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Digestibility of Leaf Proteins of *Gnetum* spp Vegetables in Rats and Effects of Some Certain Antinutrients

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Authors' contributions

This work was carried out in collaboration between all authors. Author TC designed the study and supervised the work. Authors NM and MKLB carried out all laboratories work and performed the statistical analysis. Author GI managed the analyses of the study. Author NM wrote the protocol and the first draft of the manuscript. Authors NM and NYJ managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Study digestibility of leaf proteins of *Gnetum africanum* and *Gnetum buchholzianum* and effects of some antinutritional factors on young male rats *Wistar albinos*.
Study Design: Nutritional enhancement of use of *Gnetum* spp leafy vegetables.
Place and Duration of Study: Sample: Limbe botanical Garden of Cameroon between November 2013 and February 2014.
Methodology: Proteins and antinutritional factors were determined using standard analytical

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methods. Standard diet AIN-93 was used as reference for preparation of experimental diets used for *in vivo* digestibility of these leafy vegetables. Rats were fed with diets containing the corresponding 5% (GA5P and GB5P for *G. africanum* and *G. buchholzianum* respectively) or 10% (GA10P and GB10P) of leaf proteins.

Results: Results showed that mean proteins contents was 16.70 mg/100g. Average contents of crude fibers, Neutral Detergent Fibers, Acid Detergent Fibers, crude phenolic compounds, tannins and phytates were respectively 36.17, 41.97, 37.80 g/100 g and 478.80, 244.94, 215.64 mg/100. Rats' growth was more promoted by diets containing 5% proteins. Protein Efficiency Ratio and Net Protein Efficiency Ratio were low. Digestibility Coefficient, Biological Value and Net Protein Utilization were high for 5% proteins diets. Principal Component Analyses revealed that fibers and antinutrients reduced growth and nitrogen retention from diet containing 10% proteins.

Conclusion: *Gnetum* spp. leafy vegetables have high contents of fiber, phenolic compounds and phytates which contribute to reduction of digestibility of theirs proteins.

Keywords: Gnetum spp; leafy vegetable; proteins; antinutrients; digestibility.

1. INTRODUCTION

Gnetum spp is a subspontaneous liana, largely distributed across tropical Africa forests, mainly in Cameroon, Gabon, Congo and Democratic Republic of Congo. There are about thirty species of *Gnetum* spp. In Africa, more precisely in Cameroon, two species are common: *G. africanum* and *G. buchholzianum.* They are well known by local communities as useful plants, not only because of their edible leaves but, also for a number of medicinal uses [1]. *Gnetum* spp leaves stand as one of the good vegetable source of proteins with values ranging between 16 and 18 g/100g dried mass. These proteins are of high nutritional value because of their content of essential amino acids [2,3]. It is well known that proteins are essential components of the diet needed for humans. Their basic function is to supply adequate amount of essential amino acids for body functions [4]. Since it is unlikely to obtain enough protein from animal sources to meet up with increasing demand due to population growth, adequate protein sources should be investigated in plants.

The nutritive value of a given protein depends on its content in essential amino acids and on the physiological utilization of specific amino acids after digestion [5]. Low nutritional value of vegetable proteins has long been ascribed to limiting amount of essential amino acids, poor proteins digestibility and high levels of antinutrients [6,7]. Previous study reported high contents of fibre, phytic acid and phenolic compounds in *Gnetum* spp leaves [3]. Phytates and tannins form complex with dietary proteins and lower their bioavailability and digestibility [8]. Dietary fibres bind organic compounds, decrease digestion and absorption of proteins and increase fecal bulk [9]. One of the main ways of increasing value of leafy vegetable proteins is to make a more rational use of the available leafy vegetables. For instance, it is relevant to evaluate real contribution of a given leafy vegetable proteins to diets. Fokou and Domngang [10] found that rats retained only 57 to 70% nitrogen and lost weight when fed with *G. africanum* leaves based diet. Apart from this work and as far as our knowledge is concerned, little is known about nutritive value of leaf proteins of *Gnetum* spp found in Cameroon. The aim of this work is to evaluate the digestibility of proteins of *Gnetum* spp leaves in rats and the contribution of dietary fibre, phytic acid and phenolic compounds.

2. MATERIALS AND METHODS

Leaves of *G. africanum* and *G. buchholzianum* were harvested during drying season (November 2013–February 2014) in Limbe Botany Garden (South-West Cameroon; longitude: 4 degrees 0'46" North, latitude: 9 degrees 13'13" East). Leaves were washed with tap water, sliced into small pieces using a stainless steel knife and one part of the sample was cooked (300 g of leaf per liter). Cooked and uncooked samples were then separately shade dried for one week with frequent turning to avoid fungal growth. They were later finely milled to obtain a powder using an electric blender. Samples were then frozen (-18ºC) in a labeled polystyrene container for further analysis.

2.1 Chemical Analyses

Chemical analyses were carried out on uncooked and cooked leaves of *G. africanum* and

G. buchholzianum. AOAC (Association of Official Analytical Chemists) [11] methods were used for the determination of crude proteins, crude fibre, total phenolic compounds, tannins and phytates contents. Van Soest and Wine method [12] was used for determination of Neutral Detergent Fibre (NDF). Acid Detergent Fibre (ADF) was evaluated by treating powder of dried samples with a mixture of sulfuric acid and cetyltriethylammonium bromide according to Southgate et al. method [13].

2.2 *In vivo* assays

In vivo digestibility of the cooked dried and sliced leaves of *Gnetum* spp was carried out on young male rats (*Wistar albinos*) aged 21±3 days and weighing 33 – 38 g. *Wistar albinos* were used in these experiments for the following reasons: Their growth reflects the nutritional quality of diets, they have low sensitivity to environmental and infectious factors, and they easily accept semi-synthetic diets [14].

Casein was used as protein source in the control diet. Test diets and casein based diet (Table 1) were prepared according to the AIN-93 model [15]. Cooked sample was used for *in vivo* study to favor digestibility and reduce negative effect of antinutrients.

Rats (21±3 days and weighing 33 – 38 g) were housed in individual stainless-steel metabolic cages with suspended bottom in a room temperature (23±2 degrees celcius) with alternating 12 hours period of light and dark. They were fed a reference diet (Altromin, Spezialfutter-Germany) for 3 days. They were then weighed, allocated to 6 groups (5 rats per group) and randomly assigned control diet (CAD), protein free diet (PFD) or experimental diets (GA5P, GA10P, GB5P, GB10P) (Fig. 1). Food and water were given *ad libitum.* Rats were fed for 15 days and weighed at the same time (9 am. GMT) every two days. From day 10 to day 15, feces and urines were collected and nitrogen intake recorded for evaluation of digestibility.

Table 1. Composition of control and test diets (g/Kg)

Ingredients (g/Kg)	Casein diet (10% proteins)	test diet (5%) proteins)	test diet (10% proteins)	Proteins free diet
Gnetum spp leaves	0	306.18	612.37	0
Corn oil	100	82.98	65.95	100
Cellulose	155.24	0	0	155.24
Glucose	50	35	20	50
Saccharose	100	70	40	100
Minerals(1)	53.46	10	10	53.46
Vitamines(2)	10	10	10	10
Corn starch	431.30	485.84	241.68	531.30
Casein	100	0	0	0
Distilled water (mL)	100	100	100	100

(1) : minerals (composition / 100 g): Ca_2HPO_4 (36.5 g); $CaCO_3$ (22.77 g) ; KH_2PO_4 (18.7 g) ; KCl (7.75 g) ; sodium chlorure (7.75 g) ; MgO_2 (4 g) ; Fe SO_4 (1.13 g) ; Zn SO_4 (0.9 g) ; Mn SO_4 (0.4 g) : Cu SO_4 (0.1 g) ; $CoSO_4$ (2 mg) ; IK (1 mg) ; (2):Vitamines (composition / 100 g): Vit. B_1 (1.5 mg) ; Vit. B_2 (2.5 mg) ; Vit. B_3 (15 mg) ; Vit. B_5 (5 mg) ; Vit. B_6 (1.5 mg) ; Vit. B_9 (0.5 mg) ; Vit. B_8 (150 µg) ; Vit. B_{12} (10 µg) ; Vit. A (1 mg) ; Vit. D (37 µg) ; Vit. E (40 mg); Vit. K (3 mg)

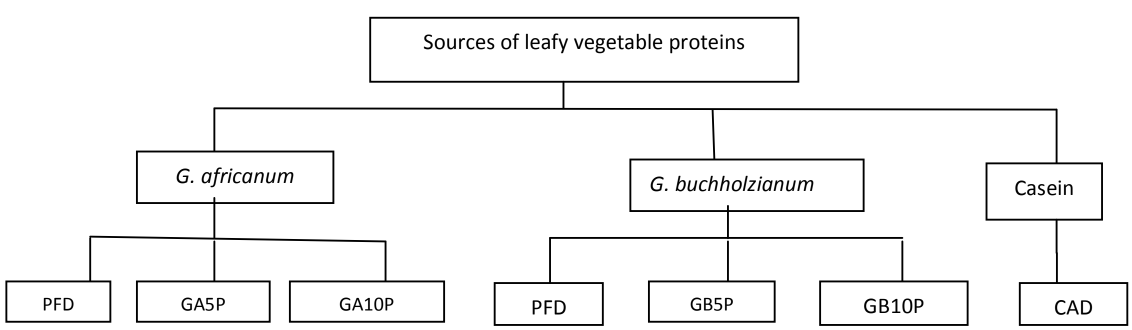

Fig. 1. Distribution of experimental groups of rats according to types of diet

PFD: Group fed proteins free diet; GA5P and GB5P are groups fed with diets containing the corresponding 5% protein while GA10P and GB10P are groups fed with diets containing the corresponding 10% proteins. CAD= group fed casein diet

Fecal collections from each rat were dried in a ventilated oven (Binder, Bergstr-Germany) and analyzed for nitrogen by Kjeldahl method [11]. Weight loss for free proteins group was used for determination of Net Protein Efficiency Ratio (NPER). Weight change and amount of food consumed during experimental period were used for Protein Efficiency Ratio (PER) determination. The protocol used was approved by the National Ethics Committee of Cameroon.

The following formulars were used for calculation of PER and NPER according to Giami and co-workers [16]:

$$PER=\frac{Wg\,(g)}{PI(g)}\times 100 \qquad NPER=\frac{Wg\,(g)+Wl\,(g)}{PI(g)}\times 100$$

Wg = weight gain; PI = Protein intake; Wl = weight loss by group which received a protein free diet.

PERCAAS and NPERCAAS which are the corresponding values of PER and NPER corrected by the Chemical Score (CS) of amino acids were obtained as a product of PER and NPER values by the lowest value of CS which was 0.17 for *G. africanum* and 0.19 for *G. buchholzianum* proteins. According to PDCASS (Protein Digestibility Corrected Amino Acid Score) method, Chemical Score (CS) of amino acids was calculated as followed [15]:

Sc = (mg of amino acid in 1g of analysed protein) / (mg of amino acids in 1g of reference protein) x 100

Kjeldahl method (AOAC, 1980) was used for determination of nitrogen contents in food, urines and faeces collected between day 10 and day 15 of rats feeding. These nitrogen parametres were used for determination of Digestibility Coefficient (DC), Biological Value (BV) and Net Protein Utilisation (NPU) and theirs corresponding values corrected by CS (DCCAAS, BVCAAS and UPNCAAS) were used for calculations [16].

$$DC=\frac{Na\,(g)}{Ni\,(g)}\times 100 \quad BV=\frac{Nr\,(g)}{Na\,(g)}\times 100 \quad NPU=\frac{Nr\,(g)}{Ni\,(g)}\times 100$$

Na = Nitrogen absorbed; Ni = Nitrogen ingested; Nr = Nitrogen retained.

2.3 Statistical Analyses

Each result is the sum of three analyses and it is expressed as mean ± standard deviation. Spearman correlation and Duncan tests were used for comparisons. Softwares used were *Statistica* 6.0 and XLSTAT version 2014.

3. RESULTS AND DISCUSSION

3.1 Chemical Analyses

Table 2 shows proximate composition of uncooked and cooked leafy vegetables of *Gnetum* spp compared to some of others local leafy vegetables. Mean values of water content in raw samples were significantly (P<0.05) higher in *G. buchholzianum* (33.57% w/w) than in *G. africanum* (29.11% w/w). These values are lower than those of some other locally consumed leafy vegetables such as *Amaranthus hybridus* (83.4%) or *Manihot esculenta* (70.46%) [7,17,18, 19]. There was no significant difference in crude proteins and NDF contents between the two *Gnetum* species. Values of crude proteins are closed to those obtained by Mialoundama [2] on the same plant in Democratic Republic of Congo. However, these values were lower than those of *A. hybridus* (24.90%) and *M. esculenta* (20-35%). High values of crude fibre (40.10% dried weight) and ADF (41.35% dried weight) were recorded especially for *G. africanum.* These vegetables could therefore be used as good sources of fibres. High values of NDF and ADF could be explained by precipitation of fermentable carbohydrates as suggested by Mertens [20]. Dietary fibres are well known for their ability to prevent or relieve constipation. They also provide other health benefits such as helping to maintain body weight, lowering risk of diabetes and heart disease [21]. High values of crude phenolic compounds, tannins and phytates were also found in both uncooked and cooked samples. These antinutrients were significantly (P< 0.05) concentrated in *G. africanum* leaves. Ekop [22] also found high values of tannins and phytic acid in *G. africanum* seeds in Nigeria. These substances are normally used by plants as secondary metabolites but could also negatively affect protein metabolism [7]. Their relative high levels in *Gnetum* spp leaves could reduce bioavailability of proteins. Only antinutrients contents showed significant decreases between raw samples and water cooked samples (Table 2).

Table 3 gives amino acids contents of leaves of *G. africanum* and *G. buchholzianum* as obtained in a previous work [3].

Table 2. Proximate composition (mg/100 of dried weight) of *Gnetum* spp leaves compared to some local leafy vegetables

Species	Water (%w.w)	Proteins	Crude fibers	NDF	ADF	Crude phenolic compounds	Tannins	Phytates
G. africanum	29.11± 0.57 [a]	16.47 ± 0.02 [a]	40.10 ± 0.88 [a]	42.06 ± 0.11 [a]	41.36 ± 1.27[a]	507.19 ± 21.53[a]	298.09 ± 13.70 [a]	240.23 ± 14.25[a]
*G. africanum**	/	15.84 ± 0.92 [a]	39.14 ± 0.52 [a]	41.23 ± 1.15[a]	39.17 ± 0.34[b]	385.23 ± 0.02[b]	198.74 ± 0.06[b]	164.05 ± 0.16[b]
G. africanum[3]	/	16.50[1]	40.00[1]	/	/	/	100.74[3]	238.26[3]
G. buchholzianum	33.57 ± 1.50 [b]	16.94 ± 0.92 [a]	33.60 ± 0.20 [b]	44.76 ± 0.19 [a]	38.88 ± 0.41[c]	460.37± 3.09[c]	222.73 ± 13.90[c]	208.00 ± 1.57[c]
G. buchholzianum *	/	15.17 ± 0.28 [a]	32.39 ± 0.17 [b]	44.31 ± 0.45[a]	37.12 ± 1.06[c]	319.86 ± 0.15[d]	176.08 ± 0.23[d]	182.03 ± 0.01[d]
G. buchholzianum[1]	/	18.50	39.50	/	/	/	/	/
A. hybridus[2]	83.40	24.90	8.61	/	/	0.84	0.49	1.32
Manihot esculenta[4]	70.46	20-35	11.50	/	/	/	6.9	107.30

Values on the same column with the same superscript letter are not significantly different (P>0.05)

**Cooked sample; [1]Mialoundama (2007); [2]Akubugwo et al. (2007); [3]Ekop (2007) (in seeds); [4]Ayodeyi and Fasuyi (2005); w.w = wet weight*

Table 3. Amino acid contents of *Gnetum* spp leaves (mg/g of dried mass)

Amino acids	*G. africanum*		*G. buchholzianum*		FAO reference [23]
	Contents	Chemical scores (%)	Contents	Chemical scores	
Tyr	6.13±0.24		6.90±1.65		-
Phe	28.84±3.52		27.92±4.01		-
Tyr + Phe	34.97	67.25	34.82	66.96	52
Thr	55.61±0.06	81.65	54.30±2.12	92.35	31
Ile	42.50±4.06	179.38	44.33±2.89	175.16	32
Leu	45.71±3.15	132.81	44.27±0.59	138.56	66
Lys	40.07±3.94	69.25	39.37±1.71[a]	67.07	57
Val	32.78±3.21	70.29	32.58±1.23	69.07	43
His	16.33±0.24	76.23	18.47±4.07	75.76	20
Met+Cys	4.80±0.13	17.77	5.20±0.17	19.25	27
Trp	26.60±0.15	312.94	24.31±0.15	286.00	8.5
Σ EAA	299.56		297.65		316.5
Asn+Asp	61.08±6.37		58.44±6.02		
Gln+Glu	61.50±0.36		60.56±1.08		
Pro	56.28±±1.03		54.25±0.70		
Ser	39.10±2.42		38.60±1.41		
Gly	67.33±3.40		60.33±1.29		
Ala	28.41±0.60		28.15±3.42		
Arg	25.86±1.56		24.20±1.71		
Σ NEAA	339.56		343.53		
Σ EAA (%)	46.85		47.83		
Σ TAA					

EAA= Essential Amino Acids; NEAA= Non Essential Amino Acids; TAA= Total Amino Acids.
Underlined amino acids are limiting, sulfur amino acids being the most limiting.

All essential amino acids were found. Apart from histidine, methionine and cysteine, there was not significant variation between *G. africanum* and *G. buchholzianum* contents in amino acids. Compared to FAO reference [23], sulfur amino acids are the most limiting in both species with chemical scores less than 20%. The highest chemical score was tryptophane followed by isoleucine and leucine. The ratio between essential amino acids and total amino acids was greater than 46.85% indicating a good chemical equilibrium between amino acids [24].

3.2 *In vivo* Assays

3.2.1 Growth measurement parameters

In vivo assays were performed using cooked leaves for both species. Weight measurement parameters and nitrogen retention in rats fed experimental and control diets were evaluated for *in vivo* assays. Fig. 2 shows that weight change is low in rats fed test diets compared to casein diet.

Weight of rats fed casein diet used as control was higher than those of rats fed experimental diets. Casein is a milk protein with relatively high content of essential amino acids and good digestibility. It can be regarded as a complete source of amino acids [14]. Weight of rats fed diets containing 10% proteins decreases (GB10P) or remains constant (GA10P) while that of GA5P and GB5P groups fed with 5% proteins increases. Growth promotion observed with these diets may be due to a good availability of theirs proteins. In fact, 5% diets contain less fibers than do 10% diets. For both groups, no significant difference (*P<0.05*) appears between *G. africanum* and *G. buchholzianum* based diets.

Defined as ratio of grams of body weight gain (in a specified time) to grams of protein consumed, PER is one of the methods available for measuring quality of proteins in food. It expresses efficiency of conversion of protein sources [25] Ability of 5% protein diets to promote rat growth is confirmed by theirs values of PER (1.93% and 1.78% respectively) and NPER (2.64% and 2.30% respectively) and their corresponding corrected values (PERCAAS and NPERCAAS (Table 4) calculated using PDCAAS (Digestibility-Corrected Amino Acid Score) method. In fact, this method allows evaluation of food protein quality based on the needs of humans as it measures the quality of a protein based on the amino acid requirements (adjusted for digestibility) of a 2 to 5-year-old child (considered the most nutritionally demanding age group) [23]. Among test groups, GA5P had the most elevated value of PER (1.93%) and NPER (2.64%). But values are low when compared to that of rats receiving casein diet (4.25% and 4.48% respectively for PER and NPER). This means they could not maintain nitrogen balance as casein instead of occurrence of all essential amino acids. PERCAAS (0.33%) and NPERCAAS (0.43%) were poor and identical for both GA5P and GB5P diets but the values obtained are more than 4 fold lower compared to casein based diet.

Poor values of PERCAAS and NPERCAAS could be explained by occurring of limiting amino acids in the leaves. Sulfur amino acids are limiting in *Gnetum* spp leaves. As a result, meals based *Gnetum* leaves could not maintain nitrogen balance if they are used as the only source of proteins. This is due to the fact that adequate amount of all essential amino acids are not provided. 10% proteins supplementation in these diets could help for weight management as shown in Fig. 2.

3.2.2 Parameters of nitrogen retention

Digestibility Coefficient (DC), Biological Value (BV) and Net Protein Utilization (NPU) and theirs values corrected by chemical score of amino acids are shown in Table 5. For test diets, values were higher in groups receiving 5% proteins. GB5P had the highest value of DC (91.80%) while BV (86%) and NPU (74.13%) were high for GA5P. The same trends were observed with DCCAAS, BVCAAS and NPUCAAS. Groups GA10P and GB10P had low nitrogen absorption and retention.

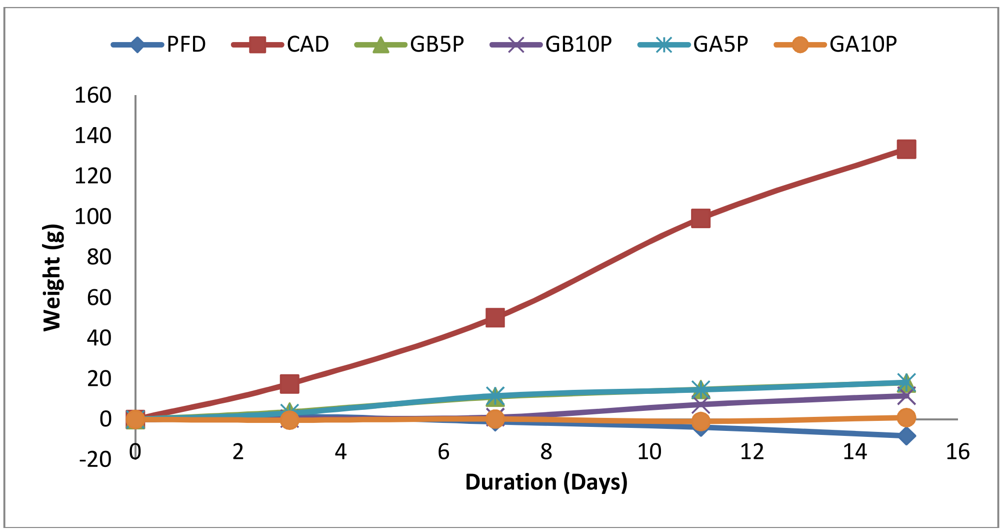

Fig. 2. Weight changes of rats fed control and test diets

PFD: proteins free diet; CAD: casein diet; GBP5 and GBP10 are test diets containing respectively 5% and 10% protein of G. buchholzianum leaves. GA5P and GA10P are test diets containing respectively 5 and 10% proteins of G. africanum leaves

Table 4. Protein Efficiency Ratio (PER), Net Protein Efficiency Ratio (NPER) and their corresponding values corrected (%) by chemical score of amino acids

Diets	PER	PERCAAS	NPER	NPERCAAS
Casein	4.25 ± 0.01^{a}	4.54	4.48 ± 0.04^{a}	4.79
G. buchholzianum 5%	1.78 ± 0.02^{b}	0.34	2.30 ± 0.04^{b}	0.43
G. buchholzianum 10%	0.54 ± 0.01^{c}	0.10	0.86 ± 0.04^{c}	0.16
G. africanum 5%	1.93 ± 0.04^{b}	0.32	2.64 ± 0.04^{b}	0.44
G. africanum 10%	0.20 ± 0.00^{c}	0.003	0.40 ± 0.03^{d}	0.06

Values on the same column with the same letter superscript are not significantly different (P>0.05)

Digestibility Coefficient (DC) gives the percentage of diet actually digested and absorbed to meet the body metabolic needs. High levels of DC indicate that an important nitrogen fraction absorbed in the diet is used by the body. Lower value of DC for 10% proteins diets could at least be explained by their relative high level of fibers. It has been shown that dietary fiber reduces digestibility coefficient and that a high-fiber meal has less total energy and nitrogen absorbed than a fiber-free meal [26].

Biological Value (BV) is the ratio of nitrogen retained on nitrogen absorbed. It is the effectiveness with which nitrogen balance can be achieved for a given amount of absorbed dietary nitrogen. It is markedly influenced by the relative amounts of essential and non essential amino acids and other nitrogen-containing compounds [27]. BV of 5% diets were greater than that of casein based diet (28.75%) and 10% test diets (Table 5). For *G. africanum* diet, BV of group GA5P (86.00%) is greater than 73% reported by Friedman [5] on soybeans but lower than that of egg (94%).When a protein contains essential amino acids in proportion required by the body, it has a high Biological Value. Some factors such as digestion, food processing, antinutrients and protein-energy relationships could affect Biological Value of a protein [28]. Leaves of *Gnetum* spp contain relative amount of phytic acids, phenolic compounds such as tannins which could bind proteins and reduce their digestibility. Low biological values of diets for groups GA10P (16.12%) and GB10P (21.13%) appear as a consequence of their elevated values of antinutrients and fibers. Nitrogen losses through urines in these groups (0.38 mg/rat/day) were high compared to those of groups fed 5% protein (0.23 mg/rat/day). BV corrected by chemical score of amino acids (BVCAAS) were low for the whole groups (Table 5) indicating the negative effects of limiting amino acids (methionine and cysteine).

Net Protein Utilization (NPU) is the ratio of amino acids converted on proteins to the amino acids supplied [5]. As observed for BV, NPU values were high for groups GA5P (74.13%) and GB5P (50.81%) compared to casein group. It may indicate good protein quality of these diets but when corrected by chemical score of amino acids, values decreased to 12.6 and 9.65% respectively (Table 5). This means that essential amino acids are not all provided in balance quantities. In *Gnetum* spp leaves, methionine and cysteine were found to be the limiting amino acids [3]. NPU is also affected by number of factors such as source of protein, food processing and digestion [28]. However diets based *G. africanum* leaves with 5% proteins promote nitrogen retention in male rats than do 10% protein diets.

3.3 Effects of Antinutrients on Protein Digestibility

Content of crude phenolic compounds, tannins, phytates, crude fibre, NDF and ADF were evaluated in the different diets given to rats and theirs effects on growth and nitrogen parameters were tested using Principal Component Analysis (PCA). From the biplot axes F1 and F2 of Fig. 3, parametres of digestibility (DC, PER, NPER, BV and UPN) are closely related to 5% proteins diets and particulary to GA5P. 10% proteins diets were closely related to antinutrients (crude phenolic compounds, fibers, phytates and tannins for GA10P, NDF and ADF for GB10P). According to the results found, more quantity of sample is required for 10% protein diet than for 5%.

Table 5. Digestibility Coefficient (DC), Biological Value (BV) and Net Protein Utilization (NPU) of tests and control diets (%)

Diets	Parameters of digestibility					
	DC%	**BV%**	**NPU %**	**DCCAAS**	**BVCAAS**	**NPUCAAS**
Casein	82.70 ± .24^a	28.75 ± .48^a	23.78 ± .78^a	88.48	30.76	25.44
5% *G. buchholzianum*	91.80 ± .50^b	56.36 ± .15^b	50.81 ± .13^b	17.44	10.70	9.65
10 % *G. buchholzianum*	13.84 ± .55^c	21.13 ± .39^c	-13.84 ± .57^c	2.62	-19.00	-2.62
5% *G. africanum*	86.20 ± .89^a	86.00 ± .63^d	74.13 ± .44^d	14.65	14.62	12.60
10 % *G. africanum*	27.67 ± .01^d	16.12 ± .40^e	4.46 ± 0.92^e	0.75	2.74	0.76

DCCASS, BVCAAS and NPUCAAS are values of DC, BV and NPU respectively corrected by chemical score of amino acids; Values on the same column with the same superscript are not significantly different (P>0.05)

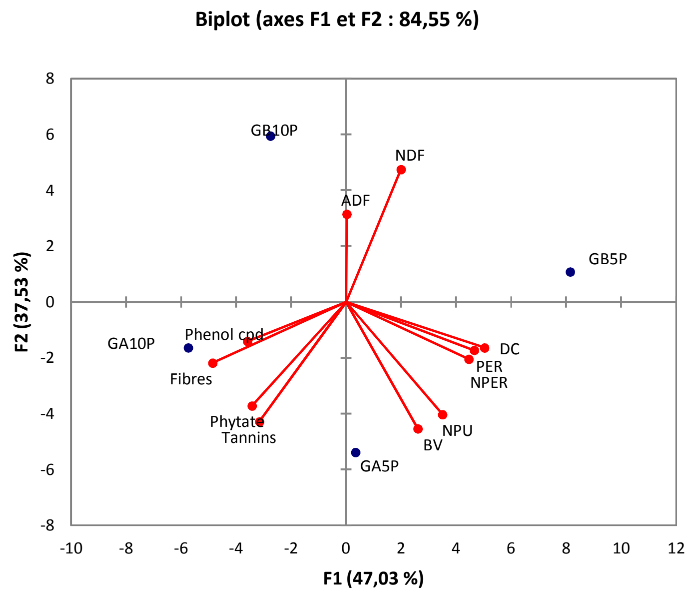

Fig. 3. Principal component analysis of effect of some antinutrients on protein digestibility in rats

GB5P and GB10P are groups of rats fed test diets containing respectively 5% and 10% proteins of G. buchholzianum. GA5P and GA10P are groups of rats fed test diets containing 5% and 10% proteins of G. africanum respectively. (Phenol. Cpd = phenolic compounds. NDF= Neutral Detergent Fibre. ADF=Acid Detergent Fiber)

Greater amount of material in 10% compare to 5% diet could explain the close relationship between antinutrients, low growth rate and 10% protein diets. It has been proved that antinutrients substances reduce nitrogen retention and promote its excretion in faeces. Because of their numerous hydroxyl groups, tannins are capable of binding proteins and thereby decreasing their solubility and bioavailability [29,30,31].

High levels of insoluble fiber and elevated contents of antinutrients in *Gnetum* spp leaves may adversely affect digestibility of proteins of these vegetables. There is therefore a need to supplement *Gnetum spp* leaves diets with other sources of proteins.

4. CONCLUSION

Gnetum spp. leafy vegetables have high contents of fiber, phenolic compounds and phytates which contribute to reduction of digestibility of theirs proteins. Digestibility parameters are affected by fibers and antinutrients. Due to their fiber contents, *Gnetum* spp leaves more than 5% in a diet could be better used to manage weight than served as a source of proteins.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ali F, Assanta MA, Robert C. *Gnetum africanum*: A wild food plant from the African forest with many nutritional and medicinal properties. J. Med. Food. 2014; 14(11):1289-1297.
2. Mialoundama F. Intérêt alimentaire et socio-économique de Gnetum. In : Le koko ou mfumbu (Gnétacées). Une plante alimentaire d'Afrique Centrale. L'Harmattan. 2007;115 – 125. Ndomou M, Mezajoug Kenfack LB, Tchiegang C. Physico-chemical properties of leaves of *Gnetum africanum* (L.) and *Gnetum buchholzianum* (L.) (Gnetaceae) from

Cameroon. American Journal of Research Communication*.* 2014;2(12):101-112.
3. Jia L, Armstrong CLH, Campbell WW. Effects of dietary protein source and quantity during weight loss on appetite energy expenditure and cardio-metabolic responses Campbell. Nutrients. 2016; 8(63):1-17.
4. Friedman M. Nutritional value of proteins from different food sources. A review J. Agric. Chem. 1996;44:6-29.
5. Evans RJ, Bauer DH. Studies of the poor utilization of the rat methionine and cysteine in heated dry bean seed (*Phaseolus vulgaris*). J. Agric. Food Chem. 1978;26:779-784.
6. Ayodeji O. Fasuyi. Nutritional evaluation of cassava (*Manihot esculenta* Crantz) leaf protein concentrates (CLPC) as alternative protein sources in rat assay. Pakistan Journal of Nutrition. 2005;4(1):50–56.
7. Graf E. Phytic acid: Chemistry and application*.* Pilatus press (USA). 1986;343.
8. Wenjuan Z, Defa L, Ling L. Jianjun Z, Qiwu D,Wenjun Y, Liying Z. The effects of dietary fibre level on nutrient digestibility in growing pigs. J. Anim. Sc. Biotechnol. 2013;4(1):17.
9. Fokou E, Domngang F. *In vivo* assesment of the nutritive value of proteins in situ in the leaves of *Solanum nigrum L*. *Xanthosoma spp*. and *Gnetum africanum*. Indian J. of Nutr. and Dietetics. 26. 1989;(12):366-373.
10. AOAC (Association of Official Analytical Chemists). Official methods of Analysis. 15th Ed. Washington D.C. CFR Section(s): 9 CFR 318.19; 1990.
11. Van Soest PJ, Wine RH. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituants. J. Ass. Offic. Anal. Chem. 1967;50:50–56.
12. Southgate DAT, Waldron K, JohnsonI T, Fenwick GR*.* Dietary Fibre: Chemical and biological aspects. Woodhead Publishing Limited. 1990;386.
13. Adrian J, Potus J, Annie P. Introduction à l'analyse des denrées alimentaires: Méthodes biologiques sur l'animal. Techniques et documentation-Lavoisier, Paris (France). 1998;256.
14. Lien EL, Boyle JM, Wrenn FG, Perry RW, Thompson CA, Borzelleca JF. Comparaison of AIN-76A and AIN-93G diets: A 13-week study in rats. Food and Chemistry Toxicology. 2001;39:385–392.
15. Giami YS. Compositional and nutritional properties of cowpea (*Vigna inguiculata l. walp*). J. Food Comp. Anal. 2005;18:665-373.
16. Rozan P, Lamghari R, Linder M, Christian V, Fanni J, Parmentier M, Méjean L. *In vivo* and *in vitro* digestibility os soybean lupine and rapeseeds meal protein after varoius technological processes. J. Agric. Food Chem. 1997;45:1762–1769.
17. Akabugwo IE, Obasi NA,Ginika SC. Nutritional potential of leaves and seeds of black nightshade *Solanum* L. *var virginicum* from afrikpo-Nigeria. Pakistan Journal of Nutrition. 2007;6(4):323–326.
18. Tchientche-Kamga R, Kouamie C, Atangana AR, Chagomoka T, Ndango R. Nutritional evaluation of five African indigenous vegetables. Journal of Horticultural Research. 2013;21(1):99–106.
19. Mertens DR. Challenges in measuring insoluble dietary fiber. J. Anim. Sci. 2003; 81:3233-3249.
20. Anderson JW, Baird P, Davis RH, Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL. Health benefits of dietary fibre. Nutr. Rev. 2009;67(4):188-205.
21. Ekop AS. Determination of chemical composition of *Gnetum africanum* (*Afang*) seeds. Pakistan Journal of Nutrition. 2007; 6(1):40–43.
22. FAO. Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation. Food and Nutrition Paper. 2013;66 Blankership DC, Alford BB. Cottonseeds, the new staff of life. A Monography of Cottonseeds Protein Research Conducted by Scientists of the Texas Woman's University Press Denton Texas. 1983;76:204.
23. Bhilave MP, Bhosale SV, Nadaf SB. Protein Efficiency Ratio (PER) of *Ctenopharengedon idella* fed on soyabean formualted feed. Biological Forum. 2012; 4(1):79-81.
24. Chu ZJ, Yu DH,Yuan YC, Qiao YW, Cai J, Shu H, Lin YC. Apparent digestibility coefficients of selected protein feed ingredients for loach *Misgurnus anguillicaudatus*. Aquaculture Nutrition. 2015;21(4):425–432.
25. WHO/FAO. Protein and aminoacids requirements in human nutrition. WHO technical reports series. No 935. Joint WHO/FAO/UNU Expert Consultation on

Protein and Amino Acid Requirements in Human Nutrition Geneva. 2002;265.

26. Vladimirovna SN, Zelenov AN, Bolshakova LS. Amino acid composition and biological value of protein of new pea morphotypes. Indian Journal of Science and Technology. 2016;9(5):1-5.
27. Zaidi F, Hassissene N, Allouache H, Kichou M, Ourdani S, Rezki K, Bellal MM, Grongnet JF, Youyou A. Les composés phénoliques: Facteur limitant du grignon d'olive chez les ruminants. Revue Méd. Vét. 2009;20(3):67–73.
28. Akande KE, Doma UD, Agu HO, Adamu HM. Major antinutrients found in plant protein sources: Their effect on nutrition. Pakistan Journal of Nutrition. 2010;9(8): 827-832.
29. Manashi DP, Jyotchna G, Dipankar K, Pronobesh CK, Sewa N, Danswrang G, Charu Lata M. Physicochemical and functional properties of rapeseed protein isolate: influence of antinutrient removal with acidified organic solvents from rapeseed Meal. J. Agric. Food Chem. 2014;62(31):7903–7914.

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