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Nutritional evaluations of clerodendrum volubile, a tropical non-conventional vegetable as sole dietary protein source in rats

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ABSTRACT

Vegetables are good source of nutrients that are essential for metabolic activities in the human body. The nutritional evaluations of *Clerodendrum volubile* were studied in male albino rats, with the aim of utilizing the results obtained in improving the use of the leafy vegetable in meeting the nutritional needs of the world growing population. The rats were fed for 28 days with test diet prepared with *Clerodendrum volubile* and as well as standard diet prepared from soybean. The faecal and urinal Nitrogen content were recorded. The Nitrogen Balance (NB); True Protein Digestibility (TD); Biological value (BV); Net Protein Utilization (NPU); and Protein Efficiency Ratio (PER) were determined for the various diets used. There was a significant decrease ($P>0.05$) in the True Digestibility; Biological value; and Protein Efficiency Ratio of the *Clerodendrum volubile*-supplemented (test) diet relative to the soybean-supplemented (standard) diet. The Net Protein Utilization of the standard diet was significantly lower than the *Clerodendrum volubile*-supplemented (test) diet. Results from this study reveal that the digestibility of the leafy vegetable protein is relatively low compared to the standard diet. The addition of limiting amino acids to the standard diet is responsible for the increase in its Biological Value, True Protein Digestibility and Protein Efficiency Ratio.

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1. Introduction

Fruits and vegetables are good sources of nutrients that are essential for metabolic activities in the human body. This has led to increasing interest in the study of nutrition and health protecting properties of indigenous and under-utilized food resources particularly leafy vegetables that constitute basic source of ingredient for rural communities in Africa. The nutritional content of vegetables varies considerably, though generally they contain a small proportion of protein and fat [1, 2], and a relatively high proportion of vitamins, provitamins, dietary minerals, fiber and carbohydrates. Many vegetables also contain phytochemicals which may have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties [3, 4]. Protein is important for building the muscles and keeping the body functioning properly. Protein deficiency is a serious cause of ill health and death in

developing countries [5]. Protein deficiency plays a part in the disease kwashiorkor. War, famine, overpopulation and other factors can increase rates of malnutrition and protein deficiency. Protein deficiency can lead to reduced intelligence or mental retardation. Measures are being taken to increase the supply and utilization of plant proteins to ultimately benefit the human population of the world.

Vegetable proteins are considered to be incomplete protein, which means they lack one or more essential amino acids required for the body to fully utilize the protein. *Clerodendrum volubile* is commonly known among other leafy vegetables in south-south Nigeria, with dearth of nutritional data base. It is commonly known among the Urhobo and Itsekiri tribes of the

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Niger-Delta as *Obenetete*. It belongs to the Family *Lamiaceae* (*Verbenaceae*). It is a climbing shrub to 3 m high, glabrous except the inflorescences, flowers numerous, 1½ cm long, and greenish-white. It grows deciduous forest and secondary jungle, across the Region, Dakar to Fernando Po [6]. Its general uses include (i) Agri-horticulture: ornamental, cultivated or partially tended; (ii) Social: religion, superstitions, magic Leaf; (iii) Food: general; and (iv) Medicines: arthritis, rheumatism; dropsy; swellings; oedema; gout; general healing; pain-killers; pregnancy; antiaborifacients; sedatives [6]. Locally the leaves are often dried and used as spices in cooking; sometimes the fresh leaves are blended and used for cooking.

This study is a continuation of our study on nutritional qualities of *Clerodendrum volubile*. In this report, the nutritional evaluations of this underutilized leafy were studied in growing male albino rats by determining its utilization, Nitrogen retention and excretion, biological values as well as its effect on their (albino rats) growth. Result from this study will contribute to the utilization of this underutilized leafy vegetable found in meeting the nutritional needs of the increasing world population.

2. Materials and method

2.1. Plant Materials

Fresh leaves of *Clerodendrum volubile* were purchased from a local market, in Benin City, Nigeria. The leaves were washed with water and air-dried at room temperature. The dried leaves were ground to fine powder, using a laboratory mill and stored in airtight containers for laboratory analysis. The nitrogen content was determined by standard method of A.O.A.C. [7] and multiplied with the factor 6.25 to obtain the crude protein value.

2.2. Preparation of diets

Three sets of diets were prepared namely: the basal diet; the test diet; and the standard diet. Table 1 shows their composition. *Clerodendrum volubile* and soybean were incorporated in the test and standard diets respectively as the only source of protein.

Table 1. Composition of experimental diets

Ingredients	Basal diet	Test-diet	Standard diet
Cassava starch	70%	70%	70%
Cotton seed oil	10%	10%	10%
Mineral Mix	4%	4%	4%
Vitamin mixture	1%	1%	1%
Lysine	-	-	15,000mg
Methionine	-	-	10,000mg
<i>Clerodendrum volubile</i>	-	15%	-
Soybean powder	-	-	15%
Cellulose powder	15%	-	-

2.3. Feeding Experiment

Weanling albino male rats were obtained from a private farm at the University of Ibadan, Ibadan Nigeria and were used as the experimental animals in the feeding trials. They were 21 to 25 days old at the onset of the experiment. They were collected and

caged with body weights ranging between 30g and 60g. They were divided into three groups and housed individually during the experimental periods in metabolic cages, 32cm long, 24cm wide and 20cm high, and made of wire mesh. The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, University of Ibadan, Ibadan, Nigeria. Feed and water were provided *ad libitum*. Feeding trials lasted for 28 days. Diet, residues, faecal matter and urine were collected daily and weight gain was recorded. At the end of the feeding trial, the rats were sacrificed using chloroform and laid according to their groups in metal tray, then dried in a hot-air oven at 105°C to a constant weight. The carcass nitrogen, diet nitrogen, faecal nitrogen and urinary nitrogen were estimated by micro kjedhal method [8].

2.4. Nutritional Evaluations

True Protein Digestibility (TD), Biological value (BV), Net Protein Utilization (NPU), and Protein efficiency ratio (PER) were computed using standard equations [8].

2.5. Statistical Analysis

Statistical significance was established using One-Way analysis of variance (ANOVA) and data were reported as mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL, USA). A significant difference was considered at a level of $p < 0.05$.

3. Results and Discussion

There is increasing interest in the nutrition and health protecting properties of indigenous and under-utilized food resources particularly leafy vegetables that constitute basic source of ingredient for rural communities in sub-Saharan Africa. In this study, the nutritional evaluations of this non-conventional leafy vegetable were carried out.

Table 2 shows the body mass, amount of consumed diet and growth rate. Significantly higher ($P < 0.05$) weight gain was observed in the group of rats fed on Standard diets, than in rats fed the test diets. Weight loses were observed on the rats fed on the Basal diet. This is not surprising as the basal diet lacked protein. Studies by Sasaki et al. [9]; Bronson [10]; Cameron and Eshelman [11] have shown that deficiencies in dietary protein slow growth and delays maturation in laboratory rodents. Diet composition clearly influences both growth rates and maturation [12]. The high weight gain in the group fed with the Standard diet can be attributed to the dietary protein content.

Table 2. Body mass, amount of consumed diet and growth rate

Parameters	Basal diet	Test-diet	Standard diet
Initial body mass (g)	62.20 ± 22.16 ^a	60.60 ± 23.10 ^b	61.20 ± 8.03 ^c
Final body mass (g)	15.40 ± 4.01 ^c	69.82 ± 23.27 ^a	76.80 ± 9.65 ^b
Average diet consumed (g/day)	3.83 ± 0.61 ^c	7.50 ± 2.23 ^b	10.90 ± 1.88 ^a
Efficiency of diet consumed	-46.8 ± 18.15 ^c	9.22 ± 0.17 ^b	15.60 ± 1.62 ^a

Note: mean \pm SD with different superscripts are significantly different with a>b>c (P<0.05).

Table 3 shows the faecal and urinal Nitrogen contents of the diets (basal, test and standard diets). The faecal Nitrogen content of the Standard diet was high, but significantly different (P<0.05) from the Test diet, the Basal diet was the highest. The urinal Nitrogen content of the standard diet was relatively high compared to that of the basal diet, the test diet had the highest. Addition of limiting amino acids to the standard diet had favourable effects, since the faecal nitrogen content is lower as compared to both the Basal and Test diets (Table 3). Orešnik and Blanchon [13] have already proven a higher reduction of nitrogen excretion after the addition of two amino acids in comparison to addition of one amino acid in Cereal Protein diets. This is in accordance with the results obtained by Pirman *et al* [8] on Pumpkin Seed Cake protein.

Table 3. Faecal and urinal nitrogen contents of the diets (Basal, Test and Standard diets).

Diets	Faecal Nitrogen	Urinal Nitrogen
Basal Diet	2.84 \pm 0.042 ^a	0.051 \pm 0.002 ^b
Test Diet	2.80 \pm 0.069 ^b	0.056 \pm 0.008 ^a
Standard Diet	2.60 \pm 0.085 ^c	0.050 \pm 0.003 ^c

Note: mean \pm SD with different superscripts are significantly different with a>b>c (P<0.05).

Table 4 shows the nutritional evaluations of the test diet (*Clerodendrum volubile*) and the standard diet in albino male rats. A higher significant (p<0.05) Nitrogen balance was observed in rats fed on the standard diet which has positive value. Positive values of NB are indications of increase in the total body pool of protein due to decrease in Nitrogen excretion. This observation corresponds to the observed results in the faecal and urinal nitrogen content of the rats fed on the experimental diets. This also implies that the protein of the leafy vegetable was not fully utilized thus resulting to the observed negative value. True Digestion for the test-diet was high but significantly lower (P>0.05) than the standard diet which was maximum. The difference might be due to poor utilization of the leafy vegetable protein by rats [14].

Table 4. Nutritional evaluations of the test diet (*Clerodendrum volubile*) and the standard diet in albino male rats.

Parameters	Test diet	Standard diet
Nitrogen Balance	-1.74 \pm 0.08 ^b	9.19 \pm 1.23 ^a
True Digestion (%)	95.46 \pm 0.43 ^b	99.63 \pm 0.44 ^a
Biological value (%)	52.86 \pm 0.24 ^b	99.79 \pm 0.18 ^a
Protein Efficiency Ratio	9.14 \pm 0.14 ^b	11.83 \pm 9.85 ^a
Net Protein Utilization (%)	99.02 \pm 5.56 ^a	8.64 \pm 0.11 ^b

Note: mean \pm SD with different superscripts are significantly different with a>b (P<0.05).

Mansour *et al.* [15] reported that the true digestibility of different products of pumpkin seeds was between 87.78 to 96.83 % and increased with the protein content in the sample. Those products contained between 720 and 960 g of crude protein in dry matter. In this present experiment the leaves of *Clerodendrum volubile* had a rather low amount of crude protein (11.20 g/kg dry matter). This corresponds to earlier reports of Pirman *et al* [8] which indicate that the crude protein content in samples inversely affect the protein digestibility.

Biological value was higher in the standard diet group and low in the test diet group at a significant difference (P<0.05). This can be attributed to the amino acid composition of the protein source and the method of preparation. Earlier reports by Mansour *et al.* (15); Orešnik and Blanchon [13]; and Pirman *et al* [8] suggest that BV increases with addition of limiting amino acids as observed in the standard diet. There have been reported flaws in the use of BV in determining nutritional values of protein owing to difference in metabolic requirements of different subjects. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is viewed as accurately measuring the correct relative nutritional value of animal and vegetable sources of protein in diets. This constrained our study due to non availability of amino acid analyzer. Further studies need to be carried out to determine the PDCAAS.

Protein efficiency ratio decreased on the test diet with significant difference (p<0.05) compared to the Standard diet. This can be attributed to the fact that the fiber contents in a diet increases, the protein utilization decreases [14].

Significant difference (P<0.05) was also observed in the Net Protein Utilization (NPU) by test diet group when compared to the standard diet, with the test diet having a higher value. This is somewhat affected by the salvage of essential amino acids within the body, but is profoundly affected by the level of limiting amino acids within a foodstuff. Hoffer [16] confirmed in a study on the 'Adaptation to Protein' the supposition that persons adapted to a surfeit protein intake will oxidize amino acids at a rate equivalent to what is consumed in a generous test meal, irrespective of other metabolic factors.

4. Conclusion

Results from this study reveal that the digestibility of the leafy vegetable (*Clerodendrum volubile*) protein is relatively low compared to the standard diet. The addition of limiting amino acids to the standard diet is responsible for the increase in its Biological Value, True Protein Digestibility and Protein Efficiency Ratio. Combination of this leafy vegetable with other foodstuffs is recommended to satisfactorily supplement for the limiting amino acids and thereby enhancing full utilization of the protein, thus meeting nutritional needs.

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