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PROTEIN QUALITY AND PATHOLOGICAL EFFECTS OF *Moringa oleifera* (*Syns Moringa pterygosperma* Gaertn) SEED AND HUSK ON ALBINO RAT ORGANS

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ABSTRACT

Moringa oleifera (Syns. Moringa pterygosperma Gaertn) is a multi-purpose useful tree whose leaf is used as vegetable, and the bark, stem and root used in medications. The seed is normally used as coagulant in water treatment with no documented information about its short or long term pathological effect on people. Seed cakes and husks are popular animal feeds especially in the Tropics, hence *Moringa oleifera* seed and husk were analysed for proximate composition and protein quality using standard methods of analysis of AOAC and weanling albino rats respectively. The histopathological effects of the prepared diets on rat organs were also studied. The seed and husk contained 18.6 and 11.2g crude protein, 6.8 and 1.1g crude lipid, 4.9 and 7.0g ash, 59.4 and 62.9g carbohydrate, 3.1 and 17.7g crude fibre, and 382.6 and 370.7 Kcal gross energy per 100g sample respectively. The seed husk protein supported rats growth at 10% level of inclusion, their weight gain being comparable with that of control group, while there was reduction in feed intake and weight loss in rats fed with the seed. Histopathological study revealed that there was no conspicuous lesion on the heart, kidney, liver and intestine of the rats on all diets, showing the safety in their consumption. *Moringa oleifera* seed and husk can serve as good source of protein of high biological value to animals especially the ruminants, and hence to humans. The use of its seed as water coagulant seems to be safe.

Keywords: *moringa oleifera*, seed, husk, protein quality, histopathology, rat organs.

INTRODUCTION

There is continuous global search for new food sources to alleviate deficiencies of micronutrients, protein, energy, and the prevention of nutritionally associated diseases of obesity, diabetes, cancers, and coronary heart disease. Dietary protein supply raw materials for the formation of digestive juices, hormones, plasma proteins, haemoglobin and enzymes, as well as functioning as buffers, helping to maintain the pH of various media such as plasma, cerebrospinal fluid, intestinal secretion at constant level, and aiding transport of nutrients [6].

Wild plant fruits constitute an important food source for native people following traditional lifestyles [17]. The fruits, seeds and leaves of many of these wild plants already form common ingredients in a variety of traditional native dishes for the rural populace in developing countries [15]. Due to high cost of obtaining good quality protein of animal origin and the importance of dietary fibre which is readily available in fruits and pulps [9, 23], there is need to source for them through wild fruits, of which *Moringa oleifera (Sysn. Moringa pterygosperma Gaertn)* fruit is one.

Moringa oleifera (Drumstick, Radish tree) plant has been used to manage many ailments such as an antihelmitic (leaf), a diuretic, antifebrile, anti-diarrhoea, and anti-asthma (bark) [3]; and its seed powder is used as flocculating agent in water purification. Most food plants contain toxic chemicals although their concentrations are often too low to present any actual hazard. Some food-borne toxicants exert their action by interfering with nutrient availability and utilization, accompanied usually with damage to the gastro-intestinal tract (GIT) and pathological lesions to organs [22].

The husk of sheabutter *(Butyrospermum paradoxum Geartn. f.)* fruit pulp was reported to remove toxic metals from waste water by binding to them [11], while the seed and husk of *Moringa oleifera* were reported to be high in crude fibre and protein; and may be promising sources of plant protein especially for ruminants and non ruminants [1, 2].

Annongu *et al*, [4] studied toxicological effects of native and industrial fermented Shea butter cake in nutrition of broilers and found poor morphology, feed intake; growth and feed conversion efficiency in all chickens fed on unfermented cake, and linked these to the toxicity of the cake. Significant decrease in blood cellular constituent was also reported, associating it to presence of saponins or tannins in untreated cake. Ingestion of saponins of Shea butter kernel cake diet was reported to cause induced liver congestion, focal necrosis and partial alterations in hepatic morphology, with pronounced rise in blood values for certain transaminases [5]. This study was therefore carried out to determine the protein quality and possible pathological effects of feeding *Moringa oleifera* seed and husk on albino rats' organs.

MATERIALS AND METHODS

Dry pods of *Moringa oleifera* fruit were obtained from and identified at Forestry Research Institute of Nigeria (FRIN) Jericho, Ibadan, Oyo State, Nigeria. The pods were shelled and winnowed to obtain the fruit, which was in turn shelled to obtain the seed. Both seed and seed husk were separately ground to powder using warring blender, and then analysed in triplicate for moisture, crude protein, lipid, fibre; and ash using standard methods of Association of Official Analytical Chemists [7]. Their carbohydrate content was found by difference. Gross energy was determined using Cal 2k - Eco ballistic bomb calorimeter (Manufacturer: TUV Rheinland Quality Services (Pty) Ltd, South Africa).

Protein quality determination

Four iso-caloric diets of which three sets were iso-nitrogenous comprising basal (0% protein), seed (10% protein), husk (10% protein) and lactalbumin (control) (10% protein) were prepared. Twenty-four weanling albino rats of Wistar Strain breed of 24-day old were purchased from Physiology Department, University of Ibadan, and were housed individually in metabolic cages at the animal house of Department of Human Nutrition, University of Ibadan, Nigeria. The rats were fed '*ad libitum*' with commercial rat pellets and clean tap water for seven days, weighed and then distributed to four groups of six rats each using systematic random sampling procedure based on their weight.

Ten grammes of prepared diet was supplied each rat on daily basis and their water was changed every other day for 14 days. The left-over of the diets were collected and weighed on daily basis. The rats were weighed on weekly basis throughout the period of the experiment [8, 16]. Feacal droppings of the rats were collected for the last seven days of the experiment, dried at 85^{o}C to constant weight, and analysed for nitrogen and gross energy. The rats were sacrificed on the twenty-first day of experiment using anaesthesia, and their internal organs (heart, kidney, liver and intestine) were extracted and subjected to histopathological study at the Histopathology laboratory of the Department of Veterinary Pathology, University of Ibadan. Analysis of variance (ANOVA), Student t-test, and correlation statistics were carried out on the results obtained and the level of significance was set at $p < 0.05$.

RESULTS

The proximate composition of *Moringa oleifera* seed and husk showed that they had low moisture and crude lipid content, relatively high in protein, high in ash and very high in carbohydrates and crude fibre compared with many other fruits and seeds (Table-1). The seed was higher in protein, crude lipid and energy, while the husk was higher in carbohydrates and crude fibre. Table-2 shows the rats diet composition, their gross energy and nitrogen content. The four diets were iso-caloric while diets 2, 3, and 4 were iso-nitrogeneous. There was highly significant difference ($p < 0.05$) in rats' feed intakes within and between the groups on daily basis and at the end of feeding experiment (Table-3). The rats on seed diet consumed the least while those on control diet had highest value of intake, closely followed by husk diet.

Table-1. Proximate nutrient composition of *Moringa oleifera* seed and husk (g/100g).

	Seed	Husk
Moisture	7.2 ± 0.02	8.8 ± 0.01
Crude Protein	18.6 ± 0.21	11.2 ± 0.01
Crude Lipid	6.8 ± 0.01	1.1 ± 0.01
Crude Fibre	3.1 ± 0.01	17.7 ± 0.02
Ash	4.9 ± 0.05	7.0 ± 0.01
*Carbohydrates	59.4 ± 0.29	62.9 ± 0.15
Gross Energy (Kcal/100g)	382.6 ± 1.70	370.7 ± 0.15

*carbohydrates found by difference.

Table-2. Rats group diet composition (g/1000g diet).

Feed component	Basal	Seed	Husk	Control
Starch	820.0	162.8	143.3	690.1
Cellulose	50.0	50.0	50.0	50.0
Vegetable oil	80.0	80.0	80.0	80.0
Vitamin Mix	10.0	10.0	10.0	10.0
Mineral mix	40.0	40.0	40.0	40.0
Seed powder	-	657.2	-	-
Seed husk	-	-	676.7	-
Lactalbumin	-	-	-	129.9
Total	1000	1000	1000	1000
Gross Energy (Kcal/100g)	405	398	396	401
Nitrogen (%)	0.61	1.75	1.68	1.71

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Table-3. Rats mean daily feed intake and weight gain/loss on weekly basis.

Rat Group	Mean feed intake	Rat weight /week (g)		
	(g/day)	Week 1	Week 2	Week 3
Basal	4.08 ± 0.59	68.63 ± 5.70	58.38 ± 4.66	54.13 ± 3.99
Seed Cake	2.56 ± 0.46	67.97 ± 8.23	59.00 ± 7.31	50.50 ± 10.47
Seed Husk	6.41 ± 0.59	66.58 ± 8.49	67.00 ± 10.42	73.60 ± 11.70
Control	6.62 ± 0.45	62.33 ± 8.48	73.18 ± 8.51	78.63 ± 9.01

There were significant differences between groups 1 and 2 (Basal and Seed diets respectively) rats' mean feed intakes for the first five days of feeding experiment, the group 1 rats recording higher feed intake. However, there was no significant difference in feed intake for the two groups for the rest of the experimental days ($p>0.05$). Also, there was significant difference in feed intake between groups 3 and 4 (control and seed husk diets) for the first three days and the last four days of feeding experiment ($p<0.05$), thereafter, there was no significant difference for both groups for the rest of the experimental days ($p>0.05$).

Positive correlation existed between rats' mean feed intake and weight gain or loss (CI = 95%, r = +0.898), weight loss increasing as feed intake decreases and weight gain increasing with increasing feed intake. Feacal nitrogen was positively correlated with weight change (CI = 95%, r = +0.505, and +0.366 for groups 1and 2, and groups 3 and 4 respectively).

Histopathological study revealed that there was no conspicuous lesion of the heart, diffuse but mild, moderate or severe degeneration and necrosis of the tubular epithelial cells of the kidney, mild/moderate vacuolar degeneration of the hepatocytes, mild diffuse sinusoidal dilatation of the liver, mild/moderate lymphoid depletion in the lymphoid nodules of the spleen, and mild/moderate/severe atrophy of intestinal villi, proliferation of epithelial goblet cells and marked mononuclear cells infilteration with moderate hyperplasia of the mucosal epithelial cells of rats in various groups.

DISCUSSIONS

The low moisture content of *Moringa Oleifera* seed and husk (Table-1) implied that they were high in dry matter and can be safely kept for a long period of time. Their crude protein was relatively high when compared with values obtained for many fruits and seeds which ranged between 0.1 to 7.5% [10, 18, 12, 14]. Omnivorous animals generally depend on plant protein to meet their protein needs; hence the seed and its husk can possibly be sources of plant protein for ruminants. The seed and husk were high in crude fibre and can be good source of dietary fibre for the ruminants. The high ash value was an indication that the seed and husk could be potential good sources of minerals, especially the macrominerals. The carbohydrate and gross energy content of the seed and husk were high. The slightly higher gross energy content of the seed compared with the husk was believed to be a result of its higher lipid content, as 1g of fat contributes more than twice the energy value for 1g carbohydrate or protein [21].

The formulated feeds for rats on group basis were all iso-caloric and three (seed (Group 2), husk (Group 3) and control (Group 4) diets) were iso-nitrogenous (Table-2). Rats in group 2 recorded poor mean feed intake which was the least among the groups (Table-3). The poor feed intake of rats in this group translated to significant weight loss compared with other groups, and made it difficult to assess the protein quality of the seed.

The observed low feed intake of rats on seed diet (Group 2) may be attributable to the fact that undefatted whole seed was used in this study. The seed oil has not been reported in the literature as fit for consumption, and there is possibility of the oil imparting unpalatable taste on the diet thereby causing the reduction in feed intake. It has been reported that feeding chickens with unfermented Shea butter cake resulted in poor feed intake, poor growth and feed conversion efficiency thereby indicating its toxicity [4]. This might have been the reason for poor feed intake of the rats on seed diet. Adepoju *et al.*, [1] reported the seed to contain saponins and tannins which reduce feed intake [5], hence this might have also contributed to reduced feed intake of the seed.

The mean feed intake of group 1 rats (Basal diet) was significantly higher than that of group 2, though significantly lower than that of husk and control groups (groups 3 and 4). However, there was consistent reduction in mean weight of rats fed the basal diet. The observed reduction in weight of rats in this group was believed to have resulted from lack of protein in the diet to support their growth in line with the findings of Goesttsch [13] who reported that animals that received low quality protein diet throughout the growth period were inferior in size to those of the control on the good diet, and the stunting effect that resulted could not be fully overcome by transferring the animals to the good diet later in the growing period. Protein is required for building of tissues, repair of worn-out tissues, defence mechanism, digestion, and growth [20, 21].

The mean rat feed intake for husk (group 3) and control (group 4) groups were similar and very close. Protein foods are always attractive and appetizing, hence the higher feed intakes recorded for the feeds compared to basal diet (diet 1). High feed intakes recorded for these groups resulted in weekly increase in weight of the rats. Lactalbumin is a standard protein of high biological value, hence the corresponding high value of feed intake and weight gain of rats on the diet. The observed high feed intake coupled with weight gain by rats on husk diet was an indication that the husk protein is of high biological value which can support rat growth at 10% inclusion level. Histopathological study revealed that there was no conspicuous lesion on the heart, kidney, liver and intestine of the rats on all diets, showing the safety in their consumption. The observed diffuse but mild, moderate or severe degeneration and necrosis of the tubular epithelial cells of the kidney, mild/moderate vacuolar degeneration of the hepatocytes, mild diffuse sinusoidal dilatation of the liver, mild/moderate lymphoid depletion in the lymphoid nodules of the spleen might be due to the presence of some antinutritional factors in the rat diets which were not taken cognizance of during the cause of the study. Plant products normally contain antinutritional factors such as phytates, oxalates, trypsin inhibitors, saponins and tannins, as these serve as means of defence for the plant tissues against microbial and insects attack. However, some of these antinutritional factors have some health benefits as phytochemicals.

The mild/moderate/severe atrophy of intestinal villi, proliferation of epithelial goblet cells and marked mononuclear cells infilteration with moderate hyperplasia of the mucosal epithelial cells of rats in the various groups might have resulted due to immaturity of the growing rats organs which were still tender.

The coagulant effect of the seed may be due to its high content of crude fibre. Fibre of Shea butter fruit pulp has been used to remove heavy metals from sewage water [11], and the use of *Moringa Oleifera* seed as water coagulant seems to be safe, as there was no case of fatality among the rats fed with the whole seed diet throughout the course of the study.

CONCLUSIONS

The feed intake of *Moringa Oleifera* seed husk by rats was highly comparable with that of control diet, and the weight gain was appreciable. This was an indication that the husk protein supported rat growth at 10% inclusion level, and hence, adjudged to be of good biological value. The taste of the seed oil coupled with the level of saponins and tannins in the seed might have resulted in reduction in its consumption by rats, hence its biological value could not be concluded in this study. The seed husk is safe for animal consumption as source of energy, fibre and dietary protein.

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