

NUTRITIONAL EVALUATION OF COWPEA AND ITS PRODUCTS BY CONVENTIONAL AND GERM FREE RATS.

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ABSTRACT

True digestibility of cowpea starch and nitrogen were studied using weanling germ free (GF) and weanling conventional rats. (CV). The rats were fed raw cowpea meal, alcohol extracted meal and cowpea meal autoclaved for 1 hour. Food consumption was high for all treatments. The weight gain of CV rats was significantly lower ($P < 0.01$) than that of GF rats for all diets. Feed efficiency in CV rats was 50% of that in GF rats. The caeca of GF rats was significantly larger ($P < 0.01$) than those of the CV animals on all diets.

Apparent digestibility of starch was in excess of 99% for CV rats, irrespective of diet. Starch digestibility in the CV rats was significantly higher ($P < 0.05$) than that in the GF rats. The quantity of starch digested by the intestinal bacteria, was generally less than 1% of the starch ingested. Autoclaving and removal of alcohol soluble components did not improve starch digestibility of the raw cowpea meal.

Apparent digestibility of nitrogen (ADN) was low in both CV and GF rats irrespective of diet. ADN was significantly higher ($P < 0.05$) in the CV rats than GF rats for raw and extracted cowpeas. In the autoclaved sample, there was no significant improvement. This indicates the probable involvement of microbial flora in the digestion of cowpea protein although the amount fermented is only a small percentage of the ingested protein (4-10%).

Key-Words: Cowpeas, Nutrient evaluation, Conventional rats, Germ free rats

INTRODUCTION

Cowpeas (*Vigna unguiculata*, L. Walp) are abundant in the tropics. They are good sources of energy, protein, B-Vitamins (thiamin and riboflavin) and certain minerals, e.g. calcium and potassium, (Walker, 1982).

In the tropics, the energy and protein requirements of weanling infants are difficult to meet and as such these infants often suffer from malnutrition. Cowpeas being good sources of protein and energy and other nutrients can form a part of a weaning food if properly exploited.

Earlier work to evaluate the digestibility of cowpea starch and protein using conventional rats revealed that the starch was almost completely digested (Ofuya, 2001). This work did not take into account any contribution the gut microbial flora might make on starch digestibility. Although attempts to assess this 'true' digestibility as opposed to the 'apparent' digestibility were made using conventional and antibiotic-treated rats, the results were inconclusive because the wide spectrum

antibiotic employed in the study was not totally effective against the gut microbial flora. There was an indication that cowpea starch was less digestible in the antibiotic-treated rats with the implication that in conventional animals, some

starch escaping digestion in the small intestine is fermented in the lower gut. Because of the uncertainties associated with the antibiotic-treated animals, any quantitative assessment of the role of the microbial flora was precluded.

True digestibility of cowpea starches would best be assessed using gnotobiotic animals. With this in mind, starch and nitrogen digestibility trials were undertaken, using germfree rats supplied with diets containing raw, autoclaved and ethanol/water extracted cowpeas. This would provide adequate information to enable cowpeas to be properly used as a component of weaning foods.

MATERIALS AND METHODS

Cowpea Seeds

Cowpea seeds were obtained from Elf Food stores in Loughborough, although they originated from California in the United States of America.

The seeds were ground in a hammer mill into a powdery form (< 1mm particle size).

To obtain the autoclaved meal, a flour-water mixture (1:5w/v) was thoroughly mixed and autoclaved at 121°C for 1 hour. The cooked material including liquor was freeze dried and milled. To obtain the extracted cowpea meal,

cowpea flour (2kg) was extracted with 80% ethanol (1:10w/v) for 48 hours with continuous stirring at room temperature. The mixture was left to settle and the supernatant containing oligosaccharides was decanted. Extraction was repeated as above two more times. The extracted flour was air dried in shallow trays under an extraction hood for 48 hours. The freeze dried and air dried materials were used directly in the preparation of the diets.

Diets

Three diets were formulated to contain ca 12% protein (Table 1) in which cowpea served as major sources of protein and energy. Diet 1 contained raw cowpea, diet 2 contained autoclaved cowpea flour and diet 3 contained extracted flour. The formulation of diets 1-3 is presented in Table 1.

The level of incorporation of vitamins was doubled (except for choline and inositol) to compensate for the destruction of some vitamins during sterilization by gamma-irradiation. Corn starch was used as a carbohydrate base for the preparation of the vitamin mixture. In addition, vitamins, E, K and B₁₂ were given to the rats fortnightly. The test diets were sterilized by gamma-radiation.

Table 1
FORMULATION OF COWPEA DIET FED TO CONVENTIONAL AND GERM-FREE RATS.

Diet	
Ingredients	g/kg
Glucose	158
Cowpea	694
Corn Oil	88
Fat and Water Soluble Vitamin ¹	10
Mineral mixture ²	50

-1 Composition of B-vitamin and fat soluble vitamin mix (g/kg): Thiamin, 2.0; Riboflavin, 2.0; Pyridoxine, 2.0; Calcium pantothenate, 12; Nicotinic acid, 40; Inositol, 40; P-Aminobenzoic acid, 120; Biotin, 0.2; Folic acid, 1.0; B₁₂ (in Mannitol), 10; Choline, 240; Menadione, 0.2; Rovimix* A500, 4.0; Rovimix* D₃ 500, 3.0; E₅₀, 30; Maize Starch, 493.6.

-2 Composition of mineral mixture (g/kg): NaCl, 296.59; KH₂PO₄, 296.40; CaCO₃, 290.67; MgSO₄.7H₂O, 90.02; MnSO₄.4H₂O, 4.39; K₂SO₄, 10.57; ZnCO₃, 0.38; CuSO₄.5H₂O, 0.36; CoCl₂.6H₂O, 0.02; FeSO₄.7H₂O, 2.06.

*Roche Products Ltd.

Feeding Trial

Female weanling conventional (CV) rats and Germfree (GF) rats weighing between 139-

170g were used. The rats were obtained from the colonies of Lister hooded rats at the AFRC Institute of Food Research, Reading. Pairs of germfree rats were housed in large stainless steel isolators and supplied with sterile diets and water *ad libitum*. Similarly, pairs of conventionalized GF rats were housed in metabolic cages supplied with sterile diets and water *ad libitum*. There were four rats per treatment. The rats were kept in a room with controlled temperature (22°C), and 12h light/dark cycle.

The test diets mixed with water prior to feeding were provided fresh daily. The food was contained in specially designed receptacles to minimize waste and faecal contamination. The rats were adapted to the test diets over a period of 3 days prior to the balance trial of 4 days duration. Faeces were collected daily throughout the balance trial. Those from the CV rats were stored at 4°C until the end of the trial, bulked and kept at -20°C until freeze dried. In the case of the GF rats, the daily collections were retained within the isolators at ambient temperature until the end of the balance period when they were removed, bulked and eventually freeze dried.

Live weights were recorded at the start of the adaptation period and at the beginning and end of the balance period. After the trial, the rats were killed by carbon dioxide asphyxiation. The abdominal cavity of each rat was opened, the caecum exposed, ligated, excised and freshly weighed. The caecal contents were rinsed out with distilled water and the empty caecum reweighed. The isolators were checked for sterility prior to the commencement of the experiment.

Nitrogen and Starch Analysis

Nitrogen was determined by a modification of the Kjeldahl-Gunnings procedure for organic nitrogen (AOAC, 1968). Amyloglucosidase Novo (AMG) (Novo Enzymes, Surrey, England) was used for hydrolyzing starch into glucose. Glucose was determined by glucose oxidase method.

Digestibility was calculated from the nutrients in the food consumed and that in the faeces.

RESULTS AND DISCUSSION

Food intake, weight gain and organ weights

Food consumption was high in all treatments but the food intake of rats on diets containing extracted cowpea flour was significantly lower (Table 2). The growth of rats

(CV and GF) on diets containing raw cowpea flour was significantly lower ($p < 0.01$) than that of rats on the other cowpea meals. This indicates an improvement in the nutritional value of raw cowpea meal on autoclaving. This improvement could be related to the destruction of antinutritional factors (ANF) in the raw cowpea meal. Oke et al., (1966) found autoclaving destroying some antinutritional factors in cowpeas.

Weight gain of the conventional rats was significantly lower ($p < 0.01$) than that of the GF rats on all diets. Wostmann (1975) claimed that GF and CV rats grow at the same rate on the same diet. Perhaps some of the weight put on by GF rats is more of fluid accumulation in the caeca than muscle mass. It has been reported that most of the caecal contents of GF rats are fluid with a dry matter percentage of approximately half of that in CV animals (Wotsman, 1975).

Although, the food intake of CV and GF rats on the three diets were similar, the feed efficiency (FE) of the GF animals was significantly higher ($p < 0.01$) than that of the conventional animals when fed autoclaved and extracted cowpea meal.

The improved feed efficiency of CV and GF rats when cowpeas were autoclaved cannot be ascribed to the inactivation of antinutritional factors since the extracted raw Cowpea Meal (CM) which still possessed a full complement of these still maintained growth equivalent to that of the autoclaved CM. The autoclaved CM by normal criteria would contain only the inactivated form.

FE in the CV rats was 50% of that in the GF rats. The GF rats would divert some of their food energy intake into growth, thereby increasing weight gain for the same food

consumption as that of their CV counterparts. The amount diverted is expected to be small. This very high FE which is associated with the doubling of weight gain by GF rats is perhaps not related to actual muscle mass gain but to the presence of more fluid in the rats caeca.

The caeca of the GF rats were significantly large ($p < 0.01$) than those of the CV animals on all diets (Tables 3 and 4). In CV rats, caecal size (caecum + contents) was affected by diet since processed (autoclaved and extracted) cowpeas elicited a significant increase in weight.

The caeca (empty plus content) of GF rats on the raw and autoclaved based diets were significantly larger than those of rats supplied with the extracted CM were similar in size, and were significantly larger than those of rats receiving the autoclaved CM diets.

It is a well known fact that the caeca of GF rats are larger than those of their conventional counterparts (Gordon, et al., 1966). This is a physiological response viz., thickening of gut wall to the germfree conditions. The response of the two types of rats to the various cowpea diets is more difficult to explain since no distinct pattern could be observed. Generally, but not completely, raw and extracted CM based diets, produced similar patterns of effects in the caeca (empty and full) of CV and GF rats. Autoclaved CM was much more variable in its effects. Particularly noteworthy is the excessively large enlargement of the full caeca of such rats. No biochemical or physiological explanations can be given for this effect.

Apparent Digestibility of Dry Matter (ADDM), Starch and Nitrogen.

ADDM in the CV rats was significantly

Table 2

Weight Gain and Food Intake of Conventional (CV) and Germfree (GF) Rats, Fed Diets Containing Raw and Processed Cowpeas

Major Dietary Component	Rat Type	Initial Wt. (g)	Final Wt. (g)	Wt. Gain Dry (g)	Food Intake Dry (g)	Feed Efficiency (FE)
Raw Cowpea Meal	CV	309.0 ± 17.0	311.5 ± 13.4	2.5 ± 0.71 ^a	86.48 ± 2.6	0.03 ± 0.04 ^a
	GF	342.0 ± 4.2	348.5 ± 7.8	6.5 ± 3.5 ^a	89.7 ± 11.6	0.07 ± 0.03 ^a
Autoclaved Cowpea	CV	301.0 ± 4.2	311.0 ± 4.2	10.6 ± 0.0 ^b	84.18 ± 13.5	0.12 ± 0.01 ^b
	GF	342.5 ± 3.5	364.0 ± 1.4	21.5 ± 4.9 ^b	95.55 ± 3.9	0.23 ± 0.06 ^b
Extracted Cowpea Meal	CV	318.5 ± 19.1	330.5 ± 19.1	12.0 ± 0.0 ^b	103.7 ± 3.3	0.12 ± 0.01 ^b
	GF	362.0 ± 22.6	383.5 ± 24.7	21.5 ± 2.1 ^b	109.28 ± 4.6	0.20 ± 0.03 ^b

Values are mean ± SD for 2 groups of rats (each group consists of 2 rats).

Values with common superscripts are not significantly different

greater ($P < 0.01$) than that in GF rats (Table 5) for all diets.

Autoclaving for 1 hour did not improve ADDM but the removal of oligosaccharides from the raw cowpea meal improved dry matters digestibility in CV rats. In the GF rats, autoclaving for 1 hour and extraction of raw cowpea meal with 80% ethanol did not cause a significant increase in dry matter digestibility.

Starch digestibility was in excess of 99% in the CV rats irrespective of diet (Table 5). In CV rats, digestibility of cowpea starch was improved by the removal of alcohol soluble components, but autoclaving had no effect.

In the GF rats, Starch digestibility was also high but always less than 99%. Autoclaving (1 hour) resulted in a decrease in starch digestibility ($P < 0.01$). The digestibility of starch in alcohol extracted cowpea whilst being almost 99% was not significantly different from that of the raw meal.

The apparent digestibility of Nitrogen (ADN) was low in both CV and GF rats irrespective of diet (Table 5). ADN was significantly higher in the CV rats than GF rats for raw and extracted cowpeas. In the autoclaved sample, there was no significant difference. This result indicates the probable involvement of microbial flora in the digestion of cowpea protein although the amount fermented is only a small percentage of the ingested protein (4-10%). It was also observed that ADN was highest for the alcohol-extracted cowpeas although this was not significantly different from the other diets for the GF rats.

Table 3

Empty Weights (g) of caeca of Conventional and Germfree Rats on Raw and Processed Cowpea Diets.

Diets	CV	GF
Raw	0.75 ± 12 ^a	11.76 ± 0.2a
Autoclaved Cowpeas Meal (1 h)	0.94 ± 1.1 ^b	11.42 ± 0.6 ^b
Extracted Cowpea Meal	0.80 ± 0.2 ^{ab}	11.65 ± 0.2 ^a

Values are mean ± SD for 4 rats.

Values with common superscripts are not significantly different.

Table 4

Fresh Weights (g) of Caeca of Conventional and Germfree Rats on Raw and Processed Cowpea Diets.

Diets	CV	GF
Raw	3.51 ± 0.6 ^a	20.6 ± 2.7 ^b
Autoclaved Cowpea Meal (1 h)	4.13 ± 0.4 ^a	23.82 ± 3.8 ^c
Extracted Cowpea Meal	3.7 ± 0.8 ^a	16.14 ± 2.9 ^a

Values are mean ± SD for 4 rats.

Values with common superscripts are not significantly different.

Table 5 Apparent Digestibility of Dry Matter, Starch and Nitrogen in Conventional and Germfree Rats Supplied Diets Containing Raw and Processed Cowpeas.

Major Dietary Component	Rat Type	Apparent Digestibility of Dry Matter (%)	Apparent Digestibility of Starch (%)	Apparent Digestibility of Nitrogen (%)
Raw Cowpea Meal	CV	87.58 ± 1.44 ^a	99.04 ± 0.11 ^a	72.0 ± 2.37 ^a
	GF	78.0 ± 1.0 ^a	98.67 ± 0.8 ^a	65.24 ± 0.33 ^a
Autoclaved Cowpea Meal (1 h)	CV	86.60 ± 2.67 ^a	99.19 ± 0.02 ^{ab}	73.4 ± 5.51 ^a
	GF	78.17 ± 1.37 ^a	97.35 ± 0.57 ^b	69.2 ± 0.14 ^{aa}
Extracted Cowpea Meal	CV	89.20 ± 0.01 ^b	99.67 ± 0.01 ^b	79.92 ± 0.31 ^b
	GF	80.06 ± 2.37 ^a	98.97 ± 0.21 ^{aa}	69.86 ± 6.9 ^a

Values are mean ± SD for 2 groups of rats (each group consists of 2 rats)
Values with common superscripts are not significantly different

The result of this experiment reveals that nitrogen (protein) digestibility is low irrespective of heat treatment of cowpeas and whether the animals are CV or GF. The small amount of nitrogen (protein) digestion (4-10% of nitrogen intake) occurring in the lower gut is of little significance. Antinutritional factors (ANF) associated with protein utilization are either of no consequence or are highly refractory and impair protein digestibility. Thus ADN may be low because of the characteristics of cowpea protein per se and /or that Protease inhibitors (PI) are exerting their effect. The contribution of PI to the low ADN is likely to be minimal since the most resistant PI are broken down in the GIT in part. The possibility of tannin contributing to this low ADN is not likely since the cowpea variety used is a white type which would

normally contain low tannin levels. It is also possible that cowpea nitrogen is peculiar. Perhaps cowpeas contain some nitrogen with a low digestibility. Some studies suggest that a soluble nitrogen fraction with a low digestibility is found in variable amounts in cooked legumes (Bressani et al., 1977, 1982).

The result shows cowpea starch and nitrogen being capable of contributing to the energy needs of the weanling if cowpeas are used as a component of weaning food. The ability of starch to contribute to gaseousness in the gastrointestinal tract of infants by extrapolation, is minimal as shown by the extremely low digestion of cowpea starch in the hind gut of the rats. So, from this study, the use of cowpea as a major component of weaning food is to be encouraged, since its energy digestibility will be high, and with the removal of cowpea oligosaccharide, very little gas would be produced in the hind gut of the weanling infant.

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