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The Effect of Cowpea Oligosaccharides on Gas Production in Adult Rats

Z.M. Ofuya

Department of Physiology, College of Health Sciences,
University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

Abstract: The effect of cowpea oligosaccharides and oligosaccharide free meal on gas production in adult female weanling rats was studied. The pattern of hydrogen produced in 24 h when some other rats were fed raw cowpea meal was also studied. In the study on the pattern of hydrogen produced in 24 h in rats fed raw cowpea meal, maximum rate of hydrogen production occurred between 2-8 h. There was considerable variability in the amount of hydrogen produced between rats. After 8 h, hydrogen production declined. When adult rats were fed a basal diet, a diet containing oligosaccharide free (OFR) meal and OFR plus the alcoholic extract; the basal diet produced an average of 1.3 mL H₂ over an 8 h period. Diets containing OFR resulted in a three-fold increase in gas production whereas the addition of cowpea alcoholic extract to the OFR resulted in a higher but variable hydrogen production. The oligosaccharides were responsible for approximately 50% of the total gas produced when whole cowpeas were ingested. The highest rate of hydrogen production was found to occur between 2-7 h after feeding.

Key words: Cowpeas (*Vigna unguiculata*), gas production, weaning

INTRODUCTION

Legumes are consumed all over the world (FAO 1975, 1981). Consumption is higher in those parts of the world where animal proteins are scarce and expensive e.g. South East Asia and Africa (COPR, 1981). In these parts of the world, they provide a large proportion of the protein required for adults and children.

Cowpea is an important source of protein in many parts of the world (Phillips and McWatters, 1991). It is an important grain legume in East and West Africa, as well as other developing countries (Dovlo *et al.*, 1976). Cowpeas are good sources of energy, protein, certain B-vitamins (thiamin and riboflavin) and certain minerals (calcium and potassium) (Walker, 1982; Uzogara and Ofuya, 1992). However, the use of cowpeas and other legumes as food is considerably limited because besides their containing antinutritional substances, they also cause gas production (flatus) in both man and other monogastric animals. This is due to the presence of raffinose groups of oligosaccharides (raffinose, stachyose and verbascose) (Christofaro *et al.*, 1974; Reddy *et al.*, 1983; Oke *et al.*, 1995; Nanna and Phillip, 1990; El Faki *et al.*, 1983; FAO, 1997). The content of oligosaccharides in legumes can range from 5-8% on a dry weight basis (Asp, 1995). Flatus is bowel gas containing carbon dioxide, hydrogen and methane (Rackis, 1975; Singh *et al.*, 1993).

Although flatulence is not a health problem, it however limits the use of legumes (El Faki *et al.*, 1983).

While the contributory role of some legume oligosaccharides to the total gas produced by beans has been studied (Fleming 1981a, b; Reddy *et al.*, 1980; Olson *et al.*, 1982), information on cowpeas is sparse. In order to reduce the flatus potential of cowpeas and thus improve the usage of cowpeas both by adults and as a possible source of weaning food in the tropics, an attempt was made in this study to understand the flatus potential of cowpeas and the quantitative role played by the oligosaccharides and the oligosaccharide free residue in the total gas produced by the legumes. This study was therefore carried out to see the extent to which cowpea oligosaccharides contribute to the total gas produced by (oligosaccharides) cowpeas. Thus the same groups of rats were fed a basal diet devoid of oligosaccharides, cowpea Oligosaccharide Free Residue (OFR) and OFR plus oligosaccharides added back and the gas produced was measured using hydrogen as an indicator. This was done in order to avoid the negative effects caused by large individual variability reported in rats (Wagner *et al.*, 1977). Also the feeding of the basal diet was in order to feed a non flatulent meal. Basal diet contained corn starch and casein which are highly digestible for a baseline result. The OFR was fed next in order to clearly see its effect and OFR plus oligosaccharides added back also in order to clearly its effect also. This method is different from the studies of Fleming (1981a) in which the oligosaccharides in beans were determined and their content correlated with the gas produced in rats.

MATERIALS AND METHODS

Preparation of experimental diets: The experimental diets (powder form) consisted of a basal formulation containing semi-purified ingredients, raw cowpeas and cowpea component diets (cowpea extract and oligosaccharide-free residue) (Table 1-3). The basal diet was formulated to contain maize starch 56.5, casein 20, corn oil 8 cellulose 5, glucose 4.9, Mineral mix 4.5, fat and water soluble vitamins 1.1% (Table 1).

Cowpea oligosaccharide free flour meal was prepared by extracting 500 g raw cowpea (1 mm particle size) with 80% ethanol (1:10 w/v) for 2 h at 80°C with continuous stirring. The mixture was left to settle and the supernatant decanted. The residue was re-extracted twice by the same method and the supernatant pooled. The extract was concentrated to 400 mL, using a rotary evaporator at less than 37°C. The total sugars present in the cowpea oligosaccharide extract were estimated by TLC technique. The oligosaccharide free meal was air dried and where appropriate crude alcoholic extract added back and freeze dried.

Estimation of cowpea oligosaccharides: Cowpea oligosaccharides were estimated by the following procedure: Whole cowpeas were milled to pass through a 1mm screen sieve (C and N) lab mill, Christy and Morris Ltd. Chelmsford, England). 1.0 g of cowpea meal was exhaustively extracted with 10 volumes of hot 80% ethanol (w/v) in a shaking water bath at 80°C for 2 h. After standing, the clear supernatant was removed by aspiration and retained. The residue was extracted twice more and the extracts combined. The alcoholic extracts were evaporated to dryness using a rotary evaporator operating at temperatures lesser than 37°C and the residue dissolved in 1.0 mL distilled water. The aqueous extract was purified by an ion exchange procedure. The aqueous extract was ran through a strongly acidic cation exchanger

Table 1: Composition of the basal diet

Ingredients	Diet (g kg ⁻¹)
Maize starch	565
Casein	200
Corn oil	80
Cellulose	50
Glucose	49
Mineral mixture ¹	45
Fat and water soluble vitamins ²	11

1. Composition of mineral mixture (g kg⁻¹): NaCl, 296.59; KH₂PO₄, 296.40; CaCO₃, 290.67; MgSO₄·7H₂O, 90.02; MnSO₄·4H₂O, 4.39; KI, 0.57; ZnCO₃, 0.38; CuSO₄·5H₂O, 0.36; CaCl₂·6H₂O, 0.02; FeSO₄·7H₂O, 20.6. 2. Composition of fat and water soluble vitamin mixture (g kg⁻¹): Thiamin, 1.0; Riboflavin, 1.0; Pyridoxine 1.0; Calcium pantothenate, 6.0; Nicotinic acid, 20.0; Inositol, 40.0; P-Aminobenzoic acids 6.0; Biotin, 0.1; B₁₂ (in Mannitol), 5.0; Choline, 120.0; Menadione, 0.1; Rovimix* A500, 2.0; Rovimix* D3500, 1.5; Rovimix* E50, 15.0; Maize Starch, 727.3, *Roche Products Limited

Table 2: Composition of cowpea meal fed in experiment 1

Ingredients	Total diet (%)
Cowpea	66.7
Casein	22.2
Glucose	11.1

Table 3: Composition of cowpea oligosaccharide free meal fed in experiment 2

Ingredients	Total diet (%)
Oligosaccharide free residue	65.1
Casein	23.3
Glucose	11.6

and a strongly basic anion exchanger in series. The cation exchanger was Dowex 50 × 4 H⁺ form, 50 mesh, while the anion exchanger was Dowex 1 × 10 formate form, 50 mesh.

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

Training of rats for meal feeding and subsequent hydrogen collection:

Adult female rats were used in this study. Adult rats were obtained from the breeding centre of the school of Agriculture, Sutton Bonington, Loughborough. The study was carried out at the Department of Applied Biochemistry and Food Science, School of Agriculture, University of Nottingham, Sutton Bonington, England. They were trained to consume adequate amounts of the experimental diets over short periods of time (meal-feeding). The rats were placed in individual metabolic cages kept in a controlled environment at 22°C with 12 h lighting.

The basal diet (Table 1) was used in the training period for meal feeding. The purpose of meal feeding was to train the rats to consume up to 6 g of the basal diet within a period of 90 min. The training was carried out as follows: 10 g of the basal diet was given to each rat. After 90 min the food was removed and that left over was weighed, split food taken into consideration. Water was supplied *ad libitum* throughout. This procedure was repeated daily until the rats consumed at least 6 g of the food within 90 min.

Hydrogen measurement: Hydrogen production from rats was monitored intermittently by the technique of Gumbmann and William (1971). The method involved the use of a closed circuit life-support system into which oxygen is bled and from which carbon dioxide is removed (Fig. 1).

Gas samples (20 mL) were taken by means of a gas-tight syringe through the sampling port every 60 min throughout the duration of the experiment. The hydrogen content of this system was determined using an exhaled hydrogen monitor (GMI Medical Ltd. Renfrew, Scotland). Before the assay, the hydrogen monitor was calibrated by injecting a 2 mL portion of GMI standard calibration gas

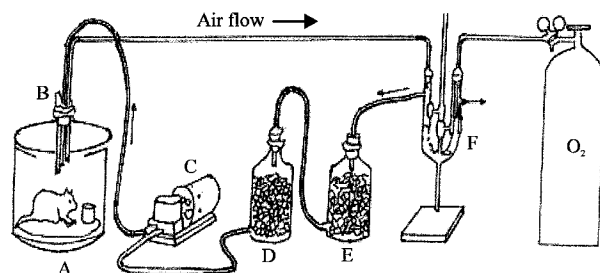


Fig. 1: A schematic diagram of the life support system. A-Desiccator, B- Gas sampling port C- Motor driven pump,, D-Sodar time (with indicator), E- Calcium sulphate, F- Manometric valve containing light vacuum pump oil

mixture into the system. Measurement of hydrogen usually commenced immediately after feeding and continued for the duration of the experiment which was usually 8 h or longer where appropriate.

Feeding procedures

Experiment 1: The pattern of hydrogen production over a 24 h period was studied using two female rats. In this experiment, cowpea diet composed of 3 g raw cowpea flour (1 mm particle size) 1 g casein and 0.5 g glucose thoroughly mixed (Table 2) The diet when supplied was completely consumed within 90 min by individually trained female rats (120 and 125 g). These rats were then placed in the life-support system for measurement of the hydrogen produced.

Experiment 2: In this experiment, hydrogen production by rats in response to oligosaccharides added back to extracted meal was studied. Eight adult female rats (112-143 g) which had been meal fed to consume 6 g of the basal diet as earlier, described were used for this study. Two rats were used per assay. On day 1, each rat was fed 4.5 g of the basal diet for a period of 90 min and placed in the life-support system and hydrogen production measured for a period of 8 h. After this time the rats were returned to their individual cages in the environmentally controlled room and only allowed water until day 2. On day 2, the rats were supplied 4.5 g of a diet containing 2.8 g of oligosaccharide-free meal (equivalent to 3.0 g raw cowpea meal) with 1.0 g casein and 0.5 g glucose which was consumed within 90 min. After feeding, the rats were placed in the life support system and hydrogen production measured for 8 h. Thereafter the rats were returned to the metabolic cages as above. On day 3, each rat was supplied a diet containing 2.8 oligosaccharide-free residue with cowpea extract (equivalent to 3.0 g whole cowpeas). Casein (1 g) and glucose (0.5 g) were added

as before, thoroughly mixed and fed. After 90 min, hydrogen production was measured over an 8 h period as described earlier.

RESULTS

The cowpea variety used in this work contained 1.4% raffinose, 2.5% stachyose and probably a small amount of verbascose. The last sugar could not be estimated

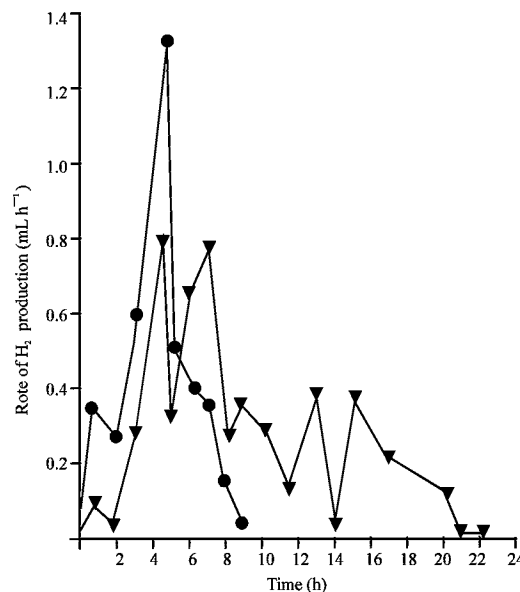


Fig. 2a: The rate of H₂ production (volume in unit time) over a 24 h period in Rats fed raw cowpeas ●—● Rot 1, ▼—▼ Rot 2

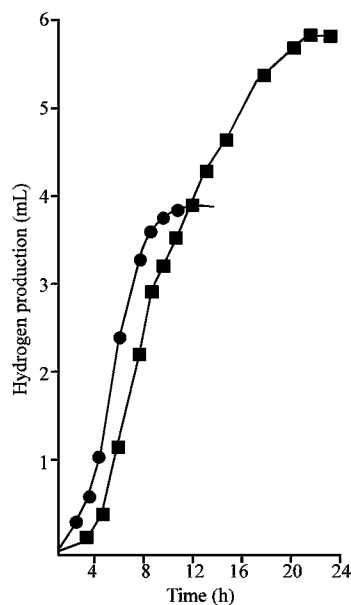


Fig. 2b: Cumulative H₂ production in Rats fed raw cowpeas over 24 h ●—● Rat 1, ■—■ Rat 2

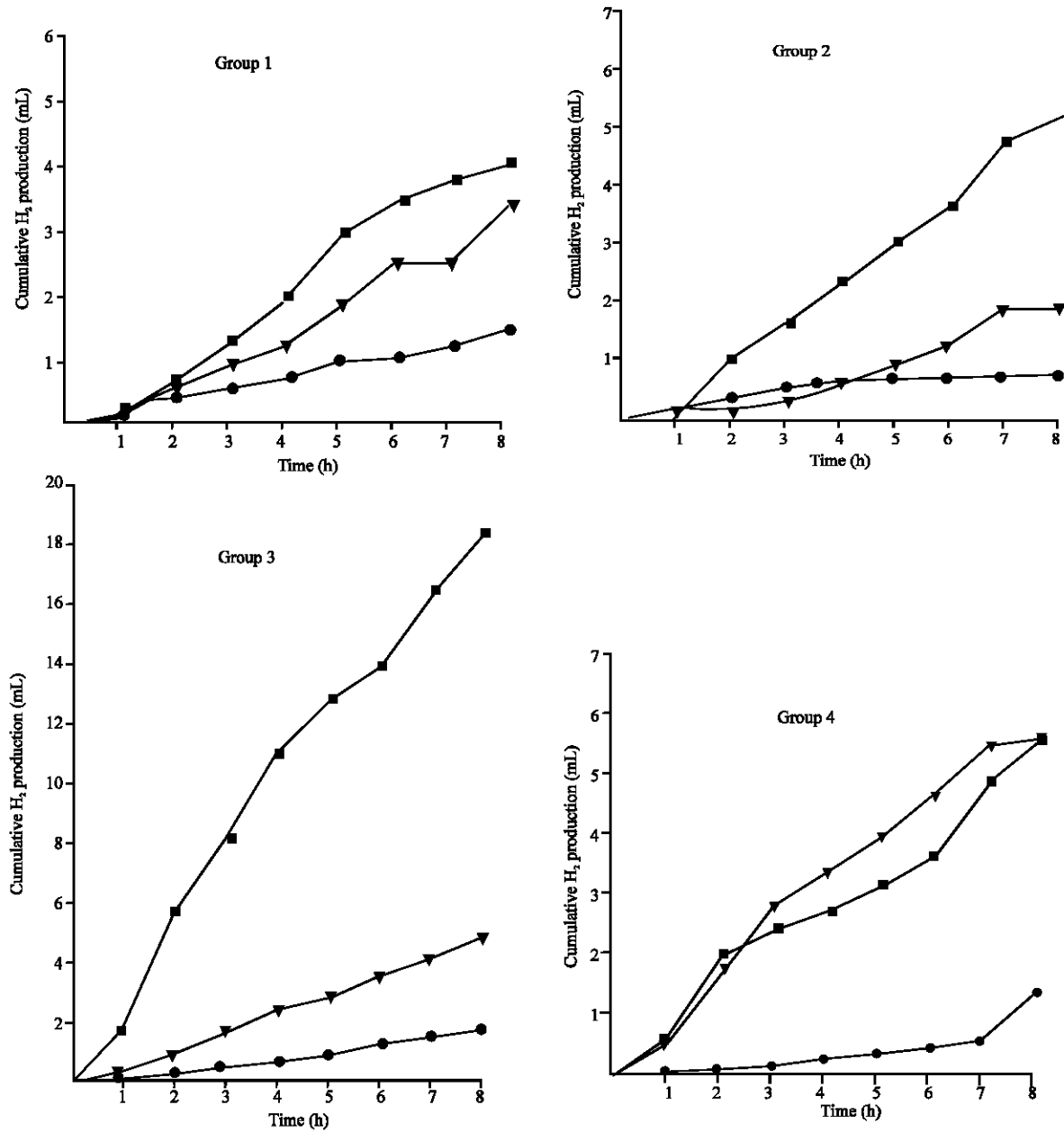


Fig. 3: Cumulative H₂ production in adult rats fed the basal diet and cowpea components (2 rats per group) ●—● Basal diet, ▼—▼ OFR, ■—■ OFR extract

quantitatively because of the unavailability of standard verbascone. Verbascone has been reported to account for around 3% of the dry weight (Reddy *et al.*, 1983) of cowpeas (Longe, 1981).

It took the rats an average of 10 days to adapt to consuming 6 g of basal diet in 90 min. Other workers have used this method to train rats in this way (El Faki *et al.*, 1983; Gumbnam and William, 1971; Venkataraman and Jaya, 1975).

Part of the hydrogen produced is passed out through the rectum while the remainder diffuses into the blood stream where it appears in the expired air.

Experiment 1: In the study of the pattern of hydrogen production over 24 h period, measurable hydrogen production could be detected within 1 h after the consumption of cowpeas (Fig. 2 a and b). Maximum rates of hydrogen production occurred between 2-8 h in both

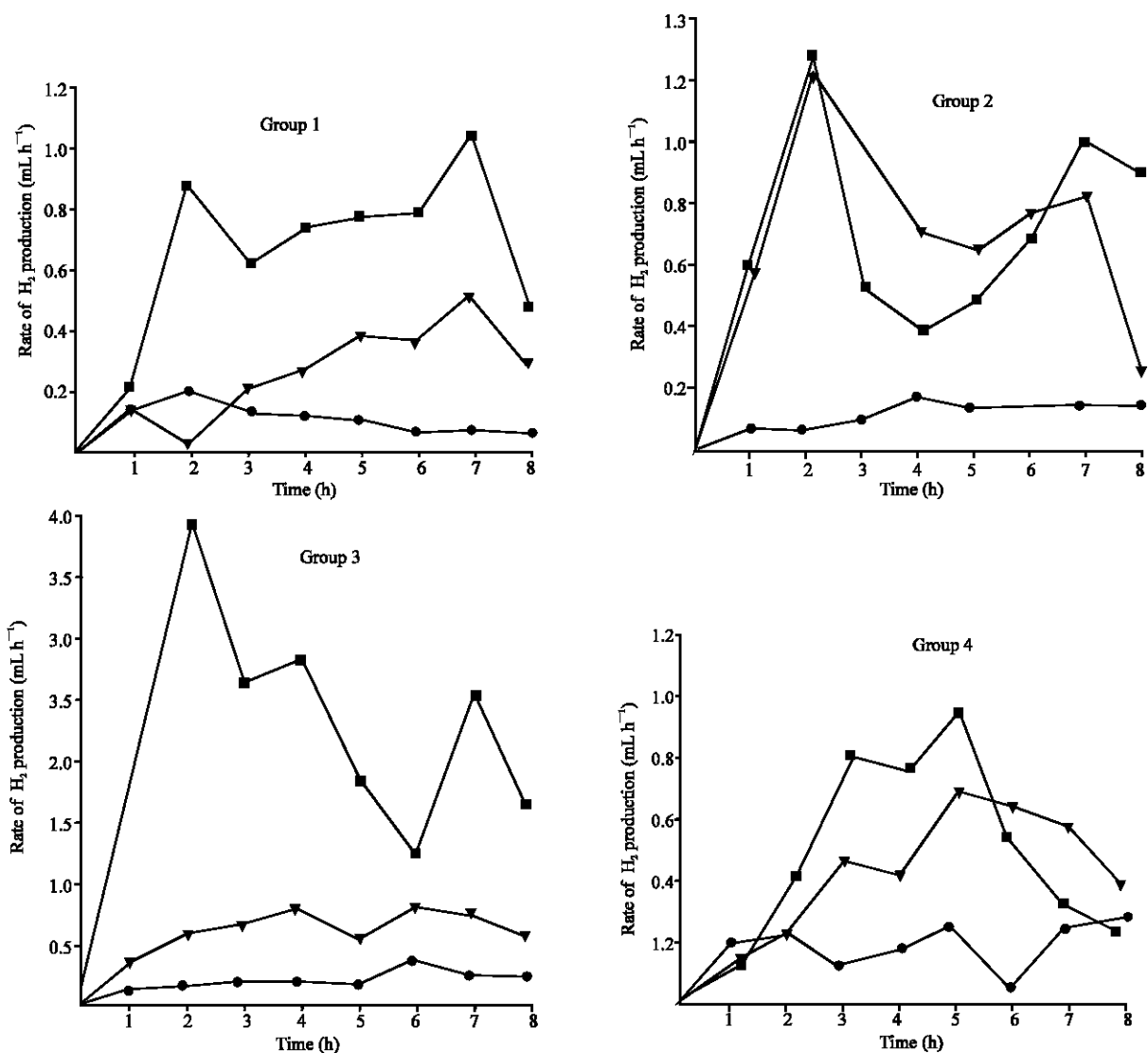


Fig. 4: The rate of H₂ production in adult rats fed the basal diet and cowpea components (2 rats per group) ●—● Basal diet, ▼—▼ OFR, ■—■ OFR extract

rats (Fig. 2a). There is considerable variability in the amount of hydrogen produced between rats. Cumulative hydrogen production was similar in both rats over the first 8 h. The rate of Hydrogen production was linear up to 4 h in both rats after which it began to drop in rat 2. In rat 1 it rose again and finally dropped after 8 h.

Experiment 2: Figure 3 and 4 show both the cumulative and rate of hydrogen production in rats fed the basal diet, OFR and OFR plus oligosaccharides. Table 4 shows an analysis of the hydrogen produced by the individual groups of rats. From the Table 4, both OFR and the oligosaccharides caused gas production. Hydrogen produced by OFR plus oligosaccharides was greater than

that produced by just the OFR while that produced by the OFR was greater than that produced by the basal diet. There was high individual variability in between rats

Table 4: Total hydrogen production in rats following the consumption of diets containing cowpea preparations

Diet	Total H ₂ produced (mL)				Mean±SD
	Replicate				
	1	2	3	4	
Basal	0.86	0.94	1.78	1.64	1.31±0.5
Oligosaccharide-Free Residue (OFR)	2.05	5.93	4.88	3.61	4.12±1.7
OFR + Cowpea extract	5.43	5.89	18.64	4.2	8.54±6.8

H₂ production was monitored over an 8 h period following feeding of 6.0 g basal diet and 4.5 cowpea diets

particularly those fed the oligosaccharides. Figure 3 shows a linear increase in cumulative gas production over 8 h while Fig. 4 shows a linear increase in the rate of hydrogen produced for 2 h after which it dropped slightly and was maintained for about the next 5 h. By the 8th h the rate dropped.

DISCUSSION

The hydrogen produced in the rats is due to bacterial fermentation of indigestible carbohydrates such as the raffinose group of oligosaccharides and dietary fibre in the hindgut of the rats. A similar response may occur in human subjects following ingestion of cowpea and its products. El Faki *et al.* (1983) found cowpea oligosaccharides causing gas production *in vitro*.

Wagner *et al.* (1977) found measurements of hydrogen production in rats to be a predictive bioassay for flatulence in man. The detection of hydrogen within one hour in experiment I most probably represents basal hydrogen production which is unrelated to the cowpea diet. The differences in hydrogen production in rats one and two of experiment I simply represent differences in the fermentative capacity of the individual rats. Fleming (1981) reported a high degree of variability in the hydrogen production among rats fed pulse legume seeds (PLS). This author also reported that rats receiving such legume diets returned to their basal level of hydrogen production within one and half hours.

The production of hydrogen by a basal diet in experiment 2 agrees with the study of (Fleming, 1981b). Fleming found a basal diet of similar composition, to elicit hydrogen production when supplied to rats. The production of hydrogen by the oligosaccharide free residue agrees with the study of Olson *et al.* (1982) and Fleming (1981a, b).

Calloway *et al.* (1971) showed that alcohol extracted beans resulted in flatus gas production three times higher than the basal diet levels but only one fifth as high as that of the control beans from which the extracted lot was made.

This study was carried out to study the contributory role of cowpea oligosaccharides to the total gas produced by cowpea so that a better understanding can be gotten on how to reduce the gas produced. From the studies carried out so far, cowpea oligosaccharides were found to contribute about 50% of the total gas produced. The oligosaccharide free residue was found to be responsible for the remaining 50%. This is in agreement with the report by Fleming (1981a, b) and Olson *et al.* (1982) in which they reported the oligosaccharides causing 50% of the gas produced by legumes. The flatulence potential of

cowpeas appear to be equally divided between the extractable and nonextractable components. Therefore the removal of cowpea oligosaccharide would reduce its flatus potential by half.

Alcohol insoluble residue would contain starch and dietary fiber as well as protein. Microbial fermentation of these products could be responsible for the hydrogen produced by this residue. Marthinse and Fleming (1982) reported gas production following the consumption of dietary fiber in humans. Noah *et al.* (1998) reported white beans (*Phaseolus vulgaris*) dietary fiber to be fermented. MacFarlane *et al.* (1992) claimed protein to be an important substrate for colonic fermentation. The protein in cowpeas might not have been completely digested. Ofuya (2002, 2006) found bacterial fermentation of a small percentage of cowpea protein in rats. Definitely the small percentage of protein fermentation cannot contribute much to gas production in the OFR residue. The role of starch in this process is also not known. Christofaro *et al.* (1974) found a negative correlation between the production of gas by legume seeds and their starch content. Ofuya (2002) reported the fermentation of a negligible percentage of starch in the gut of conventional rats in an experiment carried out using conventional and germ free rats.

Since the 1, 6 alphasgalactosidases responsible for hydrolysis of the oligosaccharides are absent from the digestive tract of man and rats, the hydrogen production from these sugars in cowpeas is by bacterial fermentation in the large intestine of the rats. *Clostridium perfringens* has been identified as the principal bacterium involved in this process.

The oligosaccharides of cowpeas have been shown to produce flatus in adult rats. Removal of such factors would be highly beneficial for those consuming cowpeas. Although alcohol was used here, other procedures can be equally effective. For example, soaking cowpeas for a number of hours and discarding the soak water could remove the majority of the oligosaccharides present. Silva and Luh (1979) reported a reduction of 90.6% in the oligosaccharides present in black-eyed beans when the beans were soaked. This result is supported by the work carried out by Onyesum *et al.* (2005). Thermal process also reduces oligosaccharides (Frias *et al.*, 1990; Onyenekwu *et al.*, 2000).

Discarding the cooking liquor has been found to reduce the level of oligosaccharides in other legumes (Ku *et al.*, 1976). In addition, germination of cowpeas is another method that could prove effective. Published data indicate that over 70% of the raffinose family of

oligosaccharides were removed from several beans on germination (Gupta and Wagle, 1980; Reddy and Salunkhe, 1980; Snauwaert and Makakis, 1976; Silva and Luh, 1979; Jaya and Venkataraman, 1981). Fermentation and gamma irradiation have also been found to be effective (Deszkiewicz-Reinhard *et al.*, 1994; Machaiah and Pednkar, 2002; Doblado *et al.*, 2003)

The most effective method for eliminating the oligosaccharides from pulse legume seeds but specifically cowpeas would be the production of oligosaccharides-free varieties by breeding.

In this study, the individual factors responsible for gas production in the oligosaccharide free residue were not investigated, there is a need for further work in order to know the contributory role of cowpea starch, protein and dietary fiber to gas production in man. While only H₂ was assayed in this study an assay of the other gases would help to give a holistic view to the total gas produced and the exact percentage of each type to the total.

Although the individual factors responsible for gas production in the oligosaccharide-free residue were not investigated directly, around 50% of gas production by cowpeas is probably due to these components. Since cowpea oligosaccharides were found to cause around 50% of hydrogen production, removal of the oligosaccharides by breeding or by chemical processing at the household level would only reduce gas production by 50%. Complete elimination of the remaining gas producing factors would require very drastic processing which would be economically unfeasible and technically difficult.

REFERENCES

- Asp, N.G., 1995. Classification and methodology of food carbohydrates as related to nutritional effects. *Am. J. Clin. Nutr.*, 61 (Suppl): 930S.
- Calloway, D.H., C.A. Hickey and E.L. Murphy, 1971. Reduction of intestinal gas forming properties of legumes by traditional and experimental food processing methods. *J. Food Sci.*, 36: 251-255.
- Christofaro, E., F. Mottu and J.J. Wuhrmann, 1974. Involvement of the Raffinose Family of Oligosaccharides in Flatulence. In: *Sugars in Nutrition*. (Eds.) Suppte, H.L. and K.W. McNull. Academic Press. London, pp: 33.
- COPR (Centre for Overseas Pest Research), 1981. *Pest Control in Tropical Grain Legumes*. 1st Edn. Crown Publisher, London.
- Deszkiewicz-Reinhard, W., E. Gupska and K. Khan, 1994. Reduction of stachyose in legume flours by lactic acid bacteria. *J. Food Sci.*, 59: 115-117.
- Doblado, R., J. Frias, R. Munoz and C. Vidal-Valverde, 2003. Fermentation of *Vigna sinensis* var. carilla by natural microflora and *Lactobacillus* species. *J. Food Protec.*, 66: 2313-2320.
- Dovlo, F.E., C.E. Williams and L. Zoaka, 1976. Cowpeas: Home preparation and use in West Africa. IDRC-055e, Ottawa, Canada.
- El Faki, H.A., T.N. Bhavanishgar, R.N. Tharanathan and H.S.R. Desikachar, 1983. Flatus effect of chickpea (*Cicer arietinum*) Cowpea (*Vigna sinensis*) and horsegram (*Dolichus biflorus*) and their isolated carbohydrate fractions. *Nutr. Rep. Intl.*, 27: 921-929.
- FAO., 1975. *FAO Year Book*, FAO, Rome.
- FAO., 1981. *FAO Year Book*, FAO, Rome.
- FAO., 1997. *Carbohydrates in human Nutrition*, FAO Food and Nutrition paper, Rome, 66: 14-18.
- Fleming, S.E., 1981a. A study of relationship between flatus potential and carbohydrate distribution in legume seeds. *J. Food Sci.*, 46: 794-798.
- Fleming, S.E., 1981b. Flatulence activity of the smooth seeded field pea as indicated by hydrogen production in the rat. *J. Food Sci.*, 47: 12-15.
- Frias, J., O. Vidal-velverdi, H. Kozłowska, J. Tebera J. Honko and C.L. Hedley, 1990. Natural fermentation of lentils. Influence of time, flour concentration and temperature on kinetics of monosaccharide, diassaccharides and alpha-galactosidase. *J. Agric. Food Chem.*, 44: 579-584.
- Gumbmann, M.R. and S.N. William, 1971. The quantitative collection and determination of H₂ gas from the rats and factors affecting its production. *Proc. Soc. Exp. Biol. Med.*, 137: 1171-1175.
- Gupta, K. and D.S. Wagle, 1980. Changes in antinutritional factors during germination in phaseolus mungoreus, a cross between *Phaseolus mungo* L (M1-1) and *Phaseolus aureus* (T1) *J. Food Sci.*, 45: 394-395.
- Jaya, T.V. and L.V. Veukataraman, 1981. Changes in the carbohydrate constituents of chickpea and green gram during germination. *Food Chem.*, 7: 95-104.
- Ku, S., L.S. Wei, M.P. Steinberg, A.L. Nelson and T. Hymowitz, 1976. Extraction of oligosaccharides during cooking of whole soybeans. *J. Food Sci.*, 41: 361-364.
- Longe, O.G., 1981. Carbohydrate composition of different varieties of cowpeas (*Vigna unguiculata*). *Food Chem.*, 6: 153-163.
- MacFarlane, G.T., G.R. Gibson, E. Beatty, Cummings, 1992. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurement. *FEMS Microbiol. Ecol.*, 101: 81-88.

- Machaiah, J.P. and M.D. Pednekar, 2002. Carbohydrate composition of low dose radiation processed legumes and reduction in flatulence factors. *Food Chem.*, 79: 293-301.
- Marthinse, D. and S.E. Fleming, 1982. Excretion of breath and flatus gases by humans consuming high-fiber diets. *J. Nutr.*, 112: 1132-1148.
- Nanna, I.A. and R.D. Phillips, 1990. Protein and starch digestibility and flatulence potential of germinated cowpeas (*Vigna unguiculata*) *J. Food Sci.*, 55: 151-153, 183.
- Noah, L., F. Guillon, B. Bouchet A. Buléon, C. Molis, M. Grates Mand Champ, 1998. Digestion of carbohydrate from white beans (*Phaseolus vulgaris* L) in healthy humans. *J. Nutr.*, 128: 977-985.
- Ofuya, Z.M., 2002. Nutritional evaluation of cowpea and its products by conventional and germ free rats. *Global J. Pure Applied Sci.*, 8: 43-47.
- Ofuya, Z.M., 2006. Effect of Microbial Flora on the digestibility of cowpea starch and nitrogen. *J. Food Sci. Technol.*, (ASFTI) India, 42: 424-427.
- Oke, D.B., O.O. Tewe and S.L. Fetuga, 1995. The nutrient composition of some cowpea varieties. *Nig. J. Ani. Prod.*, 22: 1-2, 32-35.
- Olson, A.C., G.M. Gray, N.R. Gumbmann and J.R. Wagner, 1982. Nutrient composition of and digestive response to whole and extracted dry beans. *J. Agric. Food Chem.*, 30: 26-32.
- Onyenekwu, P.C., G.C. Njoku and D.A. Amoh, 2000. Effect of cowpea (*Vigna unguiculata*) processing methods on flatus causing oligosaccharides. *Nutr. Res.*, 20: 349-358.
- Onyesum, I., A.T. Enaholo and J. Mordi, 2005. Effect of processing techniques on the content of flatulent factors and emulsion properties of cowpea (*Vigna unguiculata*). *J. Applied Sci. Environ. Manage.*, 9: 65-72.
- Phillips, R.D. and K.H. McWatters, 1991. Contribution of Cowpeas to Nutrition and Health. *Food Technol.*, 45: 127-130.
- Rackis, J.J., 1975. Oligosaccharides of Food Legumes: Alpha-galactosidase Activity and the Flatus Problem. In: *Physiological Effects of Food Carbohydrates*, Eds. Jeanes, A. and H. Hodge, pp: 207.
- Reddy, M.R. and D.K. Salunkhe, 1980. Changes in oligosaccharides during germination and cooking of black gram and fermentation of black gram/rice blend. *Cereal Chem.*, 57: 356-360.
- Reddy, N.R., D.K. Salunkhe and R.P. Sharma, 1980. Flatulence in rats following ingestion of cooked and germinated black gram and a fermented product of black gram and rice blend. *J. Food Sci.*, 45: 1161-1164.
- Reddy, N.R., D.K. Salunkhe and R.P. Sharma, 1983. Flatulence in rats following ingestion of cooked and germinated blackgram and a fermented product of black gram and rice blend. *Am. J. Clin. Nutr.*, 38: 3.
- Silva, H.C. and B.S. Luh, 1979. Changes in oligosaccharides and Starch granules in germinating beans. *Can. Inst. Food Sci. Technol. J.*, 12: 103-105.
- Singh, U., P.N. Rao, N. Subrahmanyam and K. Savena, 1993. Cooking characteristics, chemical composition and protein quality of newly developed genotypes of pigeon pea (*Cajanus cajan* L.) *J. Sci. Food Agric.*, 61: 395-400.
- Snauwaert, F. and P. Makakis, 1976. Effect of germination and gamma irradiation on the oligosaccharides of navy beans (*Phaseolus vulgarism*). *Lebensu Wiss U. Technol.*, pp: 93-95.
- Uzogara, S.G. and Z.M. Ofuya, 1992. Processing and Utilization of Cowpeas in developing countries: A review *J. Food Process. Preservation*, 16: 105-147.
- Venkataraman, I.V. and T.V. Jaya, 1975. Gastrointestinal gas production in rats fed on diets containing germinated legumes. *Nutr. Rep. Intl.* 12: 387
- Wagner, J.R., J.F. Carson, R. Becker, M.R. Gumbmann and I.E. Danhsos, 1977. Comparative flatulence activity of beans and bean fractions for man and the rat. *J. Nutr.*, 107: 689 - 689.
- Walker, A., 1982. Physiological effects of legumes in the human diet: A review. *J. Plant Food*, 4: 5-14.