nutritional status. It has also revealed that the proteins in dry *Moringa oleifera* leaf extract is of high biological value and maintains positive nitrogen balance. Dry *Moringa oleifera* leaf extract also contains bioavailable beta-carotene. However, only 3-5% of iron contained in dry *Moringa oleifera* leaf extract is bioavailable. Dry *Moringa oleifera* leaf extract may serve as an economically and suitable nutrient-rich food option, especially in the developing countries.

Recommendations

It is recommended that *Moringa oleifera* leaves be considered as a source of supplementation in households' diets. Intervention programmes have to be designed and investigated and health workers in disadvantaged communities could be educated on the attributes of *Moringa oleifera* so that these health workers could in turn educate the mothers of malnourished children and the general public. This is an inexpensive and natural method of alleviating malnutrition.

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