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Nutritional and Physiological Responses of Young Growing Rats to Diets Containing Raw Cowpea Seed Meal, Protein Isolate (Globulins), or Starch

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The nutritional and physiological effects of raw cowpea (*Vigna unguiculata* (L) Walp.) seed meal, protein isolate (globulins), or starch on the metabolism of young growing rats have been evaluated in 14-day trials. Wet and dry weight gain, feed conversion efficiency, and lipid and protein accretion were significantly reduced as a result of inclusion of seed meal, globulins, or starch in the diet, with growth retardation being most marked with the seed meal. The proportional weights of the small intestine and pancreas were increased by meal diets, and serum cholesterol levels were slightly reduced. The globulins and raw starch also increased relative small intestine weights but had no effect on the pancreas or serum constituents. The effects of cowpeas on rats appeared to be due primarily to the combined actions of globulins, resistant starches, protease inhibitors, and possibly fiber and non-starch polysaccharides on intestinal and systemic metabolism.

KEYWORDS: Cowpeas; globulins; starch; nutritional properties; intestine; pancreas; rat

INTRODUCTION

Legume seeds contain relatively high amounts of protein, energy, and micronutrients and form a primary food source for much of the world. In addition to this role as a source of essential nutrients, they may have health-promoting effects. In particular, some seeds are reported to have anticarcinogenic or hypolipidemic properties (*1*−*4*). These have been linked to a number of seed components, including fiber, slow-release starches, globulin proteins, and bioactive factors (*1*, *4*−*8*).

Raw legume seeds tend, however, to be unpalatable, and the bioavailability of the constituent protein and energy is poor (*9*, *10*). Some starch fractions, non-starch polysaccharides, and fiber are indigestible or poorly utilized by animals. In addition, the globulin (11S and 7S) proteins are generally refractory in nature, and the intact proteins or fragments derived from them can provoke mucotractive or acute immunological responses in some species. Utilization of the proteins is further compromised by deficiencies in essential amino acid content, particularly of the sulfur amino acids. Furthermore, the seeds often contain antinutritional/bioactive factors, such as enzyme (trypsin, chymotrypsin, α-amylase) inhibitors, lectins, tannins, phytates, saponins, etc., that may be bitter, may adversely affect digestion or bioavailability of other dietary constitutents, or may have debilitating effects on metabolism in the gut and associated tissues (*9*, *10*). Most legume seeds therefore require processing, usually heat treatment, before they are consumed (*9*). However, this can be energy expensive, and, although aimed at enhancing nutrient availability and palatability, it can occasionally have the opposite effect, particularly if the treatments are prolonged (*11*). In addition, it can lead to abolition of health-protective properties.

Cowpeas (*Vigna unguiculata*) are grown in many parts of the world (*12*−*15*). They adapt well to diverse environmental conditions, including arid regions and areas unsuited to growing soybeans (*13*−*15*). The seeds have high contents of protein (80−350 g/kg) and starch (500−650 g/kg) but generally contain only low or moderate levels of potentially harmful antinutritional factors (*13*, *16*−*18*, *20*−*23*). Cowpeas or products derived from them might therefore require less preprocessing than other commonly used seeds (*19*). They are already used as primary or supplemental feeds for both humans and animals and have great potential for further development (*12*−*14*).

Cowpea (*V. unguiculata* (L) Walp.) cultivars were recently tested by the Instituto Nacional de Investigaciones Forestal Agricolas y Pecuarias (Uxmal, Yucatan, Mexico) and found to grow well in the Yucatan penisula, giving high yields. The nutritional properties of the seeds have not, as yet, been tested

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Table 1. Composition of Experimental and Control Semisynthetic Diets

	experiment 1						experiment 2	
	diet 1	diet 2	diet 3	diet 4	diet 5	diet 6	diet 7	diet 8
lactalbumin	120	60		60			120	120
pedicilata meal[a]		220	442					
IT 86D-719 meal[b]				220	442			
protein isolate[b]						142		
starch[2]								500
maize starch	380	220	58	220	58	358	500	
potato starch	100	100	100	100	100	100	42	42
glucose	150	150	150	150	150	150	88	88
corn oil	150	150	150	150	150	150	150	150
minerals[c]	50	50	50	50	50	50	50	50
vitamins[d]	50	50	50	50	50	50	50	50
sodium silicate	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-tryptophan		0.26	0.50	0.26	0.50	0.50		
L-methionine		1.20	2.40	1.20	2.40	2.40		
protein ($N \times 6.25$)	100	100	100	100	100	100	100	100
available energy (MJ)	16.53	16.44	16.32	16.44	16.32	16.18	17.46	17.46
lectin[e]	0	0.05	0.10	0.05	0.10	0	0	0
protease inhibitor[f]	0	1.76	3.52	1.56	3.12	0	0	0

[a] Raw meal of *Vigna unguiculata* (L) Walp. cultivar pedicilata (composition: protein, 226 g/kg; starch, 660 g/kg; lipid, 14 g/kg; fiber, 30 g/kg). [b] Raw meal of *Vigna unguiculata* (L) Walp. cultivar IT 86D-719 (composition: protein, 223 g/kg; starch, 663 g/kg; lipid, 14 g/kg; fiber, 31 g/kg) or a protein isolate (704 g of protein/kg) or starch from it. [c] Mineral mix (1 kg) comprises copper sulfate, 400 mg; ferrous sulfate, 5000 mg; manganous sulfate, 4000 mg; zinc sulfate, 3600 mg; potassium iodate, 40 mg; potassium iodide, 40 mg; sodium fluoride, 120 mg; ammonium vanadate, 10 mg; nickel chloride, 80 mg; stannous chloride, 120 mg; sodium selenate, 6 mg; chrome alum, 960 mg; calcium carbonate, 420 g; potassium dihydrogen orthophosphate, 314 g; potassium chloride, 22 g; magnesium sulfate, 102 g; and disodium hydrogen orthophosphate, 142 g. [d] Vitamin mix (1 kg) contains thiamine, 200 mg; pyridoxine, 200 mg; riboflavin, 200 mg; *p*-aminobenzoic acid, 200 mg; nicotinic acid, 600 mg; calcium pantothenate, 400 mg; folic acid, 100 mg; biotin, 100 mg; inositol, 8000 mg; α-tocopherol, 5000 mg; retinyl acetate, 230 mg; cholecalciferol, 300 mg; cyanocobalamin, 5 mg; menadione, 100 mg; and choline chloride, 20 g, made up to 1 kg with maize starch. [e] Hemagglutinating units $\times 10^{-4}$/kg of diet. [f] Trypsin inhibited (g/kg of diet).

in vivo. In the present study, the nutritional and physiological effects of raw cowpea cultivars IT 86D-719 and pedicilata, grown in Yucatan, and a protein isolate (globulins) and starch preparation derived from cultivar IT 86D-719 on the metabolism of young growing rats have been evaluated in 14-day feeding trials.

MATERIALS AND METHODS

Materials. Cowpea (*V. unguiculata* (L) Walp.) cultivar IT 86D-719 was grown at the Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias-INIFAP (Uxmal, Yucatan, Mexico), and cultivar pedicilata was grown in the rural area of Yaxcaba (Central Yucatan, Mexico). Starch and protein isolates (globulins) were prepared from cowpea cultivar IT 86D-719 as described previously (*22*). Briefly, the seeds were soaked in 0.2% sodium sulfide overnight and coarsely ground, and the seed coat was floated off. Seed kernels were mixed in water and milled. The pH of the mixture was raised to 9 with sodium hydroxide, and the mixture was sieved 20 min later to remove fiber and large particulate matter. The sievate was allowed to settle for 3 h, and the supernatant was decanted off. The sediment (starch) was washed with water and recovered (approximately 400 g/kg). The pH of the supernatant was adjusted to 4.3 with hydrochloric acid, and the supernatant was heated at 80 °C for 10 min before recovery of the proteins by centrifugation. This heat treatment facilitated aggregation/precipitation of the proteins and rapid recovery of the protein. The isolated proteins (around 200 g/kg) were then oven-dried (40 °C). The protein isolate used in these studies contained no detectable trypsin inhibitor or lectin. However, small but variable amounts of trypsin inhibitor (<1.18 g/100 g of protein) and trace levels of lectin have been detected in some other batches of the cowpea protein isolates.

Diets. Isonitrogenous (100 g protein/kg) diets were formulated (**Table 1**) as described previously (*24*, *25*). Seed meals were included, by substitution for maize starch, as the sole source of protein (442 g of seed meal/kg of diet) or as half of the dietary protein (220 g of seed meal/kg of diet), with the remainder of the protein (50 g/kg) being provided by lactalbumin. The protein isolate was given as the sole (100 g/kg) source of protein. To evaluate the nutritional quality of the starch fraction, diets (100 g of lactalbumin protein/kg) containing 500 g of either maize or Vigna starch per kilogram were formulated (**Table 1**). A concern with the seed starch was that trace amounts of antinutritional/bioactive factors might be present in the preparation. A higher than usual starch inclusion level was thus used to maximize the likelihood that the possible effect of any antinutritional/bioactive factors in the product could be detected. Potato starch was included, as an alternative to cellulose, in all diets as a source of fiber.

The sulfur amino acids (52% of the requirement) and L-tryptophan (66% of the requirement) were the first and second limiting amino acids in the cowpea protein diets. All other essential amino acids were present at 86% or more of the requirement. Diets were supplemented with L-methionine and L-tryptophan to bring the dietary levels of these particular amino acids up to the target requirements for rats.

Animals. Male Hooded-Lister rats (Rowett strain), reared and housed in the small-animal unit of the Rowett Research Institute, were weaned at 19 d and given free access to a nonpurified diet (SDS, Witham, Essex, UK) for 10 days. They were then offered a semisynthetic (control) diet (**Table 1**) for 3−7 days before being placed on experiment. Water was freely available at all times.

Experiment 1. Rats (85 ± 2 g, four per treatment) were fed exclusively with control (diet 1) or test (diets 2−6) diets for 14 days. This was given as a fixed amount daily (approximately 90 g/kg BW d^{-1}), split into two equal feeds given at 0900 and 1800. All food was consumed. The amount given was based on the daily intakes of rats of similar age given free access to a soybean-based diet (*24*, *25*) and was equivalent to around 70% of that which would be consumed by rats given free access to high-quality control diet. Feces was collected daily and frozen. On the final day, the rats were fed 1.5 g of diet. Two hours later, they were given 1 mL of saline containing 8.69 pmol of ^{14}C-spermidine (Amersham Life Science, Buckinghamshire, UK) by intraperitoneal injection, and a further hour later they were killed by exsanguination under deep anesthesia. Blood was collected in heparinized tubes and centrifuged (800*g*, 10 min, 4 °C), and the plasma was frozen.

The stomach, small intestine, cecum, and colon were removed and their contents flushed out with ice-cold saline. The pancreas, spleen, liver, kidney, thymus, lungs, heart, and gastrocnemius hind-limb muscles were also removed. The tissues were weighed, and six sections each of 2 cm were cut from the small intestine at 3, 5, 25, and 45 cm

Table 2. Nutritional Performance of Rats Fed Diets Containing Raw Cowpea Seed Meals or Cowpea Protein Isolate or Starch for 14 Days[a]

	experiment 1						experiment 2	
	diet 1	diet 2	diet 3	diet 4	diet 5	diet 6	diet 7	diet 8
DM intake (g/d)	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4
N intake (g/d)	0.15 ± 0.04	0.15 ± 0.05	0.15 ± 0.05	0.15 ± 0.04	0.15 ± 0.05	0.15 ± 0.04	0.15 ± 0.04	0.15 ± 0.04
lipid intake (g/d)	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05
feces DM (g/d)	0.56 ± 0.07ab	0.98 ± 0.03c	1.42 ± 0.04d	0.86 ± 0.08c	1.17 ± 0.15cd	0.79 ± 0.05bc	0.44 ± 0.05e	0.68 ± 0.08f
fecal N (mg/d)	16 ± 2a	27 ± 2b	35 ± 1c	25 ± 3b	30 ± 9bc	23 ± 5ab	14 ± 2d	19 ± 2e
fecal lipid (mg/d)	22 ± 8	38 ± 18	33 ± 13	41 ± 18	53 ± 15	32 ± 10	27 ± 9	35 ± 13
wet wt gain (g/d)[b]	3.50 ± 0.24a	2.82 ± 0.11b	2.31 ± 0.14c	2.80 ± 0.18b	2.13 ± 0.27c	3.00 ± 0.08b	3.50 ± 0.13d	3.17 ± 0.15e
conversion (g/g)[c]	3.73 ± 0.27a	3.01 ± 0.14b	2.46 ± 0.18c	3.00 ± 0.22b	2.27 ± 0.24c	3.20 ± 0.11b	3.73 ± 0.15d	3.38 ± 0.17e
dry wt gain (g/d)[b]	1.16 ± 0.14a	0.83 ± 0.05b	0.66 ± 0.10c	0.79 ± 0.06b	0.55 ± 0.12c	0.83 ± 0.08b	1.13 ± 0.10d	0.83 ± 0.14e
protein gain (g/d)	0.61 ± 0.07a	0.39 ± 0.13b	0.41 ± 0.11ab	0.44 ± 0.05ab	0.27 ± 0.08b	0.39 ± 0.04b	0.56 ± 0.07c	0.45 ± 0.05d
lipid gain (g/d)	0.50 ± 0.08a	0.36 ± 0.08ab	0.23 ± 0.10b	0.34 ± 0.03ab	0.21 ± 0.10b	0.42 ± 0.08a	0.43 ± 0.04c	0.32 ± 0.05d

[a] Values are means ± SD. For each experiment, values in a row with different letters differ significantly ($p \leq 0.05$). Diets: 1, control; 2, *pediculata* meal (220 g/kg of diet); 3, *pediculata* meal (442 g/kg of diet); 4, IT86D-719 meal (220 g/kg of diet); 5, IT86D-719 meal (442 g/kg of diet); 6, IT86D-719 protein isolate; 7, control for diet 8; IT86D-719 starch. [b] Initial fresh weight, 85 ± 2 g; dry weight, 25.4 ± 0.9 g; protein ($N \times 6.25$), 16.0 ± 0.6 g; lipid, 5.1 ± 0.4 g. [c] Wet weight gain/protein intake (g/g).

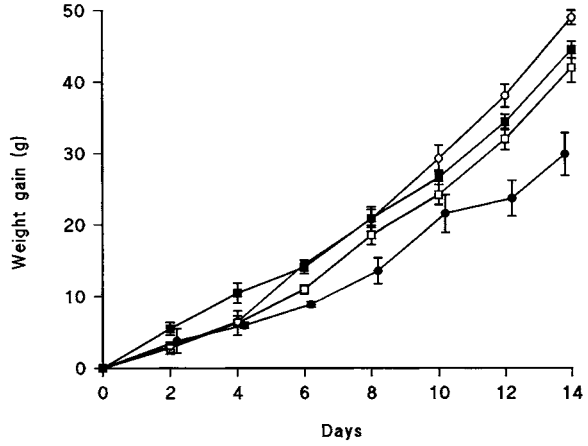

Figure 1. Growth of rats (initial weight, 85 ± 2 g) fed equivalent amounts daily of control diet 1 (○) or diets based on cowpea (*Vigna unguiculata* (L) Walp. cultivar IT 86D-719) meal (442 g/kg, diet 5, ●), protein isolate (diet 6, □), or starch (diet 8, ■) for 14 days.

from the pylorus and at 3 and 5 cm from the ileocecal junction. These and pieces of stomach, cecum, colon, pancreas, spleen, liver, kidney, and gastrocnemius muscle were weighed and were counted for radioactivity as described previously (*26*). The remainder of the tissues and carcass were freeze-dried and reweighed.

Experiment 2. Rats (85 ± 2 g, four per treatment) were fed exclusively with a control diet (diet 7) containing maize starch or a test diet (diet 8) based on raw cowpea starch for 14 days. Procedures and management were as per experiment 1, with the exception that these were not dosed with ^{14}C-spermidine on the final day.

The small-animal unit of the Rowett Research institute is licensed under the U.K. Animals (Scientific Procedures) Act 1986, and its operation is monitored by both the ethical review committee and animal welfare unit of the institute and the appropriate governmental inspectorate. All management and experimental procedures conducted during this study were approved and done in strict accordance with the requirements of the U.K. Animals (Scientific Procedures) Act 1986.

Chemical Analyses. Carcass and feces were freeze-dried and ground. N content was determined by using a Foss Heraeus Macro N automated system (Foss [Electric] UK, Bishipthorpe, Yorkshire, UK), and lipid content was determined by extraction (1:100 w/v) with chloroform/methanol (2:1 v/v) as described previously (*25*).

Intestinal and pancreatic tissue compositions were determined essentially as previously (*24*, *25*, *27*). Freeze-dried tissue was homogenized (1:20 w/v) in ice-cold perchloric acid (100 g/L), left on ice for 30 min, and centrifuged (4000*g*, 30 min, 4 °C). The pelleted material was resuspended (1:25 w/v) in 0.3 M NaOH and incubated at 37 °C for 90 min. An aliquot was taken for protein estimation by a modified Lowry method, and the remainder of the solubilized material was mixed with an equal volume of perchloric acid (100 g/L), left at 4 °C overnight, and centrifuged (4000*g*, 30 min, 4 °C). The ribose content of the supernatant was estimated by the orcinol reaction. The DNA/protein-containing pellet was resuspended in perchloric acid (50 g/L), heated at 80 °C for 60 min, cooled on ice, and centrifuged. The deoxyribose content of the supernatant was measured using a diphenylamine reagent. Bovine serum albumin, calf liver RNA, and salmon sperm DNA (Sigma-Aldrich, Poole, Dorset UK) were used as standards.

Freeze-dried pancreas samples were homogenized (1:100 w/v) in ice-cold water containing Triton X-100 (5 g/L) and centrifuged (50000*g*, 15 min). Zymogens were activated by incubation (2:5 v/v) with enterokinase at 37 °C for 30 (chymotrypsinogen) or 60 min (trypsinogen). The enterokinase was from bovine intestine (Sigma-Aldrich) and was prepared (6 g/L) in 0.05 M sodium citrate, pH 5.8. Chymotrypsin and trypsin estimations were done using *N*-glutaryl-L-phenylalanine-*p*-nitroanilide (GAPNA) and *N*-α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), respectively, as subtrates (*24*, *25*, *27*). Serum glucose, triglycerides, cholesterol, and HDL-cholesterol were determined using standard kits (Sigma-Aldrich).

Lectin (hemagglutinin) assays were carried out by a serial dilution procedure using trypsin-treated rat erythrocytes (*28*). One unit of hemagglutinating activity (HU) was defined as that contained in the amount of sample in the last dilution that caused 50% agglutination of cells. Trypsin inhibitor assays were done by determining the residual trypsin activity after formation of enzyme−inhibitor complex and using BAPNA as substrate (*28*). Inhibitory activity was determined from dilutions that gave 40−60% inhibition of enzyme activity.

Statistical Analysis. Data were analyzed by one-way analysis of variance in combination with the Tukey multiple comparison test using the Instat Statistical Package (GraphPad Software Inc, San Diego, CA).

RESULTS

Cowpea Meal. Rats fed diets containing raw cowpea (*V. unguiculata* (L) Walp. cultivars IT 86D-719 or pedicilata) meal (442 g meal/kg) as the sole (100 g/kg) source of protein grew throughout the 14-day experimental period (**Figure 1**, **Table 2**). However, the wet and dry weight gains and food conversion efficiencies achieved were well below those for animals given the same amount daily of high-quality (lactalbumin-based) control diet. Fecal dry matter and N outputs, but not lipid excretion, were greatly elevated, and accretion of both protein and lipid in the body was reduced (**Table 2**). Less marked effects were observed if the seed meals (220 g/kg) comprised only half of the dietary protein (**Table 2**). Nevertheless, even at these lower levels of inclusion, weight gains and food conversion efficiencies by rats remained lower than those with the control diet.

Table 3. Dry Body Weight (DBW), Small Intestine and Pancreas Weight, Composition, and Polyamine Uptake (pmol/h) in Rats Fed Diets Containing Raw Cowpea Seed Meals or Cowpea Protein Isolate or Starch for 14 Days[a]

	experiment 1						experiment 2	
	diet 1	diet 2	diet 3	diet 4	diet 5	diet 6	diet 7	diet 8
DBW (g)	41.8 ± 2.2a	37.4 ± 0.4b	33.9 ± 0.9b	36.8 ± 1.0b	33.6 ± 1.8b	37.4 ± 1.5b	41.2 ± 1.4a	37.4 ± 1.5b
small intestine								
weight (mg/100 g DBW)	2049 ± 41a	2226 ± 76ab	2368 ± 161b	2267 ± 67ab	2466 ± 114b	2387 ± 52b	2112 ± 68a	2374 ± 34b
weight (mg)	857 ± 39	832 ± 33	803 ± 46	835 ± 24	872 ± 24	914 ± 37	870 ± 28	888 ± 15
DNA (mg)	15 ± 2	11 ± 1	14 ± 1	16 ± 4	16 ± 3	14 ± 1	15 ± 2	13 ± 1
RNA (mg)	25 ± 5	23 ± 5	24 ± 4	29 ± 2	26 ± 5	29 ± 3	25 ± 4	26 ± 4
protein (mg)	499 ± 23	479 ± 19	463 ± 16	491 ± 15	520 ± 14	512 ± 21	511 ± 20	517 ± 11
^{14}C-SPD uptake	324 ± 6a	373 ± 18b	472 ± 33b	440 ± 18b	408 ± 2b	400 ± 30b	nd	nd
pancreas								
weight (mg/100 g DBW)	452 ± 36a	514 ± 73a	695 ± 62b	539 ± 53a	628 ± 40b	447 ± 10a	463 ± 50a	454 ± 38a
weight (mg)	189 ± 15	192 ± 28	236 ± 16	199 ± 20	211 ± 22	168 ± 6	191 ± 20	170 ± 19
DNA (mg)	1.6 ± 0.3	1.6 ± 0.2	1.8 ± 0.3	1.8 ± 0.1	1.8 ± 0.3	1.8 ± 0.2	1.9 ± 0.1	1.9 ± 0.1
RNA (mg)	19.7 ± 0.9	19.3 ± 0.9	21.8 ± 1.4	18.7 ± 1.1	21.3 ± 2.5	17.5 ± 1.7	19.7 ± 0.9	20.5 ± 0.9
protein (mg)	128 ± 12	125 ± 28	144 ± 16	120 ± 18	140 ± 21	113 ± 13	138 ± 15	125 ± 12
trypsinogen (mg)	3.4 ± 0.3	3.6 ± 0.2	3.3 ± 0.8	4.5 ± 0.4	3.7 ± 0.6	3.0 ± 0.9	3.4 ± 0.3	3.6 ± 0.3
chymotrypsinogen (mg)	7.4 ± 0.1	7.8 ± 0.8	7.2 ± 1.0	9.5 ± 0.8	7.6 ± 1.6	5.2 ± 1.8	7.2 ± 1.4	7.6 ± 0.9
^{14}C-SPD uptake	89 ± 8a	133 ± 14b	71 ± 12a	138 ± 10b	117 ± 13ab	91 ± 9a	nd	nd

[a] Values are means ± SD. Values in a horizontal row with different letters differ significantly ($p \leq 0.05$). Diets: 1, control; 2, *pediculata* meal (220 g/kg of diet); 3, *pediculata* meal (442 g/kg of diet); 4, IT86D-719 meal (220 g/kg of diet); 5, IT86D-719 meal (442 g/kg of diet); 6, IT86D-719 protein isolate; 7, control for diet 8; IT86D-719 starch. nd, not determined. Control tissue weights (mg/100 g DBW): cecum, 348 ± 47; colon, 447 ± 102; spleen, 186 ± 26; liver, 3413 ± 186; kidney, 638 ± 62; thymus, 180 ± 64; lungs, 348 ± 27; heart, 304 ± 14; gastrocnemius muscles, 741 ± 45.

The absolute weights (milligrams) and composition of the small intestine and pancreas did not appear to be significantly altered by cowpea diets (**Table 3**). However, uptake of ^{14}C-spermidine by the small intestine was slightly elevated, and the proportional weight (milligrams per 100 g of dry body weight) of the tissue was increased (**Table 3**). The proportional weight of the pancreas was also elevated (**Table 3**). Furthermore, accumulation of ^{14}C-spermidine in the pancreas was slightly increased, but only if the rats were given cowpea meal as half of their dietary protein intake (**Table 3**). In contrast, the proportional weights of the cecum, colon, spleen, liver, kidneys, thymus, lungs, heart, and gastrocnemius hind-limb muscles and the accumulation of ^{14}C-spermidine by these tissues were unaffected by cowpea meal diets (data not given).

Serum cholesterol levels were slightly lowered (control, 1857 ± 72 nmol/mL; cowpea, 1665 ± 82 nmol/mL; $p \leq 0.05$) if cowpea meal provided the only source of dietary protein for rats (442 g of meal/kg of diet). In contrast, HDL-cholesterol (control, 812 ± 22 nmol/mL; cowpea, 802 ± 27 nmol/mL), triglyceride (control, 467 ± 47 nmol/mL; cowpea, 556 ± 95 nmol/mL), and glucose levels (control, 8.8 ± 0.3 μmol/mL; cowpea, 9.4±1.2 μmol/mL) were unaffected. None of these blood parameters were altered from control values if cowpea meal was incorporated as half the dietary protein (220 g of meal/kg of diet).

Protein Isolate. Rats fed a diet based on a protein isolate (globulins) from cowpea (*V. unguiculata* (L) Walp. cultivar IT 86D-719) grew rapidly (**Figure 1**, **Table 2**). However, the wet and dry weight gains and food conversion efficiencies achieved, although much higher than those for cowpea meal, still remained below those attained on similar intakes of high-quality control diet. There was a tendency for fecal dry matter and N excretion by protein isolate-fed rats to be higher than those by controls and for protein accretion rates to be slightly lower, although these differences did not reach statistical significance.

Uptake of ^{14}C-spermidine by the small intestine and the proportional weight of the tissue were increased due to consumption of cowpea protein isolate (**Table 3**). In contrast, the diet had no effect on ^{14}C-spermidine accumulation and proportional weight of the pancreas (**Table 3**) or the cecum, colon, spleen, liver, kidneys, thymus, lungs, heart, and gastrocnemius hind-limb muscles (data not shown). In addition, blood triglyceride, glucose, and cholesterol levels did not differ from control values (data not shown).

Starch. Rats fed a diet containing raw cowpea starch gained weight, albeit at a rate slightly below that achieved by controls given an equivalent maize starch-based control diet (**Figure 1**, **Table 2**). Wet and dry weight gain, food conversion efficiency, and body lipid and protein accretion rates were lower than those attained on control diet, and fecal dry matter and N excretion were elevated. The proportional weight of the small intestine was increased (**Table 3**), but the pancreas (**Table 3**) and cecum, colon, spleen, liver, kidneys, thymus, lungs, heart, and gastrocnemius hind-limb muscle were unaffected, as were serum triglyceride, glucose, and cholesterol levels (data not shown).

DISCUSSION

Diets containing raw cowpea cultivar IT 86D-719 or pedicilata meals as the sole source of protein (100 g of protein/kg; 442 g of seed meal/kg) and supplemented with L-tryptophan and L-methionine up to target requirements did not fully support the growth of young rats. This was consistent with previous findings in rats, pigs, and poultry (*20*, *24*, *25*, *29*−*32*). The low weight gains observed in the present study were the result of reduced accretion of lipid and protein by the rats.

Moderate amounts of protease inhibitors were present in the seed meal. However, these are of a Bowman−Birk type that trigger pancreatic secretion and growth in rats but have little or no effect on body weight gain (*7*). Only low levels of lectin were detected in the seeds, and little or no α-amylase inhibitors are generally present (*20*, *21*). Dietary tannins do impair protein metabolism and nutrient utilization in vivo (*9*). However, cowpea tannins are reported to have only limited effects on growth and metabolism of rats in vivo (*33*). The poor growth of rats fed cowpea-based diets is thus unlikely to be due to the actions of these antinutritional/bioactive factors.

The protein isolate (globulin) fraction of cowpea comprised the bulk of the extractable protein (*22*, *34*). It contained no detectable lectin or protease inhibitors but had a tendency to

be less digestible by rats than a high-quality protein, lactalbumin. This might, in part, have been a result of racemization during the extraction process (*10*) or bacterial growth on non-starch polysaccharides present in the protein isolate (*35*, *45*). However, it is also consistent with the general view that legume globulin proteins are refractory in nature (*36*). Nonetheless, the differences in apparent fecal N digestibility were very small (approximately 84% for the protein isolate versus around 89% for lactalbumin) and insufficient to explain the low protein accretion rates observed in rats fed the cowpea protein isolate.

Retention of absorbed N by rats fed the cowpea protein isolate was very low (around 53% of N was retained). This would be consistent with findings with faba bean, lupin, lentil, and soybean globulins (*37*−*39*). Urinary N (primarily urea N) excretion by rats fed these globulins was very high. As in the present study, this did not appear to be due primarily to impaired digestion of the dietary proteins or deficiencies in their amino acid profiles. It has been suggested that the native proteins or products derived from them may interfere with intestinal or systemic metabolism and thereby limit N retention in the body (*9*, *37*−*41*). The mechanisms by which legume globulins modulate gut or systemic metabolism remain unknown. However, some are mucotractive, in that they trigger excessive synthesis and secretion of mucus in the intestine (*40*, *41*). This leads to high losses of endogenous N and dry matter in the feces. It may also distort nutrient needs, in particular the profile of amino acids required by the animal (*37*), and thereby impair the efficacy of nutrient utilization. In the present study, fecal dry matter and N excretion by rats given cowpea protein isolate tended to be higher than those in control animals. In addition, endogenous N excretion by rats fed cowpea meal has been shown to be elevated (*42*). Mucotractive effects of cowpea globulins might therefore be a factor limiting the nutritional performance of rats on cowpea diets.

Crude cowpea starch preparations are reported to be comprised primarily of readily available starch. However, they may contain variable amounts (between 8 and 22% of the total) of resistant starch (*5*, *43*, *44*). In the present study, raw cowpea starch was substituted directly for high-quality maize starch, and the rats were fed the same amount daily of their respective diets. Assuming the resistant starch was not available, the energy intake of rats fed the raw cowpea starch diet may have been 133−142 kJ/rat/d, as compared to 148 kJ/rat/d for those fed the maize starch-based control diet. Thus, the reduced rates of growth and protein and lipid deposition observed with rats given the diet based on crude cowpea starch were probably a result of lower intake of available energy. Fecal dry matter excretion by the rats was increased by around 0.24 g/rat/d, whereas the intake of resistant starch would have been approximately 0.34−0.94 g/rat/d.

The growth retardation caused by diets based on cowpea meal (442 g of seed meal/kg of diet) is thus likely to be due, in large part, to the combined actions of the globulin proteins and some starch constituents on lipid and protein accretion by rats. However, their combined effects appear insufficient to account for all the growth inhibition observed. Reduced energy availability due to the presence of insoluble fiber and non-starch polysaccharides or the actions of other components, such as the albumin proteins (*38*), are also likely to be contributory factors to the overall effects of cowpea meal on the metabolism of rats or nutrient availability.

Small Intestine. The proportional weight of the rat small intestine and ^{14}C-spermidine uptake by the tissue was increased by consumption of cowpea protein isolate. Polyamine accumulation indicates an elevated rate of metabolic activity in the tissue (*26*). However, the tissue composition (DNA, RNA, and protein content) altered little compared to that for controls, suggesting that these changes were not the result of a growth response in the intestine, such as would be triggered by significant amounts of a trophic dietary lectin (*8*). Cowpea globulins may be mucotractive and stimulate increased synthesis of mucin by goblet cells. Mucin has a high carbohydrate content but a low protein content (*46*). Thus, higher amounts of mucin present in intestinal goblet cells could slightly increase the overall weight of the intestine without greatly altering its overall protein, RNA, and DNA content (*39*−*41*). It is thus possible that the higher metabolic activity in the intestine and the increase in its proportional weight are a result of mucotractive effects of cowpea globulins on the gut.

Acute inflammatory responses, disruption to small intestine epithelial structure and integrity, and severe fluid loss have been observed in newly weaned piglets fed cowpea-based diets (*31*, *32*). These responses have strong similarities to those observed in piglets and calves fed diets containing untreated glycinin and β-conglycinin, the major globulins of soybean (*9*). They may thus be a result of adverse interactions of the cowpea globulins with the local (gut) immune system of the piglets. Chronic intestinal responses to cowpea or soybean globulins (*39*) have not been observed in rats. This may indicate that there are species differences in responsiveness to these proteins, with the pig being particularly susceptible. Alternatively, it may be that the age of the animal is important. The piglets used were very young and possibly not fully immunocompetent, whereas the rats used in these studies were young (>30 days old), fully developed adults. Indeed, adult pigs are not adversely affected by soybean or soybean globulins (*9*).

Raw cowpea starch also caused an increase in the proportional weight of the small intestine. Goblet cell numbers and mucosal mass of the small intestine are influenced by the level of fiber in the diet and tend to increase if the fiber content of the diet is high (*47*, *48*). The control diet contained only 42 g of potato starch/kg as dietary fiber. The test diet would have contained this plus 40−110 g of resistant starch/kg. This additional fiber may have been sufficient to cause the observed changes.

The changes in small intestine weight caused by cowpea meals are probably due to the combined actions of the globulins, resistant starches, and possibly insoluble fiber and non-starch polysaccharides on intestinal metabolism. The consequences for gut function and health remain unclear. However, no chronic or cumulative deleterious effects on gut metabolism were previously noted when rats were fed cowpea-based diets for 700 d (*25*).

Pancreas. Slight enlargement of the pancreas was evident in rats fed cowpea meal (442 g meal/kg of diet). This was consistent with the presence of Bowman−Birk-type inhibitor in these seeds (*7*, *24*). These protease inhibitors can induce deleterious changes in the pancreas of rats in the long term (*24*) but may have lesser or no effects in other species (*7*). No enlargement of the pancreas was evident when cowpea meal was incorporated at 220 g of meal/kg in the diet, although small amounts of protease inhibitor were present in the diet.

No protease inhibitor or, indeed, lectin was detected in the cowpea protein isolate and starch fractions, and they did not cause pancreatic growth in rats.

Cholesterol. Serum cholesterol levels of rats were slightly reduced if they were fed cowpea meal (442 g of meal/kg of diet). This change was not evident in rats given the protein isolate or the starch fractions. It may thus have been due to the

actions of the insoluble fiber or non-starch polysaccharides or bioactive components in the raw meal (*3*, *45*).

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