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THE ABILITY OF RAW AND PROCESSED COWPEAS TO SUPPORT GROWTH IN WEANLING WISTAR STRAIN RATS

Z. M. OFUYA

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ABSTRACT

The ability of raw and processed (boiled and autoclaved) cowpeas (*vigna anguiculala walp.*) to promote growth in female weanling wistar rats was studied with the hope that they can be used as a component of weaning food. The result showed that all the test diets supported growth as indicated by the weight gain in the rats. The raw and processed cowpea diets caused a slight pancreatic enlargêment. Apparent digestibility of dry matter (96.8%) of the control group of rats fed a casein-maize starch diet was significantly higher ($P < 0.01$) than that of the other test diets. Energy Digestibility (DE) for the control diet was 97.58%, whereas that for the various cowpea diets ranged from $87.4 \pm 0.44 - 88.88 \pm 0.88\%$. The digestibility of starch in the rats fed raw and autoclaved cowpeas was 99%. Nitrogen was poorly digested ($83.33 \pm 2.5 - 84.75 \pm 1.2\%$) in all the test diets while digestibility in the control diet was 93.4%. Based on these results, cowpeas promoted growth in rats and possibly can contribute to the energy and protein needs of the weanling infant if properly processed.

KEY WORDS: Cowpeas (*Vigna unguiculata*), Apparent Digestibility of dry matter, Energy, Protein and starch.

INTRODUCTION

Cowpeas (*Vigna unguiculata*) are widely grown in the tropics. Like other legume seeds, they are good sources of energy, protein, B-vitamins (thiamin and riboflavin) and certain minerals, such as calcium and potassium (Walker, 1982). Weanling infants have high energy and protein requirements, which are often difficult to meet in the developing countries. From its nutrient content, cowpeas can be a good source of nutrient particularly energy and protein if exploited positively. Legume seed starch is regarded as being intermediate between the highly digestible cereal and the poorly digestible banana, plantain and potato starches (Dreher et al, 1982). Although legume starch digestibility has been extensively studied, some degree of processing or cooking is considered necessary to gelatinize the starch and so optimize its digestibility (Dreher et al, 1982). Improvement in the digestibility of the starch of brown Dutch beans (*Phaseolus vulgaris*) in vitro has been reported following autoclaving or boiling (Hellendoorn, 1969).

El Faki et al, 1983 studied the in-vivo digestibility of starch extracted from cowpeas using weanling rats. The starch extracted from cowpeas had a digestibility of 88.3% in the weanling rats. Information available on the in-vivo digestibility of native or processed cowpea starch is scarce. In less developed countries, cowpeas constitute one of the foods that can be fed to infants under one year of age. If the use of cowpeas in the preparation of infant food is to be exploited to a large extent, a complete evaluation would appear an essential pre-requisite. There is a paucity of information on energy and protein digestibility in rats. The objective of this study therefore is to determine the ability of raw and processed cowpeas to support growth in weanling rats (rats being used as a model for the weanling infants). In doing so, the apparent digestibility of dry matter, starch and protein were determined.

MATERIALS AND METHODS

cowpea seeds

Cowpea (*Vigna unguiculata*) seeds were obtained from ELF Food Stores in Loughborough, United Kingdom, although they originated from California, United States of America.

The seeds were washed and dried and then ground with a hammer mill into a powdery form (<1mm particle size). Proximate analysis of cowpea flour was first carried out (see below) before the flour was processed by boiling and autoclaving. To obtain the boiled product, a flour-water mixture (1:3 w/v) was boiled in a steam jacketed boiler for 30 minutes with constant stirring. The dry autoclaved meal was obtained by placing the flour in a shallow tray to a depth of 1cm covered with aluminium foil and heated in an autoclave at 121°C for 30 minutes. The wet

Z. M. OFUYA, Department of Human Physiology, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nig.

autoclaved mixture was prepared by thoroughly mixing a flour-water mixture (1:3 w/v) and autoclaving it at 121°C for 30 minutes. This quantity of water would enable complete dissolution of the cowpea flour for effective heat penetration.

The boiled and wet autoclaved cooked materials were freeze dried in their entirety (Solid and Liquor) and then milled (<1mm particle size) before they were incorporated into semi-purified diets for rats.

PROXIMATE ANALYSIS

Crude fat and ash were determined according to the method of AOAC (1975). See below for energy, starch and nitrogen determination

Oligosaccharides were determined as follows: The oligosaccharides, sucrose, raffinose and stachyose were extracted with 80% ethanol, and then evaporated to dryness. The extract was purified by ion exchange procedure. The aqueous extract was run through a strongly acidic cation exchanger and a strongly basic anion exchanger in series. The cation exchanger was Dowex 50 x $4H^+$ form, 50 mesh; while the anion exchanger was Dowex 1 x 10 formate form, 50 mesh.

The purified and deionised extracts were run on thin layer chromatography. Authentic sugars were used as standards for identification.

DIETS:

Diets were formulated to contain Ca 24% protein with 10% derived from casein. Five experimental diets were prepared as follows:-

1. Control diets containing casein and maize starch (Table 1).
2. Test diet containing raw cowpea flour as a major source of protein and energy.
3. Test diet based on boiled cowpea fluor.
4. Test diet based on dry autoclaved cowpea flour.
5. Test diet based on wet autoclaved cowpea flour.

All the test diets contained corn oil 5%, vitamin B mixture 1.1%, vitamin A, D, E, and K mixture 0.1%, mineral mix 5%. The formulations of the cowpea diets are shown in Table 2. The energy contents of the control and cowpea diets were similar.

FEEDING TRIAL

Weanling, female, wistar rats weighing 72.4 – 90.2g were obtained from the breeding centre of the School of Agriculture, Sutton Bonington England. They were housed in individual wire bottomed cages permitting feacal collection. They were maintained in an environment, which was controlled for temperature and relative humidity. Lighting was for 12 hours daily. The experimental diets were supplied in a total of over 14 days with the balance trial being taken during the last three days of this period. All the rats were given the same bulk of food. Water was given *ad-libitun* Daily food intake was recorded, and the feaces collected over the three days trial period, were pooled and dried. The daily food intake was regarded as the difference between the food given and the weight of the left over food obtained the following day. The rats were weighed at the beginning and at end of the 14 days period. At the completion of the trial, the rats were killed by carbon dioxide inhalation and the pancreas and caecum of each rat excised and dried to constant weight, in an air circulating oven at 105°C. Weighing was done after 24 hours and after a further one hour. Feed efficiency ratio (FER) was also determined.

ENERGY, NITROGEN AND STARCH DETERMINATION

Nitrogen was determined by a modificastion of the Kjeldahl Gunnings procedures for organic nitrogen (AOAC, 1975). Energy was determined by the use of an adiabatic bomb calorimeter (Parr instrument Co. Moline, USA), which estimates the energy content of organic substances. Starch was analysed by a modified method

Table 1 : Formulation of the Control Casein-maize Starch Diet

Ingredients	G/kg diet
Casein	237.2
Dextrinised Starch (Cerelose)	250.8
Maize Starch	400
Corn oil	50
Vitamin B mixture[1]	11
Vitamins A, D, E and K mixture[2]	1
Mineral Mix	50

1. Composition of B Vitamin mixture dispersed in 1ml maize oil in g/kg Thiamine, 0.31, Riboflavin, 1.0; Pyridoxine, 0.4 Calcium pantathenate, 6.0; Nicotinic acid, 20.0; Inositol, 40.0; P-Aminobenzoic acid, 60.0; Biotin, 0.02; B_{12} 0.1 tritirate, 5.0 Choline, 120.0; Maize starch, 747.1.
2. Composition of fat soluble vitamin mixture: Vitamin A (5,000 iu), 172mg retenyl acetate, Vitamin D (250 iu), 6.25 ug Cholecalciferol; Menaphtone 1.0mg, Vitamin E (45 iu), 30.0mg α - tocopherol.
3. Composition of mineral mixture (g/kg): Nacl, 296.59; KH_2PO4 296.4, $CaC0_3$, 290.67; $MgSO_4.7H_20$, 90.02; $MnSO_4$, 39; KI, 0.57; $ZnC0_3$, 0.38; $CuSO_4.5H_2O$,0.36; $CoCl_2.6H_20$, 0 02 $FeSO_4.7H_20$, 20.6.

Table 2 :Formulation of Cowpea – Based Diets

Ingredients	G/kg Diet
Casein	100
Corn oil	88
Vitamin B Mixture[1]	11
Vitamins A, D, E and K Mixture[2]	1
Mineral Mx[3]	50
Cowpea Flour	750

1. Composition of B Vitamin mixture dispersed in 1ml maize oil in g/kg: Thiamine, 0.31, Riboflavin, 1.0; Pyridoxine. 0.4; Calcium pantathenate, 6.0; Nicotinic acid, 20.0; Inositol, 40.0; P-Aminobenzoic acid, 60.0; Biotin, 0.02; B_{12} 0.1 tritirate, 5.0 Choline, 120.0; Maize starch, 747.1.
2. Composition of fat soluble vitamin mixture: Vitamin A (5,000 iu), 172mg retenyl acetate; Vitamin D (250 iu), 6.25 ug Cholecalciferol; Menaphtone 1.0mg, Vitamin E (45 iu), 30.0mg α - tocopherol.
3. Composition of mineral mixture (g/kg): Nacl, 296.59; K_2PO_4, 296.40; $CaC0_3$, 290.67; $MgSO_4.7H_20$, 90.02; $MnSO_4$, 39; KI, 0.57; $ZnC0_3$, 0.38; $CuS0_4.5H_20$, 0.36; $CoCl_2.6H0$, 0.02; $FeS0_4.7H_20$,20.6.

Macrae and Armstrong (1968): Agidex (Glaxo laboratories kelmersdalel) was the enzyme used for hydrolysis while glucose was estimated by the glucose oxidase method.

RESULT AND DISCUSSION

Analysis of the cowpeas used in this study showed a starch content of 42%, crude protein (Nitrogen x 5.8) 19.86 ± 0.51%, Ash 3.97 ± 0.08%, Crude fat 0.05%, sucrose 2.67%, raffinose, 1.97% and stachose 2.8%. These values agree with published results (Rosario et al, 1981; Molina et al, 1976; Ologhobo and Fetuga, 1982; Reddy, et al, 1984; Oke, et al, 1995.)

Verbascose was probably present in small amounts in the oligosaccharide fraction as there was a third sugar on the TLC plate, but due to the absence of an authentic standard, confirmation of this was not possible.

Both the control and the test diets supported the growth of rats (Table 3).

The differences in weight gain though significant (PL 0.05) is most likely a reflection of the types of food consumed by these rats. In addition, none of the rats was mal-nourished as indicated by the fact that all the rats gained weight. The ability of autoclaved samples to support growth in rats could be attributed to the fact that autoclaving destroys the antinutrients in cowpeas. This is supported by the study carried out by Umoren, et al (1997).

The raw cowpea meal proved to be innocuous, since none of the rats lost weight or died during the experimental period.

Feed efficiency ratio [FER) [weight gain/food intake] obtained for the various diets followed a similar pattern to that obtained for weight gain. (Table3) FER was highest for test diet 4 (dry autoclaved cowpea meal) and lowest for test diet 3 (boiled cowpea meal). The reason for the variability in FER values obtained may be related to the amounts of food consumed by the rats.

All the cowpea diets proved to be palatable compared with the control diet although no effect of the diet on feacal bulk was noted. In addition, irrespective of diet, no gastrointestinal tract disturbances were observed since the feaces was not watery. The result is different from that obtained for another pulse legume seed, brown Dutch bean (Phaseoulus vulgaris). Hellendoorn (1969) found that rats fed raw or heated (to boiling) brown Dutch bean developed serious diarrhoea.

All cowpea diets caused 2 – 3 fold increase in the size of the caecum compared with the control (Table 4). Processing the cowpeas had no effect on full caecal weight. Fleming and Vose (1979) and Shurpalekar et al (1979) observed caecal enlargement on feeding legume starches and carbohydrates to rats. The enlargement of the caecum obtained in this study could be due to the presence of indigestible seed components. These may include dietary fibre, other indigestible carbohydrates, and probably proteins.

Pancreas weights of rats fed the control diet were significantly lower (P<0.01) than those of rats fed the test cowpea diets with the exception of diet 4 (dry autoclaved cowpea meal) (Table 4). Thus, slight pancreatic enlargement occurred in rats fed diets containing raw cowpea meal, wet autocaved cowpea meal and boiled cowpea meal. In view of the fact that no loss in

Table 3: Food Intake and Weight Gain of Weanling Rats Fed with Control and Processed Cowpea Diets for a period of 14 Days.

Major Dietary Component	Weight gain (g)	Food Intake Weight (g) Dry weight Basis	Food Efficiency Ratio
1. Control (basal Casein-maize Starch diet)	15.0 ± 3.1[a]	119.7 ± 3.7	0.13 ± 0.3[c]
2. Raw Cowpea	21.4 ± 1.7[b]	135.0 ± 3.4	0.16 ± 0.01[ab]
3. Boiled Cowpea Meal	12.9 ± 3.7[a]	112.5 ± 3.4	0.12 ± 0.03[c]
4. Autoclaved Cowpea Meal (CM) (dry)	24.54 ± 3.8[b]	139.4 ± 6.4	0.15 ± 0.01[b]
5. Autoclaved Cowpea Meal (CM)(Wet)	19.4 ± 1.6[c]	128.9 ± 5.8	0.15 ± 0.01[a]

Values are mean ± standard deviation for 5 or 6 replicates.

Values with Common superscript are not significantly different.

weight was observed in rats fed cowpea meals, pancreatic enlargement cannot be attributed to the presence of antinutritional factors in cowpeas. This could be explained by the fact that the rats on these diets consumed more food than those on the basal diet and diet 4.

The apparent digestibility of dry matter (ADDM) of the control diets (96.8%) was significantly higher (P< 0.05) than those on all the test diets (P<O 05) (Table 5). The digestibility of the test diets ranged from 87.0 – 87.6% with processing surprisingly having no effect on the digestibility of raw cowpeas.

The digestible energy of the various cowpea diets was unaffected by processing (87.4 – 88.8%) (Table 5). This indicated that probably all the starch and nitrogen were digested to the same extent in the raw and processed cowpeas. Similar results have been reported by Cabezas et al (1982) who found the percentage energy digestibility of cowpea to vary from 87.2% to 89.2%. These authors also claimed that energy digestibility was little influenced by processing. The energy digestibility of the control diet was 97.6%. This was high due to the ready digestibility of the control diet.

Cowpea nitrogen was poorly digested compared with the control (Table 5). Processing by heating with water (treatment 3 and 5) significantly increased the digestibility over the raw and the dry heated cowpeas (Treatment 2 and 4). The mechanism by which this increase was effected is not known. The apparent digestibility of nitrogen of the basal diet (93.4%) is closely related to the value (94%) obtained for a similar diet by Jones (1984).

In general, nitrogen digestibility of cowpea was low irrespective of processing. Although, certain processes effected marginal increases in nitrogen digestibility, this was still low compared with the control. Since the rats were not fed *ad libitum* direct comparisons are difficult to make.

Table 4: Pancreas and Caecal Weights of Rats Fed Control and Processed Cowpea Diets

Test diet	Dry organ weight (g)	
	Pancreas	Caecum and contents
1. Control (basal Casein-maize Starch diet)	0.18 ± 0.02[a]	0.19 ± 0.02[a]
2. Raw Cowpea meal	0.23± 0.03[b]	0.40 ± 0.02[b]
3. Boiled Cowpea Meal	0.21± 0.02[b]	0.46 ± 0.0[b]
4. Autoclaved Cowpea Meal (dry)	0.19± 0.02[ab]	0.46 ± 0.1[b]
5. Autoclaved Cowpea Meal (Wet)	0.20± 0.03[b]	0.47 ± 0.8[b]

Values with common superscripts are not significantly different (p> 0.05)

Values are mean ± SD for 5 to 6 replicates.

Table 5: Apparent Digestibility of Dry Matter, Energy, nitrogen and starch

Test diet	Apparent digestibility of dry matter (%)	Apparent digestibility of energy (%)	Apparent digestibility of nitrogen (%)	Apparent digestibility of Starch (%)
1. Control (basal Casein-maize Starch diet)	96.8 ± 0.1[a]	97.58 ± 0.1[a]	93.4 ± 0.5[a]	99.90 ± 0.01[a]
2. Raw Cowpea meal	87.0 ± 0.3[b]	87.4 ± 0.44	83.33 ± 2.5[c]	99.47 ± 0.03[b]
3. Boiled Cowpea Meal	87.63 ± 0.2[b]	88.66 ± 0.22[b]	86.70 ± 0.94[b]	98.52± 0.35[a]
4. Autoclaved Cowpea Meal (dry)	87.21 ± 1.6[b]	88.88 ± 0.08[b]	83.75 ± 0.47[c]	99.63± 0.09[a]
5. Autoclaved Cowpea Meal (Wet)	87.5 ± 0.7[b]	88.89 ± 0.1[b]	84.75 ± 1.2[c]	98.92± 0.07[a]

Values are mean ± standard deviation of 5 to 6 replicates

Values with common superscripts are not significantly different

Cowpea starch (raw) was found to be almost completely digested and no significant differences were obtained between the test diets and the control diet (Table 5). Clearly, processing did not cause any further increase in the digestibility of raw cowpea starch.

The results is similar to that obtained for cowpeas starch by other workers using *in-vitro* method and α -amylase (Kumer and Venkataraman, 1976) and that obtained by *in-viro* method (El Faki et al 1983). Of the pulse legumes studied by Kumar and Venkataraman (1976), cowpea starch was the most readily digestible.

The digestibility of cowpea starch reported by El Faki et al (1983) was lower (88.3%) than that obtained in this study. Much younger rats were used by El Faki and co-workers and the digestive capacity of the young animals could be much lower than that of the rats used in this study. The results obtained from this study shows cowpeas capable of contributing to the growth of weanling rats.

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