Low Nutritional Quality of Unconventional Tropical Crop Seeds in Rats[1]

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ABSTRACT As the search for alternative sources of food to alleviate hunger continues, this study was undertaken to determine the biological value in growing rats (BV) of proteins of some lesser known tropical seeds gathered in Nigeria. Antinutritional factors (trypsin inhibitors, phytic acid, oxalate, tannin, alkaloids) and amino acid compositions were also determined, and protein digestibility-corrected amino acid score (PDCAAS) was calculated using the amino acid requirement pattern of the preschool child and individual seed-specific correction factors for crude protein. A rat growth and balance study was conducted to determine digestibility, nitrogen-, and energy balance by feeding as the only unsupplemented protein source milled and heat-treated seeds of *Adansonia digitata* (Bombacaceae) and *Prosopis africana, Lonchocarpus sericeus, Enterolobium cyclocarpium, Sesbania pachycarpa* and *Pterocarpus osun* (Leguminosae) in comparison to casein fortified with methionine (control). Diets containing *P. africana* and *L. sericeus* seeds caused poor feed intake and weight loss in rats and were excluded from the nitrogen-balance test. Among the seed samples, *S. pachycarpa* followed by *A. digitata* showed the most advantageous nutritional quality [amino acid composition, digestibility, BV and net protein utilization (NPU)]. True digestibility was 82.9 and 74.5 vs. 98.5, BV was 64.6 and 70.0 vs. 90.4, and NPU was 53.5 and 52.1 vs. 89.0 for *S. pachycarpa* and *A. digitata* vs. casein (control), respectively. In terms of PDCAAS, lysine was the first limiting amino acid for *S. pachycarpa* (88%) and for *A. digitata* (58%). The PDCAAS of all essential amino acids was below 100% for *E. cyclocarpium* (e.g., cysteine + methionine: 37%) and for *P. africana* (e.g., threonine: 46%, except valine and a very high content of cycteine and methionine). In conclusion, all seeds tested in the rat balance trial were of inferior quality compared to casein. Before these tropical seeds could be used as food components or feed supplements, safety studies and proper processing to remove antinutritional factors and possible toxic constituents were required. J. Nutr. 128: 2014–2022, 1998.

KEY WORDS: • *rats* • *tropical crop seeds* • *protein quality* • *nitrogen balance* • *antinutritional factors* • *amino acid score*

According to the World Hunger Project, 50% of the world's hungry people live in five countries, including Nigeria. A combination of several factors, e.g., the onset of the Sahalian drought, the neglect of the agricultural sector by the government, rising population and import bans on some cereal staples such as rice and corn, resulted in limited food production (Igbedioh 1996). Whereas the percentage of underweight children has declined in all continents over 15 yr (1975–1990), the numbers in Africa actually have increased from 19.7 to 27.4 million (Pellett 1996). During hungry periods, energy and protein intakes decreased by 20–30% or more, although the average protein intake per day in 13- to 19-yr-olds can be as low as 20 g (Igbedioh 1996). However, energy availability may also affect protein utilization because of the interrelationship of protein and energy metabolism (Elwyn 1993). Furthermore, because the diets in developing regions depend mainly on cereals for both protein and dietary energy, they insufficiently provide indispensable amino acids (Young and Pellett 1990, Pellett 1996), especially for the most vulnerable population groups such as children and pregnant women. In particular, lysine and the sulfur amino acids are likely to be limited in cereals and soybeans, respectively. Compared to the lysine requirement estimated for a 18–30-yr-old male with a body weight of 65 kg (2840 mg/d), Nigerian diets are deficient in lysine by 810 mg/d (Pellett 1996). Because animal protein production may not meet indispensable amino acid requirements, there is worldwide interest in the search for new plant species capable of supplementing traditional crops and staples. Consequently, the exploitation of presently neglected and lesser known plants of natural bushes and forests may be one approach to banning hunger (Becker 1986).

Comparing the amino acid patterns of major food protein sources, we found that legumes generally contain lysine amounts slightly lower than those found in beef and milk (Young and Pellett 1994). Moreover, a few reports on the chemical composition of African legume seeds indicate the potential usefulness of wild plants as food and feed (Badifu 1993, Apata and Ologhobo 1994, Madubuike et al. 1994, Ezeagu et al., 1996, Petzke et al. 1997). Because those plant

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TABLE 1

Characterization of selected tropical crop seeds

Genus and species	Family	Described[1]	Toxicity[2]	Use as food[3,2]
Adansonia digitata	Bombacaceae	yes	no	Humans, baobab (i.e., milk of fruits, gubdi "or soup of leaves, miyar kuka") (Obizoba and Anyika 1994)
Prosopis africana	Leguminosae	yes	no	Humans, okpiye, condiment prepared by fermentation of seeds (Achi 1992)
Lonchocarpus sericeus	Leguminosae	no	yes	unknown
Enterolobium cyclocarpium	Leguminosae	no	unknown	Humans (fruits)
Sesbania pachycarpa	Leguminosae	yes	no	Humans
Pterocarpus osun	Leguminosae	no	no	Humans

[1] Keay 1989.
[2] I.E. Ezeagu, unpublished data.
[3] Ezeagu et al. (1996).

seeds may contain antinutritional factors and other harmful substances, they should be examined in animal diets before being used in human diets.

Hence, based on the classical methods, our study investigates in vivo the food potential of several legumes and one other crop gathered in the Nigerian wild, and consumed as the sole protein source by growing rats. We also provide data on the chemical composition and antinutritional factors in these seeds used as unconventional food sources.

MATERIALS AND METHODS

Collection of seed samples. Mature wild seeds (35–50 kg) were harvested during the dry period (October 1993–January 1994) from villages around the city of Ibadan, Nigeria, with the help of the natives. Collections were taken from several plants to get a representative sample for the Ibadan region. The samples were identified at the Forestry Research Institute, Ibadan. **Table 1** provides a characterization of the tropical crop seeds analyzed. A soybean sample (*Glycine max,* TGX 1660-15F) was provided by The International Institute of Tropical Agriculture, Ibadan, Nigeria, and was analyzed to compare the amino acid profile with that of the wild seed samples. An aliquot of the raw seeds was milled to flours in a Wiley mill (Rekord A, Gbr. Jehmlich GmbH, Nossen, Germany) to pass a 0.5-mm mesh sieve and stored in air-tight containers at 4°C until analysis.

Analytical procedures of seed samples. Moisture content was determined by drying at 110°C using an oven (T 6030, Heraeus Instruments, Hanau, Germany) until reaching weight constancy (at least for 24 h). Nitrogen was determined by the standard micro-Kjeldahl method (AOAC 1990) using a digestion apparatus (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany) and a titration system (T110-TR160-TA10-TM120, Schott-Geräte GmbH, Hofheim, Germany). The crude protein content was calculated by multiplying percentage nitrogen by factor 5.71, which takes into account the nonprotein nature of part of the nitrogen and has been approved for calculating crude protein content in soybeans (Pellett and Young 1980, Petzke et al. 1997). Crude lipid content was assayed by extraction with petroleum ether (b.p. 40–60°C) in a Soxhlet extractor (AOAC 1990). Gross energy was determined by the use of adiabatic bomb calorimeter (IKA-Calorimeter C4000, Janke & Kunkel, IKA Analysentechnik, Heitersheim, Germany). Carbohydrate content was calculated by difference. Trypsin inhibitor activity was determined using benzoyl-DL-arginine-p-nitroanilide as substrate (Kakade et al. 1974), and tannin was estimated by the Folin-Denis method spectrophotometrically (UVIKON 932, Kontron Instruments GmbH, Neufahrn, Germany) as outlined by AOAC (1990). The presence of alkaloids was qualitatively estimated by the combined method of Seaforth (1964) and Hultin and Torssell (1965). Samples (0.5 g) were extracted in 20 mL of 0.275 mol/L of hydrochloric acid. The extract was treated with Mayer's reagent visually compared to strychnine solution (0.1 mg/100 mL) and classified as weak, moderate, strong and very strong. Ash, phytate and oxalates (total and soluble) were estimated by methods of AOAC (1990), Davies and Reid (1979) and Baker (1952), respectively. Using the method of Carpenter (1960) modified by Booth (1971), available lysine was estimated as fluorodinitrobenzene-reactive lysine. The in vitro digestibility of the samples was assayed by the multienzyme technique of Hsu et al. (1977). Total dietary fiber was determined by an enzymatic-gravimetric method (AOAC 1990).

To determine the amino acid composition, seed samples were prepared based on recommendations in the Report of the Joint FAO/WHO Expert Consultation (FAO/WHO 1991), hydrolyzed with 6 mol/L hydrochloric acid (2.5 mg of nitrogen/150 mL of hydrochloric acid, 24 hr under reflux by a continuous flow of nitrogen, and after drying (40°C), washed twice with distilled water to remove residual hydrochloric acid and dried again. Norleucine served as an internal standard. Cysteine and methionine, which can be partially destroyed during the acid hydrolysis, were converted to acid-stable derivatives (cysteic acid and methionine sulfone, respectively) by performic acid oxidation (Weidner and Eggum 1966). The oxidized samples were then hydrolyzed with 6 mol/L of hydrochloric acid as described above. For tryptophan determination, alkaline hydrolysis was performed according to Rowan et al. (1989) using 4.3 mol/L of NaOH in Teflon containers which were flushed with nitrogen and placed in an oven (T 6030, Heraeus Instruments, Hanau, Germany) maintained at 110°C for 24 h. 5-Methyltryptophan was used as an internal standard. The hydrolyzed samples were stored at −18°C in citrate buffer at pH 2.2 prior to analysis. Amino acids were analyzed by ion-exchange chromatography with post-column ninhydrin detection using a high performance liquid chromatographic system (System Gold, Beckman Instruments, Inc., Fullerton, CA).

The protein digestibility-corrected amino acid score (PDCAAS)[3] of indispensable amino acids was calculated according to the recommendations of the Joint FAO/WHO Expert Consultation based on amino acid requirements of the preschool child, an individual nitrogen-to-protein factor based on amino acid nitrogen content for each seed sample and the protein digestibility value that was determined in this study (Clugston et al. 1996, FAO/WHO 1991). The following equation was used:

$$\text{PDCAAS (\%)} = [\text{AAC} \times \text{Cf} \times \text{D}]/\text{AAP} \qquad (1)$$

where AAC is the amino acid content in food protein (mg/g crude protein), Cf is the correction factor for crude protein [100/(nitrogen percentage determined as amino acid nitrogen)], D is the true digestibility or in vitro protein digestibility, and AAP is the amino acid

[3]Abbreviations used: AAC, amino acid content; AAP, amino acid content in 1985 FAO/WHO/UNU requirement pattern for ages 2–5 yr (mg/g crude protein); BMG, body mass gain; BV, biological value; Cf, correction factor; D, digestibility; EFE, endogenous fecal energy; EFN, endogenous fecal nitrogen; EI, energy intake; EUN, endogenous urinary nitrogen; FE, fecal energy; FI, feed intake; FN, fecal nitrogen; NI, nitrogen intake; NPU, net protein utilization; PDCAAS, protein digestibility-corrected amino acid score; UN, urinary nitrogen.

TABLE 2

Composition, energy and nitrogen contents of the experimental diets used in the pretest and the nitrogen-balance experiment

Diet	Egg[1]	Casein + methionine (control)[1]	*Adansonia digitata*[1]	*Prosopis africana*[2]	*Lonchocarpus sericeus*[2]	*Enterolobium cyclocarpium*[1,2]	*Sesbania pachycarpa*[1,2]	*Pterocarpus osun*[2]
				g/kg				
Ingredients								
Seed (milled, heat treated)	—	—	570	450	360	450	300	350
Casein[3]	—	115	—	—	—	—	—	—
L-Methionine[4]	—	5	—	—	—	—	—	—
Whole egg[5]	48.2	—	—	—	—	—	—	—
Wheat starch[6]	753.7	660	305	365	530	385	540	510
Sunflower seed oil[7]	78.1	100	15	75	0	55	50	30
Cellulose[8]	50	50	50	50	50	50	50	50
Vitamin mix[9]	20	20	20	20	20	20	20	20
Salt mix[9]	50	50	40	40	40	40	40	40
Nitrogen	7.5	17.5	16.4	16.8	16.5	17.0	16.3	16.0
Energy (MJ/kg)	18.2	18.4	18.1	18.4	17.9	17.3	17.4	19.1

[1] Diets in the main nitrogen-balance experiment.
[2] Seeds have been used in a pretest (except *A. digitata*) over 6 d in groups of three rats.
[3] Dauermilchwerk Peiting GmbH, Landshut, Germany, contained 86% crude protein (nitrogen % × 6.38).
[4] Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany.
[5] Rex Deneke GmbH, Berlin, Germany.
[6] Heller u. Strauß, Berlin, Germany.
[7] Thomy GmbH, Karlsruhe, Germany.
[8] Rettenmeier, Ellwangen, Germany.
[9] Hernández-Triana et al. (1996).

content in 1985 FAO/WHO/UNU requirement pattern for ages 2–5 yr (mg/g crude protein).

All determinations of chemical composition were duplicated with chemicals purchased from several suppliers (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany; Merck KGaA, Darmstadt, Germany; Fluka Chemie AG, Buchs, Switzerland) and of analytical grade.

Animals, diets and feeding experiments. Weanling Wistar rats (Tierzucht Schönwalde GmbH, Schönwalde, Germany) were fed with free access to a stock diet (Altromin, Lage, Germany; 190 g/kg of crude protein, 40 g/kg of crude fat, 11.9 MJ/kg of metabolizable energy) prior to the experiments. Because these seeds, might be toxic, we examined five samples in a 6-d pretest to investigate acceptability, feed intake and growth. When about 5 wk old (body mass ≈88 g), rats in five groups of three rats each were randomly selected to participate in this pretest (**Table 2**). Seeds used in the nitrogen-balance experiment were selected according to the pretest results. In the main balance experiment, three seeds were fed as the sole source of protein for 10 d (4-d adaptation period, 6-d urine and feces collection) to randomly selected groups of six animals each (Table 2). The experimental protocol has been evaluated and approved by the ethics committee of the Ministry of Nutrition, Agriculture and Forestry of the Government of the Federal State of Brandenburg, Germany.

The food composition of test diets is shown in Table 2. In addition to the casein-fed control group [fortified with L-methionine (5 g/100/g of protein)], a separate group fed 4 g of crude egg protein/100 g diet was introduced to correct for endogenous nitrogen losses. Urinary and fecal nitrogen should be of endogenous origin assuming complete absorption and retention of the 4 g of crude egg protein diet nitrogen by rats (Pellett and Young 1980). Prior to the preparation of the diets, the seeds were autoclaved at 105°C and 210 kPa for 20 min as recently described (Barth et al. 1993) to inactivate heat-labile constituents as lectins or enzyme (trypsin) inhibitors that were suspected to be active in all leguminose seeds (McGuiness et al. 1982, Phillips 1993). The amounts of seed flour and sunflower oil in the diets were varied to obtain isonitrogenous and isoenergetic mixtures. During the experiments the animals were kept individually in specially constructed wire-mesh-bottom metabolic cages to allow quantitative collection of urine and feces. The room temperature was 24.6 ± 1.3°C at controlled humidity and a fixed 12-h light-dark cycle (0700–1900 h of light). Feed intake (FI) was recorded daily, body mass on days 0 and 6 (pretest) and on d 0, 4 and 10 (main nitrogen-balance experiment).

Sampling, analysis and calculation of results. Feed and nitrogen efficiency were calculated according to Equations 2 and 3.

$$\text{Feed efficiency} = \text{BMG/FI} \quad (2)$$

$$\text{Nitrogen efficiency} = \text{BMG/NI} \quad (3)$$

where BMG is the body mass gain and NI is the nitrogen intake in grams. Urine and feces were collected in 0.5 mol/L of sulfuric acid over a 24-h period. Fecal samples were dried and ground. The nitrogen content was determined by a micro-Kjeldahl method as mentioned above. For carcass analysis, the rats were killed by ether inhalation after 3 h of food deprivation. The bodies were autoclaved in 1.37 mol/L of hydrochloric acid for 2 h, dried at 105°C and ground. The protein content was calculated by multiplying nitrogen percentage by the factor 6.25. The energy content was determined as mentioned above. The nonprotein energy (gross energy − protein content × 23.6 kJ) given includes fat, all minor nonfat components, carbohydrates and organic acids. To calculate the retained nutrients during the balance experiment, six animals (mean body mass 90 g) which did not receive the experimental diets were used to determine the carcass composition at the beginning of the balance test period. Estimation of the endogenous excretion of nitrogen and energy was performed using fecal nitrogen (FN), urinary nitrogen (UN) and fecal energy (FE) values of the group fed a 4 g/100/g crude egg protein diet during nitrogen-balance collection period d 5–10 (FN = 76.5 ± 0.8 mg; UN = 121.7 ± 7.0 mg; FE = 82.0 ± 2.6 kJ).

Parameters of digestibility were calculated using the following equations:

$$\text{Apparent nitrogen digestibility} = [\text{NI} - \text{FN}]/\text{NI} \quad (4)$$

$$\text{True nitrogen digestibility} = [\text{NI} - (\text{FN} - \text{EFN})]/\text{NI} \quad (5)$$

$$\text{Apparent energy digestibility} = [\text{EI} - \text{FE}]/\text{EI} \quad (6)$$

$$\text{True energy digestibility} = [\text{EI} - (\text{FE} - \text{EFE})]/\text{EI} \quad (7)$$

TABLE 3

Chemical characteristics of selected tropical crop seeds[1]

Seed	*Adansonia digitata*	*Prosopis africana*	*Lonchocarpus sericeus*	*Enterolobium cyclocarpium*	*Sesbania pachycarpa*	*Pterocarpus osun*
Nitrogen, *g/kg*	28.1	35.9	44.8	35.3	52.7	45.6
Amino acid nitrogen, %	82.0	93.8	66.4	74.6	67.9	49.6
Crude protein[2], *g/kg*	160.5	205.0	255.8	201.6	300.9	260.4
Crude protein, corrected[3], *g/kg*	144.0	210.0	185.4	164.6	222.6	141.1
Fat, *g/kg*	161.4	50.9	341.5	103.2	161.9	202.7
Total carbohydrates, *g/kg*	567.5	620.7	310.1	578.1	439.7	440.7
Ash, *g/kg*	49.5	36.7	37.7	40.0	28.9	38.5
Moisture, *g/kg*	61.1	86.7	54.9	77.1	68.6	57.7
Gross energy, *MJ/kg*	19.35	17.05	24.37	16.99	18.66	23.89
Trypsin inhibitor, raw seeds, *IU/mg*	0	11.9	7.7	25.5	12.8	40.8
Trypsin inhibitor, heat-treated seeds[4], *IU/mg*	0	3.4	0	8.5	0	nd[5]
Phytic acid, *g/kg*	14.0	12.5	12.5	11.6	7.2	9.2
Oxalate, total, *g/kg*	4.2	33.4	14.4	9.3	12.6	11.7
Oxalate, soluble, *g/kg*	2.6	23.3	12.8	5.9	9.8	7.5
Tannin, *g/kg*	1.2	2.5	3.8	1.8	2.4	6.9
Alkaloids (qualitative)[6]	(+)	+++	++	+++	(+)	+

[1] Per unit of fresh weight.
[2] Protein conversion factor 5.71.
[3] Using individual correction factors for each seed sample based on amino acid nitrogen content according to the formula: (N × 6.25)/(Cf), where (Cf) = 100/(percentage nitrogen determined as amino acid nitrogen).
[4] Autoclaved at 105°C and 210 kPa for 20 min.
[5] Not determined
[6] (+) weak, + moderate, ++ strong, +++ very strong.

where EFN is the endogenous fecal nitrogen in mg, EI is the energy intake, and EFE is the endogenous fecal energy in kJ.

The biological value (BV) and net protein utilization (NPU) were calculated according to Equations 8 and 9:

$$BV = [NI - (UN + FN) + EUN + EFN]/[NI - (FN - EFN)] \quad (8)$$

$$NPU = [NI - (UN + FN) + EUN + EFN]/NI \quad (9)$$

where EUN is the endogenous urinary nitrogen.

Statistical analysis. Values are means ± SEM. Significant differences between mean values were determined by analysis of variance followed by comparisons using the Newman-Keuls multiple range test (Snedecor and Cochran, 1989). $P < 0.05$ was considered as significance limit.

RESULTS AND DISCUSSION

Food composition. The chemical composition of the seeds under investigation are given in **Table 3.** The corrected protein concentration varied from 140 to 220 g/kg, the fat concentration from 51 to 203 g/kg. Among the antinutritive factors, the high trypsin inhibitor activity in seeds of *Enterolobium cyclocarpium* and *Pterocarpus osun*, the tannin content of *P. osun* and the high oxalate content of *Prosopis africana* were striking; *P. africana* and *E. cyclocarpium* contained high amounts of alkaloids.

The amino acid composition, the available lysine content and the PDCAAS values are summarized in **Tables 4** and **5.** To our knowledge, this is the first report on the amino acid composition of these tropical seeds with the exception of *Adansonia digitata* and *P. africana*. While the amino acid composition of *A. digitata* seeds (Table 4) corresponds to the composition reported earlier (FAO 1970), the data for *P. africana* differs considerably for methionine and valine, which were found to be much higher in the seeds of our study. Values for cysteine and tryptophan were not given in the literature (FAO 1970). An extraordinary high content of sulfur amino acids was determined in seeds of *P. africana*, which results in a PDCAAS value of 460% for cysteine + methionine (Table 5). This is unusual for naturally occurring leguminous proteins which are reported to have a low content of sulfur-containing amino acids (Zarkadas et al. 1997). Therefore, growth depression and reduced food intake in the pretest group consuming *P. africana* seeds as the only protein source may be related to the excess of sulfur amino acids. In toxicity studies of amino acids, consumption of methionine at four times its requirement results in growth depression and tissue damage when incorporated into a diet low in protein (Harper et al. 1970). The cysteine/methionine molar ratios were found to be relatively high but comparable to other vegetable protein sources—soy, rapeseed, wheat and pea—and amounts to 1.7, 1.0, 2.6, 1.5, 1.8, 4.7, and 2.2 for *A. digitata, P. africana, Lonchocarpus sericeus, E. cyclocarpium, S. pachycarpa, P. osun* and *G. max*, respectively (Sarwar 1997).

Based on the amino acid requirement pattern of the preschool child (FAO/WHO 1991) and the true protein digestibility (Table 5), in seed samples of *A. digitata, S. pachycarpa* and *P. osun*, lysine was estimated to be the first limiting amino acid. The sulfur amino acids were found to be first-limiting in seed proteins of *L. sericeus* and *E. cyclocarpium* and threonine in *P. africana*, respectively (Table 5). For calculating the PDCAAS of *P. africana* and *L. sericeus*, the in vitro protein digestibility values of the pretest seed samples were used. In vitro protein digestibility compares favorably with values obtained by in vivo methods applied in rats (Maga et al. 1973, Saunders et al. 1973). Furthermore, relatively low PDCAAS values of nearly all indispensable amino acids in *P. africana, E. cyclocarpium* and *P. osun* were observed (Table 5), although the low amino acid nitrogen-to-total-nitrogen ratio (Table 4) was taken into account by using individual nitrogen-to-protein factors for each seed sample (Petzke et al. 1997). Furthermore, PDCAAS may overestimate the quality of heat-treated milled

TABLE 4

Amino acid composition, available lysine, and percentage amino acid nitrogen in selected tropical crop seeds compared to a tropical soybean[1,2]

Seed	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Valine	Isoleucine	Leucine	Tyrosine
						(mg/gN)					
Adansonia digitata	540	182	298	1363	307	287	265	312	221	409	170
Prosopis africana	607	134	272	1229	244	509	185	457	151	418	177
Lonchocarpus sericeus	561	202	259	719	257	226	210	236	204	441	207
Enterolobium cyclocarpium	637	203	332	906	314	290	260	265	232	488	247
Sesbania pachycarpa	459	170	223	878	246	269	193	214	194	338	190
Pterocarpus osun	370	187	197	473	248	195	194	202	158	292	219
Glycine max	778	256	336	1249	426	293	277	317	293	507	265

Seed	Phenylalanine	Histidine	Lysine	Arginine	Cysteine	Methionine	Tryptophan	Available lysine		Nitrogen determined as amino acid nitrogen
	(mg/g N)								*% of total lysine*	*%*
Adansonia digitata	284	124	233	588	120	78	86	178	69	82.8
Prosopis africana	173	108	377	616	490	550	55	241	64	93.8
Lonchocarpus sericeus	307	117	375	337	91	37	58	243	65	66.4
Enterolobium cyclocarpium	223	146	347	380	50	35	46	387	102	74.6
Sesbania pachycarpa	236	143	260	466	121	73	71	204	72	67.6
Pterocarpus osun	178	81	181	253	125	28	71	179	99	49.6
Glycine max	334	170	422	499	144	69	100	331	78	91.9

[1] Values of amino acid composition are means of two hydrolyzations each injected twice.
[2] Values of available lysine are means of two determinations.

seed proteins when considerable amounts of antinutritional factors remain and digestibility is low compared to a highly digestible protein like casein (Sarwar 1997). Nevertheless, a positive correlation was obtained relating the data on NPU (Table 7) for *A. digitata*, *E. cyclocarpium*, *S. pachycarpa* and *P. osun* (37.7 ± 3.1) to PDCAAS for cysteine + methionine ($r = 0.96$) even if PDCAAS is > 100%. There was no correlation between PDCAAS for lysine and NPU ($r = 0.32$).

Sample matrix factors related to the carbohydrate constituents may interfere with the fluorodinitrobenzene reactivity of lysine in tropical crop seeds, thus questioning the so-determined nutritional availability of lysine (Booth 1971, Pellett and Young 1980, Petzke et al. 1997). Our observation of artificially high values of about 100% for *E. cyclocarpium* and *P. osun* (Table 4) might be explained in this context. However, since we have not characterized carbohydrates in this study a definitive final explanation cannot be given. For the remaining seed samples, the available lysine ranged from 64 to

TABLE 5

Protein digestibility-corrected amino acid score of indispensable amino acids including histidine of selected tropical crop seeds compared to a tropical soybean variety and to casein (fortified with L-methionine)[1]

Seed	Threonine	Valine	Isoleucine	Leucine	Tyrosine + phenylalanine	Histidine	Lysine	Cysteine + Methionine	Tryptophan
					%				
Adansonia digitata	78	129	115	90	105	95	58[2]	115	113
Prosopis africana	46[2]	154	64	75	65	65	77	490	59
Lonchocarpus sericeus	96	109	118	108	132	99	104	83[2]	86
Enterolobium cyclocarpium	66	83	91	81	82	84	66	37[2]	65
Sesbania pachycarpa	98	120	136	101	133	147	88[2]	152	125
Pterocarpus osun	87	91	89	70	100	67	49[2]	97	102
Glycine max	98	118	137	100	124	117	95[2]	111	119
Casein (+ L-methionine)	124	161	162	136	166	150	131	130 (327)	105

[1] PDCAAS: Based on amino acid requirement of the preschool child, an individual seed-specific correction factor for crude protein based on amino acid nitrogen content, and on true protein digestibility (except for *P. africana, L. sericeus* and *G. max* using in vitro protein digestibility values of 0.69, 0.67 and 0.75, respectively).
[2] Indicating the value of the first limiting amino acid.

TABLE 6

Body mass, feed intake and feed efficiency in rats after a 10-d feeding of diets containing selected heat-treated tropical crop seeds[1,2]

Protein source		Casein + methionine (control)	*Adansonia digitata*	*Enterolobium cyclocarpium*	*Sesbania pachycarpa*
Feed intake, *g*	d 1–10	94.2 ± 0.9[a]	92.2 ± 3.3[a]	64.6 ± 9.7[c]	84.4 ± 4.9[b]
	d 5–10[3]	59.2 ± 0.2[a]	57.9 ± 2.6[a]	46.5 ± 4.8[b]	57.8 ± 1.3[a]
Body mass, *g*	initial	90.5 ± 8.5	90.2 ± 8.2	86.7 ± 7.1	86.6 ± 7.3
	final	120.1 ± 6.3	93.9 ± 6.9	76.6 ± 7.1	97.5 ± 7.4
Body mass gain, *g*	d 1–10	29.8 ± 5.6[a]	3.7 ± 3.5[c]	−10.0 ± 4.2[d]	10.9 ± 4.1[b]
	d 5–10[3]	25.5 ± 3.0[a]	10.6 ± 2.6[c]	−0.3 ± 2.6[d]	15.4 ± 2.2[b]
Feed efficiency, *g gain/g feed intake*	d 1–10	0.31 ± 0.06[a]	0.04 ± 0.04[b]	−0.14 ± 0.06[c]	0.12 ± 0.04[b]
	d 5–10[3]	0.43 ± 0.05[a]	0.18 ± 0.04[c]	−0.01 ± 0.06[d]	0.26 ± 0.04[b]
Protein efficiency, *g gain/g protein intake*	d 1–10	2.83 ± 0.52[a]	0.41 ± 0.40[c]	−1.59 ± 0.64[d]	1.36 ± 0.46[b]
	d 5–10[3]	3.93 ± 0.34[a]	1.96 ± 0.42[c]	−0.08 ± 0.65[d]	2.86 ± 0.40[b]

[1] Values are means ± SEM, $n = 6$. Means within a row not sharing a common superscript letter are significantly different, $P < 0.05$.
[2] For food composition see Table 2.
[3] Nitrogen-balance collection period.

72% which was lower than the soybean sample (78%) (Table 4). In general the fluorodinitrobenzene-reactive lysine method is a mere chemical assay which can not be translated directly into terms of in vivo conditions. Recently, a new bioassay for determining digestible reactive lysine was suggested (Rutherford et al. 1997), which is basically the combination of a true lysine digestibility assay and the determination of the availability of lysine by guanidation.

In vivo protein quality. Seeds were selected for the nitrogen-balance experiment on the basis of the pretest results. Due to the poor feed intake and the weight loss of the rats, *P. africana, L. sericeus* and *P. osun* were excluded from the main nitrogen-balance test. In the groups consuming *E. cyclocarpium* and *S. pachycarpa,* increased food intake was observed after the first 3 or 4 d of feeding, indicating a gradual adaptation to the diets. The main nitrogen-balance experiment was performed using *A. digitata* in addition to *E. cyclocarpium* and *S. pachycarpa* (Table 2), the results of which are summarized in **Tables 6–9.** In Table 6 BMG and feed efficiency during the total (d 1–10) and the balance (d 5–10) periods are given separately. Compared to casein (reference protein), the rats receiving *A. digitata* and *S. pachycarpa* consumed the same quantity of food in the balance period, whereas intake was significantly lower than the diet containing *E. cyclocarpium.* Weight gain, however, was significantly lower in all experimental groups compared to the casein group. With seed, *E. cyclocarpium* rats did not grow at all during the balance period. After feeding the diets for 3 or 4 d (prebalance period), rats adapted to the food: food consumption increased and weight gain occurred. Similar to the weight development, feed and protein efficiency were also lower for all diets compared to casein. The diet containing *S. pachycarpa* gave the most positive results among the investigated seeds.

Table 7 shows the in vivo protein evaluation by the nitrogen-balance method. Besides the distinctly higher UN excretion, a remarkably reduced protein digestibility of all seeds, especially for *E. cyclocarpium,* was observed. It is striking that the FN excretion and the ratio FN/UN excretion of all groups are far higher than in the casein group. The low protein digestibility may be attributed to increases of endogenous protein losses and to high FN excretion. Using a ^{15}N-dilution method, Huisman et al. (1992) estimated that endogenous protein is both enhanced after feeding legumes (raw and toasted peas and beans) in piglets and increased by the admin-

TABLE 7

Protein quality evaluation by nitrogen-balance in rats fed diets containing selected heat-treated tropical crop seeds[1,2,3]

Protein source	Casein + methionine (control)	*Adansonia digitata*	*Enterolobium cyclocarpium*	*Sesbania pachycarpa*
Nitrogen intake, *mg*	1036.0 ± 3.0[a]	947.8 ± 42.9[b]	791.3 ± 81.6[c]	942.8 ± 20.8[b]
Urinary nitrogen, *mg*	220.3 ± 33.4[c]	333.3 ± 14.8[b]	361.6 ± 36.7[ab]	399.2 ± 25.9[a]
Fecal nitrogen, *mg*	91.6 ± 7.9[d]	319.9 ± 32.7[b]	462.0 ± 56.5[a]	237.3 ± 6.2[c]
Fecal nitrogen/urinary nitrogen	0.42	0.96	1.28	0.59
Nitrogen balance, *mg*	724.1 ± 35.1[a]	294.6 ± 23.8[b]	−32.4 ± 55.4[c]	306.3 ± 11.8[b]
Apparent digestibility, %	91.2 ± 0.8[a]	66.3 ± 2.1[c]	41.5 ± 6.3[d]	74.8 ± 1.0[b]
True digestibility[4], %	98.5 ± 0.8[a]	74.4 ± 2.4[c]	51.2 ± 6.0[d]	82.9 ± 0.9[b]
BV[4], %	90.4 ± 3.9[a]	70.0 ± 2.4[b]	40.1 ± 9.6[d]	64.6 ± 2.4[c]
NPU[4], %	89.0 ± 3.3[a]	52.1 ± 2.3[b]	20.9 ± 6.7[c]	53.5 ± 1.7[b]

[1] Values are means ± SEM, $n = 6$. Means within a row not sharing a common superscript letter are significantly different, $P < 0.05$.
[2] Nitrogen-balance collection period d 5–10.
[3] For food composition see Table 2.
[4] Based on a 4% egg protein diet group ($n = 6$).

TABLE 8

Energy intakes and digestible energy in rats fed diets containing selected heat-treated tropical crop seeds[1,2,3]

Protein source	Casein + methionine (control)	*Adansonia digitata*	*Enterolobium cyclocarpium*	*Sesbania pachycarpa*
Energy intake, *kJ*	1088 ± 4[a]	1046 ± 46[ab]	805 ± 83[c]	1008 ± 22[b]
Feces, *g*	5.3 ± 0.5[c]	22.8 ± 1.0[a]	11.1 ± 1.6[b]	11.2 ± 0.5[b]
Feces, *kJ*	79 ± 6[c]	376 ± 19[a]	185 ± 26[b]	179 ± 8[b]
Digestible energy[4], %	92.8 ± 0.6[a]	64.0 ± 0.9[d]	77.0 ± 2.1[c]	82.3 ± 0.6[b]
True digestible energy, %	100.4 ± 0.6[a]	71.9 ± 1.0[d]	87.3 ± 2.6[c]	90.4 ± 0.7[b]

[1] Values are means ± SEM, $n = 6$. Means within a row not sharing a common superscript letter are significantly different, $P < 0.05$.
[2] Nitrogen-balance period d 5–10.
[3] For food composition see Table 2.
[4] Based on a 4% egg protein diet group ($n = 6$).

istration of isolated trypsin inhibitors and raw soybeans (Barth et al. 1993). In seeds of *E. cyclocarpium* the trypsin inhibitor was not destroyed completely (Table 3) and, consequently, the digestion of seed proteins may have been impaired as shown, e.g., for raw soybeans in minipigs (Barth et al. 1993, Pusztai et al. 1992). This observation confirms the need for suitable processing and control of antinutritional factors when these wild seeds are fed and that protein digestibility and protein quality may differ considerably for various unconventional seeds (Chitra et al. 1995, FAO 1989, Mnembuka and Eggum 1995, Phillips 1993, Siddiqui et al. 1994).

The BV of the seeds (Table 7) ranges from 0.40 to 0.76, thus reflecting the differences in excretion of nitrogen in urine and feces. Feeding of *S. pachycarpa* resulted in the most positive nitrogen balance, whereas *E. cyclocarpium* causes a slightly negative nitrogen balance. The NPU for *A. digitata* and *S. pachycarpa* was comparable (0.52 and 0.54, respectively), but as a consequence of the high nitrogen excretion, especially in feces, these values are far lower than in the casein group (0.89). Differences in nitrogen retention are most likely influenced by the indigestible carbohydrate fraction of the seeds in which, e.g., the total and soluble starch content is quite variable (Ezeagu et al. 1996). Considerable amounts of total dietary fiber were determined for *P. africana* (77.6%), *L. sericeus* (32.9%), *E. cyclocarpium* (51.6%), and *S. pachycarpa* (55.5%). Their fiber specific effect on exogenous and endogenous nitrogen losses remains to be elucidated. However, when taking into account the actual increased endogenous protein excretion as reported for legumes, the NPU values observed may be artificially high. Relating NPU to nitrogen excretion, the nitrogen retention (apparent NPU) data were lower than those presented in Table 7. Therefore, an unknown portion of endogenous nitrogen remains unconsidered. Although the NPU as the classical approach has limitations when using diets containing complete seeds, it still allows a first in vivo evaluation of protein quality when amino acid data are also provided.

The digestible energy of the different diets (Table 8) varies substantially and is negatively correlated to the fecal mass and to the phytic acid content of the seeds (Table 3). Because of the weak relationship between digestible energy values and nitrogen excretion (Tables 7 and 8), other compounds, like fat, carbohydrates or dietary fiber, seem to be responsible for the different energy excretion in the groups. So Tovar et al. (1992) found an incomplete digestion of legume starches in balance studies with rats. *S. pachycarpa* showed the highest apparent and true digestibility of energy, followed by *E. cyclocarpium*. In contrast, seeds of *A. digitata* had the highest content of phytic acid and the lowest energy digestibility, the latter resulting from a feces production four-times higher than observed in the casein group.

To relate the feeding experiment results to a distinct seed component is difficult. However, it seems reasonable to conclude that the relatively well-balanced amino acid pattern of the proteins of *S. pachycarpa* (Tables 4, 5) is reflected in a better BMG or protein efficiency as compared to rats fed the

TABLE 9

Carcass analysis data of rats after feeding for 10 d diets containing selected heat-treated tropical crop seeds[1,2]

Protein source	Casein + methionine (control)	*Adansonia digitata*	*Enterolobium cyclocarpium*	*Sesbania pachycarpa*
Body mass, *g*	120.3 ± 6.2	93.9 ± 6.9	76.6 ± 7.1	97.5 ± 7.4
Dry mass, *g/100 g*	32.8 ± 1.1[ab]	31.0 ± 0.7[c]	32.2 ± 1.0[b]	33.8 ± 0.9[a]
H_2O, *g/100 g*	67.2 ± 1.1[bc]	69.0 ± 0.7[a]	67.8 ± 1.0[b]	66.2 ± 0.9[c]
Protein, *g/100 g*	17.5 ± 0.6[a]	17.4 ± 0.6[a]	17.3 ± 0.5[a]	16.7 ± 0.7[b]
Fat, *g/100 g*	8.2 ± 1.5[a]	5.5 ± 0.6[b]	6.3 ± 1.4[b]	9.2 ± 1.0[a]
Energy, *kJ/100 g*	731 ± 53[a]	625 ± 23[b]	654 ± 54[b]	749 ± 36[a]
Weight gain, *g*	29.8 ± 5.6[a]	3.7 ± 3.5[c]	−10.0 ± 4.2[d]	10.9 ± 4.1[b]
Retained H_2O[3], *g*	18.9 ± 4.5[a]	3.0 ± 3.0[b]	−7.4 ± 2.8[c]	5.2 ± 3.2[b]
Retained protein[3], *g*	5.3 ± 0.7[a]	0.8 ± 0.6[b]	−1.8 ± 0.6[c]	1.3 ± 0.8[b]
Retained fat[3], *g*	3.2 ± 2.0[a]	−1.4 ± 0.6[b]	−1.5 ± 1.4[b]	2.6 ± 1.0[a]
Retained energy[3], *kJ*	253 ± 70[a]	−39 ± 22[c]	−99 ± 57[c]	130 ± 41[b]

[1] Values are means ± SEM, $n = 6$. Means within a row not sharing a common superscript letter are significantly different, $P < 0.05$.
[2] For food composition see Table 2.
[3] Related to a zero-group with the following starting parameters: Body mass, 90.1 ± 0.9 g; H_2O, 68.5 ± 0.9%; protein 17.3 ± 0.5%; fat, 7.3 ± 0.4%; gross energy, 694 ± 22 kJ/100/g.

other seeds. Nevertheless, effects of antinutritive factors (Table 3) on food consumption and feed efficiency should be considered. The diet resulting in the lowest food intake and growth (*E. cyclocarpium*) had the highest alkaloid content and some residual trypsin inhibitor activity after heat treatment (Tables 3 and 6). A high variability of antinutritive factors was reported for other legume seeds also (Sotelo et al. 1995a, b, Chitra et al. 1995). Thus, wild species of *Vigna* spp generally showed a higher protein content, antitryptic activity and tannin content as well as lower protein digestibility than cultivated species (Carnovale et al. 1991). Wild species of *Phaseolus vulgaris* were reported to contain higher amounts of trypsin inhibitors and lectins (Sotelo et al. 1995b). However, tannins isolated from cowpeas appeared not to change growth rate, protein efficiency ratio and nitrogen excretion when given to rats in increasing concentrations (Chang et al. 1994). On the other hand in young pigs a high tannin content in the diet (hulls of faba beans) reduced the apparent and true ileal digestibility of crude protein and amino acids and increased the excretion of endogenous protein (Jansman et al. 1995).

In addition the body composition of the rats, especially the protein and fat content, should give information about the nutrient retention of the diets after the 10-d feeding period (**Table 9**). Compared to the casein group, the carcasses of rats fed *A. digitata* contained more water, whereas feeding *S. pachycarpa* resulted in a significantly reduced protein and water content. The carcasses of rats receiving *A. digitata* and *E. cyclocarpium* showed a lower fat and energy content. Rats consuming *S. pachycarpa* retained more nutrients and energy in comparison to other seeds. This is mainly attributed to a higher body weight gain explained by better protein and energy digestibility of this diet. The results are consistent with those of Rubio et al. (1991), showing a reduced body weight gain and lower body fat and protein content after feeding *Vicia faba* meal to rats in comparison to lactalbumin.

In conclusion, none of the seeds investigated resulted in a high biological value in comparison to the casein + methionine control. Use as food component or feed supplement seems to be possible for *S. pachycarpa* which showed the most advantageous nutritional parameters of the seeds examined, followed by *A. digitata*. Clearly, the mere information on chemical composition can only be a first indicator of a potential usefulness. From a nutritional standpoint these seeds may be used for human and animal nutrition as supplementary protein and energy sources to indigenous food and feed supply. A possible use could be the production of food supplements made from traditionally fermented beans which may also decrease additionally antinutritional factors (Jideani and Okeke 1991, Achi 1992). Prior to this, however, additional safety studies are needed to address possible toxic, carcinogenic and mutagenic effects of these seeds.

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