"NUTRIENT CONTENT AND FEEDING VALUE OF SOME KENYAN GRAIN LEGUMES"

BY

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D E D I C A T I O N

This work is dedicated to my parents, brothers and sisters.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

CHRISTINE BOGERE

This thesis has been submitted for examination with our approval as University Supervisors.

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ABSTRACT

The chemical composition of four types of beans (P. Vulgaris) Viz: Mwezi Moja, Canadian Wonder, Mexican 142 and Rose coco; cow peas (V. Unguiculata) and pigeon peas (C. Cajan) was studied by chemical analysis for amino acids, proximate composition, minerals, gross energy, trypsin inhibitor and phytohemagglutinin activities. The amino acid contents of the legumes were compared with that of FAO/WHO (1973) according to the essential amino acid Index and chemical score methods. All the legume seeds studied were deficient in the sulphur containing amino acids except pigeon pea which was deficient in valine. Tryptophan was not analysed for.

In the extracts of raw Rose coco, Mwezi Moja, Mexican 142, Canadian Wonder beans, pigeon pea and cow pea, activities of hemagglutinins which was shown to be a protein material precipitable by saturation with ammonium sulphate $(NH_4)_2SO_4$ were detected at levels of 17066.7, 952.6, 213.30, 114.0, 9.8, and 2.5 HA/ml respectively. This material was completely destroyed at 121^{o}C for 30 minutes. Trypsin inhibitor activities were also detected in the extracts of raw Rose coco, Mexican 142, Canadian Wonder, Mwezi Moja beans Cow peas and Pigeon peas at levels of 45.1, 34.6, 24.8, 24.3, 21.7, 12.8 TUI/ml respectively. However, it was partially destroyed at 121^{o}C for 30 minutes and the percentage destruction of the TIA in Mexican 142, Mwezi Moja, Rose coco, Cow pea and Pigeon pea were 76%, 71, 65, 55, 54, and 14, respectively.

Pigeon peas and Cow peas were relatively non-toxic in the raw state and were little affected by heat treatment. Addition of tryptophan to autoclaved pigeon peas did not significantly improve the PER. However, supplementing cow peas with methionine improved its nutritional value and PER from 0.4 to 2.4 which was as good ($P > 0.01$) as casein. Pigeon peas and cow peas also caused panceatic hypertrophy when fed to rats. The various cow pea meals when fed to pigs were not significantly improved by heat and methionine supplementation.

Histopathological alterations caused by the various legume diets on some organs of the rats were also monitored. Generally, the PHA and TIA induced severe congestion, haemorhage and coagulative necrosis of the liver. The kidney was characterized with pyknotic nuclei in the epithelium and in the pancreas, there was marked pancreatic acinar atrophy.

These experiments showed that raw beans when fed as the sole source of protein are toxic to rats. The deleterious effects of raw beans were partially destroyed by heating. The supplementation of autoclaved beans with DL-methionine improved performance suggesting this amino acid to be limiting. Raw and cooked pigeon peas failed to support good growth of rats even after tryptophan deficiency was corrected. The nutritional value of cow peas, when properly processed and supplemented with methionine, is much higher than the nutritional value of beans and pigeon peas and is almost as good as that of casein.

The nutritive values of the legumes were determined with weanling male rats using growth rate, protein efficiency ratio, feed efficiency and apparent protein digestibility methods in the diets containing 10% protein supplied wholly by the appropriate legume. Three different diets were given to rats; raw legumes soaked and autoclaved legume and soaked, autoclaved legumes supplemented with the first limiting amino acid. The control groups had a standard diet with casein as the protein source. Only cow pea supplemented with maize to supply 16% protein level was fed to growing pigs from average weight of 23 kg to 45 kg live weight. They were fed raw and autoclaved cow peas with or without 0.1 % methionine supplementation. The control group had a commercial sow and weaner-diet.

The diets prepared with ground raw beans were highly toxic when fed to rats and caused rapid loss of weight, weakness, shivering, prostration, anorexia, marked abdominal distention, fierceness, growth inhibition, debilitation, emaciation, diarrhea and death within 4-10 days. Autoclaving the beans destroyed the injurious effect although there was still severe growth depression. Addition of 0.3 % D-L methionine to Mexican 142; Mwezi Moja, Canadian Wonder and Rose coco beans significantly enhanced the growth performance of rats and increased the PER from 0 to 1.6, 1.4, 1.3 and 1.2 respectively. They were all inferior ($P < 0.01$) to the casein diet which produced a PER of 2.5 when fed to rats.

1. INTRODUCTION

The potential role of legumes as a source of proteins to improve human and animal nutrition has long been recognised. Food legumes have on the average twice as much proteins as cereals and various species are consumed all over the world.

The consumption of grain legumes ranges from insignificant amounts in Europe and North America to fairly large quantities in Asia, South America and Africa. In several countries in East and Central Africa, food legumes mainly beans and peas, are a daily component of the diet.. It has, for instance, been estimated that the average daily consumption of legumes ranges from about 65 gm in Kenya to nearly 75 gm in Uganda. (Protein Advisory Group (PAG) 1973).

In addition, and especially where they are incorporated at relatively high levels in the diets, legumes also contribute substantially to the energy, mineral and vitamin daily requirement of human beings and other simple-stomached farm animals.

However, in order to maximise on their feeding values, grain legumes should be adequately processed to destroy the various toxic factors such as trypsin inhibitors, hemagglutinins etc. which are normally present in the seed (Bowman 1944; Honovar et al., 1962; Kakade and Evans 1965 a,b; Liener and Kakade 1969; Jaffe' 1973 a,b; Liener 1977).

Secondly, since they are deficient especially in sulphur containing amino acids, the amino acid deficiency should be corrected. This is normally accomplished by supplementing legumes with other feedstuffs rich in sulphur containing amino acids or as often done under experimental conditions by adding synthetic amino acids.

However, in spite of the knowledge and several publications from various parts of the world on the nutrient contents and feeding values of legumes, which show a high degree of variability not only between and within species, but also to environmental and processing conditions, data are lacking under Kenya conditions on the nutrient contents and feeding value of the various legumes produced in the country.

This study has been conducted to determine the nutrient contents and feeding values of four beans (Phaseolus Vulgaris) Viz; Mwezi Moja, Mexican 142, Rose coco and Canadian Wonder, and two types of peas; Cow peas (Vigna unguiculata/Sinensis (L) Walp.) and Pigeon peas Cajanus cajan (L) Mill sp.) when offered as the sole protein source in a raw, autoclaved or when supplemented with methionine or tryptophan on the growth performance, digestibility and their effects on the pancrease, liver and kidney of young weanling rats. The feeding value of raw or autoclaved cow peas, with or without methionine supplementation was also evaluated for growing pigs.

2. LITERATURE REVIEW

2.1 Production and Consumption of Grain Legumes in Kenya.

2.1.1 Production

According to Schoenherr and Mbugua (1976), grain legumes constitute next to maize, the most important group of food crops in the diets of people in Kenya. Beans (*P. vulgaris*), pigeon peas (*C. cajan*) and cow peas (*V. unguiculata/sinensis*) are among the most important legumes produced in the country. They estimated the total area devoted to cultivation of grain legumes to be nearly 480,000 hectares annually, of which 320,000 hectares or 67 % are under common beans (*P. vulgaris*). On individual bean variety basis the same workers (Schonherr and Mbugua, 1976) reported that 33 % of the total acreage under beans was devoted to the cultivation of Rose coco seed type, 20 % to Mwezi Moja, 19 % to Canadian Wonder and 11 % to Mexican 142. Twelve or so other minor seed types were reported to make up the remaining 16 % of the land under the bean crop.

Most of the cultivation of beans is in the medium potential areas of Central and Eastern provinces where the average area under the bean crop is estimated at two hectares per household (Schonherr and Mbugua, 1976).

Estimates of the yield of the bean crop have been reported by many groups. According to Anon, 1972/73 yields of the beans estimated for three production levels

viz; low, medium and high potential areas were 180, 540 and 1350 kg per hectare, respectively. Mukunya and Keya (1975) obtained yields of nearly 500 kg per hectare but claimed the potential yield of beans to be much higher, at 1500 kg per hectare. Working in Krinyaga District, Van Eijnatten (1975) reported production levels varying from 720 kg to nearly 2250 kg per hectare.

Data on the yield of the individual bean varieties are comparatively few. According to Mukunya and Keya (1975) Canadian Wonder and Mexican 142 grown under experimental conditions can produce as much as 3000 kg dry seeds per hectare. Van Eijnatten (1975) reported the yield of Mexican 142 to be as high as 2700 kg per hectare in Kirinyaga District.

The yield levels reported are known to be markedly affected by crop husbandry practices and severe infestation of the crop with pests and diseases have been reported to significantly reduce yields (Schonherr and Mbugua, 1976).

Another legume of relative importance in Kenya is the cow pea (V. unguiculata/sinensis). Cow pea, a crop which probably originated in Nigeria (Rachie et al., 1975; Dovlo et al., 1976) is reported to be the most important and widely grown pulse crop in the humid tropical parts of Africa (Rachie, 1972), but in Kenya the production of this legume is less than that of beans (Schonherr and Mbugua 1976). The major producing area in Kenya is centered in Kitui district.

Estimates on the yield of this crop are lacking under Kenya conditions. However, reports from other parts of the world indicate production levels of between 0.15 and 0.30 metric tons per hectare (Rachie et al., 1975). Despite the low yields, its economic value as a subsidiary crop in the lives of most African families has long been recognised (Aykroyd and Doughty, 1964). Furthermore, the crop is highly adapted to the different soil types and is less susceptible to pests and diseases than the common beans (Whyte et al., 1953; Bressani, 1973).

Another grain legume also important in Kenya is pigeon peas (C. Cajan). While on a global basis it is the fifth most important pulse crop after field beans, cow peas, chick peas and broad beans (Morton, 1976), in Kenya it is the third most important grain legume and it has already a well-developed export market, mainly as dry grain canned seeds to the U.S.A. and the Carribean (Rachie and Wurster, 1971).

Pigeon peas is a drought resistant crop and grows quite well in the drier parts of Kenya mainly in Machakos and Kitui districts and a little in the Coast Province (Anon, 1970). Apart from being drought resistant, the crop is also known for maintaining soil fertility in dry areas by root nodulation and bringing nutrients from deeper soil horizons to the surface through its deep rooting system (Gaywala, 1938).

Although no published data on the yield levels of pigeon peas are available in Kenya in countries where pigeon pea

production is a tradition, for example in India, yield levels from breeding lines have been reported to range between 1500-2800 kg per hectare (Anon 1978/9).

2.1.2 Consumption

While it is generally agreed that grain legumes are an important daily component in the food of the people of Kenya, published information on the quantity and pattern of consumption is lacking.

A comprehensive review of legume consumption in Africa undertaken by the Bukavu Technical Meeting and reported by Aykroyd and Doughty (1964) indicated that in the 97 surveys from 50 areas in some 13 African countries, there was a wide range in the quantity of legumes consumed. Daily consumption ranged from under 10 gm per person to over 150 gm per caput per day. The average daily consumption for all surveys was 40 gm per caput per day and Kenya exhibited a fairly higher 65 gm per caput per day.

Recently, Schonherr and Mbugua (1976) estimated the average daily consumption of pulses in Kenya to be 10 gm per person. If the estimate by Schonherr and Mbugua (1976) is correct then it means that in those Kenyan societies which are dependent almost exclusively on grain legumes for their source of protein are heavily under nourished since the Food and Agricultural Organisation (FAO, 1973) estimates for adequate protein intake are put at 20 gm per day for a sixth month old baby and 53 gm per day for an adult, assuming that legume protein is 70 % utilisable.

2.2 Nutrient content of grain legumes

Legumes are important in the nutrition of humans as well as livestock and poultry mainly because of their high protein content. In addition they are rich in carbohydrates but relatively low in fat, except for soybean and groundnuts. The high carbohydrate content makes them a rich source of energy. Grain legumes also contain variable quantities of minerals and vitamins (Stanton et al., 1966).

Various tables containing the nutrient content of grain legumes and other feedstuffs have been compiled (FAO 1970 , US-Canadian Feed Atlas, 1971; Latin America Feed Tables, 1974). Individual researchers have also presented data on the nutrient content of most grain legumes (Block and Weiss 1956 , Orr and Watter 1957 , Patwardhan 1962 , Aykroyd and Doughty 1964 , Venkat-Rao et al. 1964 , Evans and Bandemer 1967). However, some reports are confusing at times because scientific names have not always been used while describing the grain legumes analysed (Patwardhan, 1962).

In this section data will be presented pertaining to the protein, amino acid, carbohydrate, fat, energy and mineral contents of common beans (P. Vulgaris), cow peas (V. Unguiculata/ Sinensis) and pigeon peas (C. Cajan).

2.2.1 Common Beans (P. Vulgaris)

Common beans (P. Vulgaris) contain high and variable levels of protein. Kelly (1973) reported protein content of common beans to vary between 17 and 32 %. Tandon et al. (1957)

analysed 25 varieties of common beans and reported protein levels varying between 20.1 and 27.9 %. Bressani (1969) analysed a relatively large sample of common beans and reported protein values of between 16.8 and 28.2 %. Recently, Ritchey et al. (1976) reported a protein range from 18.8 to 21.5 % in the different bean varieties they analysed. Similar variability in protein content of beans is apparent from the many compiled Feed Tables (US-Canadian Feed Atlas, 1971; Latin America Feed Table 1974).

The variations in the protein content of the beans appear to be due to differences in varieties, environmental factors such as soil, rainfall etc. and to crop husbandry practices, especially fertiliser application (Bressani et al., 1973). Rutger (1971) and Leleji et al. (1972) showed that yield and protein content were negatively correlated in beans, but yield and gross protein production were positively correlated.

Of more importance than the total protein content of beans is the amino acid composition and especially those generally considered to be essential. Data presented by most research workers indicate beans to be very deficient in sulphur containing amino acids - methionine and cystine but quite high in lysine (Dickson and Hackler, 1973; Kelly and Bliss, 1975; Moreira et al. 1976).

Variations occur in both the essential and non-essential amino acid contents of beans. Moreira et al. (1976) analysed 47 bean varieties from South America and found a wide range in methionine content, varying from 0.91 mg/16 gN to 1.97 mg/16 gN. No correlation between methionine content and protein level was observed. Kelly and Bliss (1975) reported methionine contents ranging from 0.87 to 1.32 g/16 gN in bean samples obtained from Europe, North America and South America. Similar variability in the amino acid contents of common beans are apparent from the many compiled Feed Tables (US-Canadian Feed Atlas, 1971; Latin America Feed Table 1974; National Academy of Sciences, National Research Council (NAS-NRC) 1974).

The variations in the amino acid content of beans have been suggested to be due to differences in varieties and to variations in ecological conditions (Lantz et al., 1958). These variations in the amino acid contents may be of significance where breeding programmes for the selection of high protein quality beans are desired.

Beans are quite rich in carbohydrates. According to Ritchey et al. (1976), common beans contain between 56 and 62 % nitrogen free extracts and nearly seven percent fibre. They are, however, considerably low in fat (1-2 %) and contain only between 3 to 4 % ash.

The gross energy of common beans is reported to be quite high, approximately 4.4 Mcal/kg (Aykroyd and Doughty, 1964). This means that beans are also an important source

of energy for man and farm animals.

The mineral contents of common beans was recently reported by Ritchey et al.(1976). The beans were reported to be high but variable in calcium (59-181 mg/100 g), phosphorus (374-510 mg/100 g) magnesium (123-178 mg/100 g) and iron (5.4-7.5 mg/100 g). While the beans studied differed significantly in all the four minerals analysed except, phosphorus, no one bean type was consistently lower or higher in mineral content than the others. Data on calcium and phosphorus content of common beans reported North America, South America and Asia also show similar variability (de Moraes and Angelucci, 1971; Rockland et al., 1973; Sinha and Tripathi, 1973; Kadwe et al., 1974). de Moraes and Angelucci (1971) reported iron content of P. vulgaris beans to be about 2.76 mg/100 g dry seeds.

2.2.2 Cow pea (V. Unguiculata/Sinensis)

The protein content of cow pea varies from as low as 19 % to 34.6 % (Russel et al., 1946; Esh, 1959; Idusogie, 1971; Owusu-Domfeh et al., 1970; Boulter et al., 1973). Most cow pea varieties, however, contain approximately 24 % protein (Maner and Pond, 1971). The difference in the protein contents reported appear to be due to differences in varieties and agronomic practices (Bressani, 1973).

Reports on amino acid composition of cow pea indicate that while they are a rich source of lysine, they are very deficient in methionine and tryptophan (Orr and Watt, 1957;

Bressani et al., 1961; Owusu-Domfeh et al., 1970; Godfrey-Sam-Aggrey et al., 1976).

According to Godfrey-Sam-Aggrey et al. (1976) although the overall quantity of protein in a crop of cow peas can be increased by nitrogen fertilisation, the quality of the protein in terms of the relative levels of the essential amino acids-lysine, methionine and cystine, is not improved. These researchers observed a decrease in methionine and cystine contents of cow peas with increased nitrogen fertiliser application. Boulter et al. (1973) also noted that as the amount of protein increased in the seed the percentage of sulphur amino acids in the protein decreased. These observations appear to be related to the fact that storage protein formed at a late stage of seed development is relatively low in methionine and cystine, hence the observed negative correlation (Godfrey-Sam-Aggrey et al., 1976).

Data presented by Ritchey et al. (1976) indicate cow peas to be low in fat (1.3 %) and ash (3.1 %), fairly high in crude fibre (6.0 %) and very high in nitrogen free extract (53.2 %). Owusu-Domfeh et al. (1970) reported cow peas to contain 1.6 % ether extract, 3.8 % ash, 4.6 % cellulose and to have 4.30 Kcal/gm gross energy.

Cow peas contain variable quantities of minerals. Ritchey et al. (1976) reported cow peas to contain 69, 518, 206 and 8.0 mg/100 gm of calcium, phosphorus, magnesium and iron respectively. According to Owusu-Domfeh et al.

(1970) cow peas produced in Ghana contain 90 and 150 mg/100 gm calcium and phosphorus, respectively. Kadwe et al. (1974) reported a value of 21 mg/100 gm iron in cow peas they analysed.

2.2.3 Pigeon peas (C. Cajan)

Pigeon peas are held to be high in protein. According to Khan and Rachie (1972) pigeon peas contain between 19 and 28 % protein. Ahmad and Shah (1975) reported a protein content of about 25 % while Akbar et al. (1973) reported a value of 21.4 % protein. Most of the different types of pigeon peas studied, however, contained between 20 to 23 % protein (Pond and Maner, 1974).

The amino acid profile of pigeon peas although adequately balanced in lysine is very deficient in sulphur containing amino acids and tryptophan (Jansen 1973; Ahmad and Shah 1975). According to Orr and Watt (1957) tryptophan is more limiting than methionine.

Early studies by Krauss (1932), Ramiah and Satyanarayana (1938), and Fresie (1938) indicated pigeon peas to be rich in carbohydrates (61-65%). Ahmad and Shah (1975) found pegeon peas to contain about 1 % fat, nearly 3 % fibre and about 3 % ash. Ramiah and Satyanarayana (1938), however, reported a slightly higher fat (1.6-2.7 %) level in the five Indian pigeon peas varieties they analysed.

Data on the mineral content of pigeon peas are few. According to Munsell et al. (1949) the iron content of pigeon peas varies between 1.6 and 4.1 mg/100 gm dry seeds.

2.3 Anti-nutritional Factors in Grain Legumes

It has been recognised for many years that legumes when offered in a raw form to simple stomached animals have very low feeding values (Liener 1962). The poor feeding values of raw grain legumes have been attributed to a number of factors such as trypsin inhibitors, hemagglutinins, flatulence, amylase inhibitors, hydrocyanic acid, phytates, oxalates and to the poor balance especially of sulphur containing amino acids (Liener 1974). In this section evidence will be provided pertaining mainly to the occurence, nature and toxicity of the more common anti growth factors in legumes-trypsin inhibitors and hemagglutinins while recognising that the other mentioned factors contribute to varying degrees to the poor utilisation of grain legumes by human beings, rats and simple stomached farm animals.

2.3.1 Trypsin inhibitors

It has been recognised for many years that the nutritional value and protein digestibility of legumes are very poor unless subjected to cooking or some other form of heat treatment (Osborne and Mendel 1917; Liener 1962). This depression in protein value and digestibility has been generally attributed to the presence of protease inhibitors which are common constituents of most edible grain legumes (Liener and Kakade 1969).

Read and Haas (1938) appear to have been the first to recognise the presence of an inhibitor of trypsin in plant material. Bowman (1944, 1946, 1948) obtained partially purified fractions of trypsin ihibitors from soybeans. The purified form of the inhibitor was finally isolated and crystallised by Kunitz (1945, 1946).

The existence of trypsin inhibitors is now widely recognised among grain legumes. They have shown to be present in almost all the common bean varieties (P. vulgaris in pigeon peas and in cow peas (Sohonie and Bhandarkar 1955; Tawde 1961; Kakade and Evans 1965 a,b; Ventura and Filho 1967).

Trypisin inhibitors are found in various parts of the plant with the highest concentration occuring in the seed Jaffe' 1950 a). Within the seed they appear to be located in the cotyledon and especially the outer part of the cotyledon mass (Zimmerman et al. 1967).

Purified trypsin inhihitors from beans, cow peas and pigeon peas have recently been studied for their physical and chemical properties. Pusztai (1968) reported on a bluish-pink protein isolated from kidney beans. The protein had a molecular weight in the range of 10,000-15,000 and was very rich in cystine (14%). Wagner and Reihm (1967) studied the physical and chemical properties of a trypsin inhibitor isolated from navy beans. The molecular weight of this particular inhibitor was also found to be quite high. It

was also shown to be high in cystine (15 %). According to Tawde (1961) the minimum molecular weight of trypsin inhibitor in pigeon peas is about 15,660. It is also quite high in cystine and its resitance to heat denaturation resembled that of the lima beans trypsin inhibitor. The inhibitor in cow peas has a molecular weight of about 16,000 and is composed of 150 amino acid residues. While it is also high in cystine, cow pea trypsin inhibitor apparently does not contain methionine (Ventura and Filho 1967).

The mechanism of action between the inhibitor and the enzyme has been a subject of interest for the last thirty years. Kunitz (1947) has shown that the combination of the inhibitor with trypsin was accompanied by a decrease in the sum of the free amino groups, thus suggesting that the interaction had occured through ionic groups. Attempts to determine which amino acid residues are involved in the enzyme-inhibitor complex have also been reported (Feinstein and Feeney 1966). According to these researchers, inactivation of serine and histidine residues located at the active site prevents the inhibitor from complexing with trypsin. Feinstein and Feeney (1966) further postulated that conformational changes in the modified enzyme were responsible for the inability of the inhibitor to complex with the enzyme. Other physical measurements based on ultra violet spectra, fluorenscence and optical rotation produced evidence to the effect that tryptophan and tyrosine residues lie at or near the zone of contact

between the interacting species (Edelhoch and Steiner, 1965).

The reaction between the inhibitor and the enzyme appears first to involve the cleavage of the arginyl-isoleucine bond that lies between the disulphide loop of the inhibitor (Finkenstadt and Laskowski 1965; 1967; Ozawa and Laskowski 1966). The inhibitor in such a modified form appears still to be active but when the nearly formed C-terminal residue is removed by treatment with carboxy-peptidase B, an inactive derivative is formed (Figure 1).

The amount of inhibition produced is related to the quantity of the inhibitor in the seed and this appears to vary not only within the different varieites but also between varieties (Liener 1969). Kunitz (1947) showed that soybean trypsin inhibitor neutralised approximately an equal weight of crystalline trypsin. The reaction is almost instantaneous, the half time of the reaction being almost four seconds with a second order velocity constant of 2×10^7 litres/mole/sec (Green 1957). The inhibitor in navy beans appears to act in a competitive manner, one mole of the inhibitor combines with two moles of trypsin (Wagner and Reihm 1967). Pusztai (1968), working with isolated inhibitor from kidney beans observed that at low concentrations the inhibitor formed a 1:1 enzyme-inhibitor complex with trypsin, chymotrypsin, pancreatic elastase and human plasmin. It was also apparent that the inhibitor

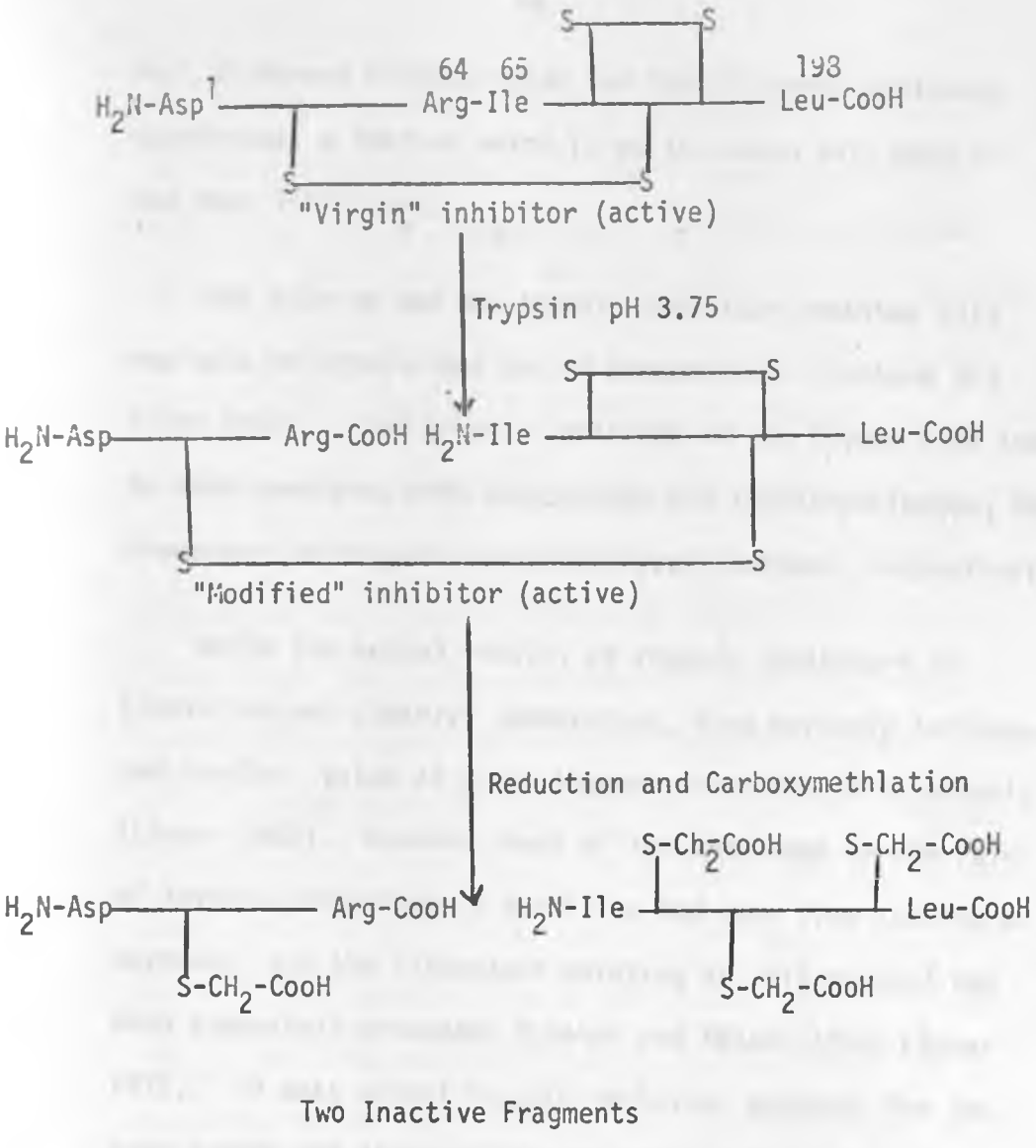

Fig. 1. Postulated mechanism of action between the inhibitor and trypsin. (Liener 1969).

had different binding sites for the different mentioned substrates; a feature which is quite common with many of the bean inhibitors.

One mole of cow pea trypsin inhibitor combines with one mole of trypsin and two of chymotrypsin (Ventura and Filho 1967). The trypsin inhibitor of the legume also seems to form complexes with trypsinogen and chymotrypsinogen, the precursors of trypsin and chymotrypsin enzymes, respectively.

While the actual role(s) of trypsin inhibitors in plants are not clearly understood, they markedly influence the feeding value of grain legumes when offered to animals (Liener 1962). However, much of the knowledge on the role of trypsin inhibitors in nutrition has come from studies on soybeans and the literature relating to this subject has been adequately presented (Liener and Kakade 1969; Liener 1972). To what extent trypsin inhibitor accounts for the poor growth and digestibility of other legumes is not certain at the present time. The situation with bean legumes is particularly puzzling since beans are also known to contain other growth inhibitors such as hemagglutinins, amylase inhibitors and flatulance causing factors (Liener 1976).

The mechanism by which trypsin inhibitors cause growth inhibition is not yet clearly understood. There seems, however, to be little doubt that the hypertrophy of the pancreas represents one of the primary physiological effects produced by feeding raw legumes or the isolated inhibitor (Rackis 1974). Booth et al. (1960) are of the

opinion that pancreas hypertrophy leads to an excessive loss of endogenous protein secreted by the pancreas. Since this protein, consisting largely of pancreatic enzymes, is quite rich in cystine (30-40 %), the resulting effect is a net loss of sulphur containing amino acids from the body (Kakade et al. 1969 b). This would explain why the need for methionine, which is inherently limiting in most legumes, is rendered even more accute in diets containing raw legumes. Secondly, it appears that even the sulphur containing amino acids present in the raw legume have a very low availability to the animal. Liener (1976) tested this hypothesis and observed that the cystine of the unheated navy bean was much less available to the chick than the cystine provided by the heat inactivated bean. It thus appears that trypsin inhibitors of legumes have a double-edged effect; they not only reduce the availability of the protein in the legume but also cause a significant loss of sulphur containing amino acids through increased pancreatic enzyme synthesis. This loss of sulphur containing amino acids serves to accentuate an already critical situation with respect to cystine and methionine in the legume and may help explain the poor growth and protein digestibility normally encountered when raw beans and other legumes are offered to animals (Liener 1976).

2.3.2 Phytohemagglutinins

Another anti physiologic factor commonly distributed in the plant kingdom is the protein with the unique property of agglutinating red blood cells - the so called phytohemagglutinins (Jaffe' 1969). Agglutinin containing plants have been found in many botanical groups including mono-and di-cotyledons, molds and lichens, but most frequently they have been detected in Leguminosae and Euphorbiaceae (Tobiska, 1964).

The name agglutinin was first proposed by Elfstrang (1897) for phytohemagglutinins and only later was its use extended to immunoagglutinins. In some literature the term lectins is also used to refer to phytohemagglutinins. In this work the names lectins, hemagglutinins and phytchemagglutinins will be used interchangeably.

About ten or so different types of phytohemagglutinins have been described (Jaffe' 1969). Although they are proteins and contain sugars and lipids, differences exist in their physical as well as chemical properties (Rigas and Osgood 1955; Ohama 1960; Jaffe' and Hanning 1965; Takahashi et al., 1967).

Phytohemagglutinins appear to be a significant factor contributing to the poor nutritive value of many kinds of beans which enjoy popular consumption in some lesser developed countries (Jaffe' 1969). Data presented by Honavar et al. (1962) indicated that the growth-promoting

properties of black and kidney beans, varieties which contain very high phytohemagglutinin levels, were only improved following heat treatment. According to these investigators black and kidney beans contained 2450 and 3560 hemagglutinating units/ml of extract, respectively. Cow peas and pigeon peas were devoid of phytohemagglutinin activity. When the phytohemagglutinins from the black and kidney beans were isolated in pure form and fed at various levels to rats in a basal ration containing 10% casein, inhibition in growth was obtained at levels as low as 0.5% of the diet, the kidney bean hemagglutinin being much more effective in this respect than black bean hemagglutinin. At a level of 0.5%, it caused 100% mortality after about 2 weeks, whereas 1.2% of black bean hemagglutinin was necessary to produce a similar mortality rate.

Jaffe' and Gomez (1975) extracted hemagglutinins from four bean cultivars representing four different hemagglutinin specifity types and injected them into mice. The lethal doses LD_{50} when injected by intraperitoneal route were for type A (red kidney bean) 470 mg/kg; type B (vainica seavegra) 1500 mg/kg; type C (cubagua subline) 590 mg/kg; and type D (mountain half runner) 3000 mg/kg; calculated as injected bean protein per kg of body weight. When applied intravenously the D extract was also the least toxic. Intradermal injection of the four extracts produced local lesions which were most severe with A extracts and very light with D type extracts.

These so called A, B, C, and D types of hemagglutinins

have been defined with respect to action with different blood preparations (Jaffe' et al. 1972).

Based on such classification, it has been possible to study the actual responses of rats or mice to different hemagglutinins. Thus, while the four different hemagglutinin groups could not be distinguished with rabbit blood, only two types - A and C were found to be toxic when injected into mice, (Jaffe' and Brucher 1972). It was realised that only those extracts which agglutinated cow cells were toxic when injected into rats. Feeding tests confirmed the fact that those varieties which displayed agglutinating activity towards trypsinated cow cells were also toxic and inhibited growth when incorporated into the diets of rats, whereas those varieties which were non agglutinating or agglutinated only rabbits cells were non toxic, (Jaffe' and Vegalette 1968; Jaffe' and Brucher 1972). These results serve to emphasise the importance of testing the hemagglutinating activity of the seed extracts against several species of blood cells before one is justified in concluding that a particular bean is toxic or not. The use of trypsinated cow cells would appear to offer an important tool for detecting beans which are potentially toxic.

In addition to the lethal toxic effects of hemagglutinins, pathological lesions have been reported when kidney bean extracts are fed to animals (Szperl-Seyfriedowa 1951). Parenchymatons and fatty degeneration and oedema are found in various organs. The liver shows focal necrosis and fatty changes while haemorrhages occur in the stomach, the intestinal wall and other organs. The kidney and mycardium

show distentions of the capillary vessels with numerous thrombi. According to Kakade et al. (1965 a) the morphological changes in the rat fed navy beans (P. vulgaris) consist of increased weight of kidney and heart, pancreatic acinar atrophy and fatty metamorphosis of the liver. Hintz et al. (1967) reported multiple histological lesions in the brains of the rats fed raw kidney bean.

The mechanism by which hemagglutinins exhibit toxicity is not fully understood. Jaffe' (1960) reported a low food absorption and nitrogen retention in rats fed isolated bean agglutinin. The absorption of glucose from a ligated intestinal loop in anaesthetised rats previously fed a bean agglutinin by stomach tube was much reduced (Jaffe' and Camejo 1961). The hypoglucemia observed by Hintz et al. (1967) in rats fed a bean diet may also be indicative of reduced intestinal absorption of glucose. The experiments of Kakade and Evans (1966) demonstrated reduced absorption of amino acids from raw beans, an effect which may be related to the action of the hemagglutinin although other factors may also be involved. It has been suggested in addition that phytohemagglutinins are adsorbed on erythrocytes or stroma (Jaffe' 1960) and this appear to be pH dependent (Rigas et al. 1966). According to Rigas et al. (1966) there are over 400,000 binding sites for kidney bean agglutinin on the surface of each erythrocyte. Jaffe' (1960) postulated that the action of hemagglutinin was to combine with cells lining the intestinal wall and interfere with normal activity of the cell membranes,

thus producing the toxic effect. This effect of course will be reflected in the extent to which the protein is apparently digested and this in turn depends on the concentration of trypsin inhibitor and hemagglutinin contents of the seed legume (Liener, 1976).

In the plant, haemagglutinins have been suggested to act as plant antibodies and also to function as carbohydrate fixers and to translocate carbohydrate materials in the growing plant (Ensgraber 1958).

2.3.3 Amylase inhibitors

The presence of amylase inhibitors has been shown in many legume seeds (Jaffe' 1973 a). Among the 42 samples of beans examined by Jaffe' (1973 a) only three were free from this inhibitor. The fact that undigested starch may appear in considerable concentration in faeces of rats fed diets prepared with beans rich in amylase inhibitor indicates that it may have some practical importance (Jaffe' and Vegalette 1968). Thus the use of crude legume seeds containing amylase inhibitor for the preparation of animal feed mixes should be viewed with caution. Furthermore, it seems important to study the relation between amylase inhibitor and digestible energy in animal feed. Experiments are also necessary to clarify whether the small residual activity in beans which resists cooking has any nutritional significance (Jaffe' 1973 a).

2.3.4 Flatulence

Dry beans have been known to cause gastrointestinal distress when eaten by humans. Blair et al. (1947) appear to be among the first authors to quantitate the production of flatus in man and to show that soybeans increased gas production and carbondioxide content in the gastrointestinal tract. Although currently it is believed that bacterial fermentation of food residues assimilation of available nutrients is the source of intestinal gases causing gastrointestinal distress, the identity of all the substances fermented is not complete (Olson et al. 1974). Steggerda (1968) suggested that the α-galactosides, raffinose and stachyose, were principle sources of gastrointestinal gases from soyabeans ingested by either man or dog. The involvement of this family of sugars in the flatulence problem has been recently reviewed by Christofaro et al. (1974) and Rackis (1975). These carbohydrates are not digested because mammalian intestinal mucosa lacks α-galactosidase and the α-galactosides themselves are not absorbed into the blood. Bacteria in the lower intestinal tract metabolise them to form methane, hydrogen, and carbondioxide.

Recently, Olson et al. (1974) reported on the flatulence effect of dry beans offered to rats. Their results indicated that the α-galactosides in dry beans contribute to rat hydrogen production and that there are other factors particularly in oligosaccharide-free bean residue that also contribute to flatulence. A stimulating effect, which may be

a synergistic effect, was also demonstrated when the rats were fed stachyose and an α-galactoside-free bean extract.

2.4 Some Methods of Improving the Feeding Value of Grain Legumes.

Although legumes contain numerous examples of the so-called anti-growth factors, they have nevertheless provided man over the centuries with a valuable source of protein. This is attributed in part to the fact that man has learned how to detoxify the so-called anti-nutritional factors by using suitable preparative measures.

Some of the methods that have been found to be effective in improving the feeding values of grain legumes include soaking, heating, supplementation with feed ingredients rich in sulphur containing amino acids or simply by adding synthetic sulphur amino acids especially under experimental conditions. It may be worthwhile to note that some of these methods are combined together during the preparation of grain legumes for human consumption. Thus, most grain legumes are soaked in water before boiling or cooking and eating them with a cereal source. Cereals are generally high in sulphur amino acids but low in lysine while legumes are high in lysine but low in sulphur amino acids.

The feeding value of most grain legumes is improved by heating (Liener and Kakade 1969). The extent to which the deleterious substances are destroyed, hence the feeding value improved, is a function of temperature,

duration of heating, moisture conditions and particle size (Liener 1958).

While most of the studied reported on the effects of heat in improving the feeding value of grain legumes have dealt with soybeans (Borchers et al. 1965; Rackis 1966; Albrecht et al. 1966), there are some few reports on beans and peas. Kakade and Evans (1965 b) found that autoclaving navy beans for 5 minutes at 121^{o}C destroyed 80% of the trypsin inhibitor activity. Jaffe' (1973 b) noted that trypsin inhibitor activity in common beans was readily destroyed by heating at 90^{o}C and this was accompanied by improvement in nutrient digestibility. Similar improvements in the feeding value of heated beans were also reported by Kakade et al. (1969a).

Generally, proper heat treatment will eliminate the activity of the protease inhibitors and the toxic effects of the hemagglutinins although it should be recognised that conditions may sometimes prevail whereby complete destruction of the phytohemagglutinins may not be achieved. For example, Korte (1972) has observed that in mixtures of ground beans and ground cereal prepared under field conditions prevailing in Africa, the hemagglutinin activity was not always destroyed and the cooked products produced diarrnea and otner signs of toxicity. A reduction in the boiling point of water in mountainous regions could also conceivably result in incomplete destruction of the hemagglutinins. Occassionally

outbreaks of massive poisoning after the consumption of partially cooked bean flakes have been reported (Griebel 1950). Perhaps it is also of interest to note that the marked resistance of phytohemagglutinins to inactivation by dry heat (de Muelenaere, 1964) should caution against the indiscriminate use of bean flour in foods which have been prepared by dry heat instead of cooking, such as has been proposed for bread and cakes (Marcas and Boctor, 1959).

2.5 Some of the methods used for evaluation of Protein Quality.

Several methods have been devised for determining the nutritional value of proteins. They allow to have a good idea of the chemical and biological values of proteins. In this study, however, only some few selected ones are reviewed. Since protein is to be used for a biological purpose, a biological assessment is more accurate and prefered, but the chemical method is usually quicker, less labourious and provides more information, (Bender 1961).

2.5.1 Chemical Methods

2.5.1.1 Total Protein Analysis

Total or crude protein content is normally determined by the kjeldahl method as specified by the Association of Official Analytical Chemists (1975). This procedure consists, in brief, of a conversion of all amino acids and amide nitrogen to ammonia by oxidative digestion in the presence

of boiling concentrated sulphuric acid, which is diluted with water after cooling. The ammonia is liberated from the resulting solution of ammonium sulphate by neutralization with a strong alkali, and is quantitatively steam-distilled into a solution of dilute acid of known strength. The amount of acid equivalent to the ammonia is determined by back-titration using dilute alkali of known strength. To arrive at the protein content of a legume, the nitrogen content as determined is multiplied by a conversion factor (6.25) to give the percentage protein. The procedure of using 6.25 as a factor for pulses is of doubtful validity because it can lead to very high or very low values for the protein figure (Tkachuk 1977). The kjeldahl test is widely accepted as the standard method for the estimation of protein in grains. It is comparatively cheap and provides a means of testing large numbers of samples. This is normally the first step in evaluation of protein.

2.5.1.2 Amino Acids Analysis

The nutritive value of a food protein depends in a large measure on the relative proportions of the essential amino acids it contains (Eggum 1969; Burr 1975). All methods of protein quality evaluation are directly or indirectly measuring the relative efficiency of protein sources in satisfying essential amino acid requirements. Strictly speaking, all the amino acids are essential units for the synthesis of the protein molecule. However the

body can manufacture many amino acids if it has an adequate nitrogen source, but it cannot produce certain others in adequate amounts to meet body needs. Those amino acids which can not be synthesized in sufficient amounts by the body and which must be provided in the diet are essential (Robinson 1967). The essential amino acid composition of grain legumes has been determined by several workers (Block and Weiss 1956; Patwardhan 1962; Vankat Rao et al., 1964; Aykrod and Doughty 1964; Evans and Bandemer 1967).

Automated column chromatography is nowadays the method of choice for amino acid determination and has attained a high degree of sophistication and accuracy (Light and Smith 1963; and Jacobs 1970). There are however still some problems of variability when different systems are used (Potter et al. 1968). In spite of these achievements, there is the fundamental shortcoming of amino acid analysis in food stuffs, especially as it relates to the destruction of the sulphur amino acids, during hydrolysis. Some destruction of serine and threonine occurs, and the branched chain amino acids are not always completely liberated. There is also complete loss of Tryptophan (Light and Smith 1963). For tryptophan, no method yet appears to be completely satisfactory (Mauron 1973). Another drawback of the method is that the determination of amino acids in acid hydrolysates of closely related proteins may reveal only small differences in amino acid content. Whereas biological tests with the unhydrolysed proteins may show large differences in apparent or available amino acid contents (Haenel 1973). This

method is however widely accepted because it has the advantage of being strictly analytical and from which it is possible to predict the nutritional quality of a protein.

2.5.1.3 Limiting Amino Acid

The quality of a food protein is dependent largely on the amount of the limiting amino acids present in the protein. Limiting amino acid has been defined as the essential amino acid of a protein which shows low deficit in comparison with a standard (NAS-NRC 1963).

Since all amino acids must be present for protein synthesis to occur, deficiency of any amino acid should have adverse effects upon protein synthesis (Mitchell and Block 1946). The sulphur-containing amino acids, methionine and cystine are generally reported to be the first limiting in the majority of legumes (FAO/CCTA 1959; Patwardhan 1962). The next important deficiency appears to be that of tryptophan. These amino acids seem to limit the nutritive value of most legumes (FAO/UN 1970; Owusu-Domfeh et al. 1970; Burr 1975). The effect of these limitations appears to be more marked on growth than on protein requirements for maintenance (Patwardhan 1962).

2.5.1.4 Chemical Score

The first effort to evaluate proteins directly on the basis of their ability to meet amino acid requirements was the chemical score procedure developed by Block and

Mitchell (1946). They used whole egg protein as their standard since it is utilized with an efficiency close to 100 % in rat essays and is considered to be one of the most effective food sources of proteins. The essence of the procedure was to calculate the percentage deficit of each amino acid in the test protein with respect to the amount in the proteins of the whole egg. The amino acid that was in greatest deficit could then be identified and the amount expressed as percentage of the whole egg gave the value for the chemical score. The lower the chemical score, the poorer the protein as a source of amino acids, the higher the chemical score, the better the protein. Although Block and Mitchell (1946) in some cases found that the chemical score underestimated the biological value, on the whole they found a good correlation between their chemical score and biological value. Mcluaghlan et al. (1959); Bender (1961); Rao et al. (1964) found however that the egg had very high contents of Methionine and cystine. Its use as the reference standard therefore exaggerates any deficiency of these amino acids in proteins under test.

An alternative pattern for scoring protein was proposed by the Joint FAO/WHO (1973). It places less emphasis on the amounts of sulphur containing amino acid than do any of the previously recommended reference patterns. However, according to Mclaughlan and Campbell (1974) and Schelling (1975) since chemical score is calculated from the total amino acids and not available amino acids, it generally

reflects a higher biological value particularly in the heat processed foods than in fact is the case.

The chemical score works fairly well for proteins that differ widely in quality (Mclaughlan and Campbell 1974). Several studies have also demonstrated that protein quality can be predicted with moderate accuracy by chemical score (Miller and Payne 1961; Rao et al. 1964; Payne 1968; Pellett and Srouji 1970).

2.5.1.5 Essential Amino Acid Index

The essential amino acid index (EAAI) developed by Oser (1951) is based on the ratios of the amounts of essential amino acids in a test protein relative to their amounts in standard whole egg protein as shown in the formula below:

$$EAAI = \sqrt[10]{\frac{100a}{100a_e} \times \frac{100b}{100b_e} \cdots\cdots \frac{100j}{100j_e}}$$

Where a, b ..... j are percent of essential amino acids in the food protein and a_e, b_e ..... j_e are the percent of the respective amino acids in whole egg protein. Oser's (1951) method computes values that integrate the amino acid content of proteins expressed as percentages of the corresponding values of a standard protein chosen for its excellence in nutrition. By this method, the amino acid deficiencies that limit the nutritive values of a protein tend to be obscured if the amino acid composition compares well with that of the chosen standard.

The correlation of EAAI with biological value was found to be superior to that found with the original chemical score of Block and Mitchell (1946), however, the computed values of the EAAI tend to overestimate biological values. Essential amino acid index method therefore appears to predict the maximum potential value of a protein (Mauron 1973). Oser's (1951) integrated EAAI as modified by Mitchell (1954) overestimates biological value as much as the chemical score underestimates it.

2.5.2 Biological Methods

2.5.2.1 Protein Efficiency Ratio (PER)

Quite naturally, growth was one of the first parameters to be considered but it is largely due to a refinement of the simple growth method by Osborne and Mendel (1919) that progress has been made in this field. These investigators introduced the concept of the protein efficiency ratio defined as the weight gain by an animal per unit weight of protein consumed. Standardized procedures are set out in AOAC (1975) in which the results are computed in comparison with a standardized casein sample to which is assigned a PER value of 2.5. The standardized procedures prescribe the age of the male rats to be used, a 4 week period in an ad-libitum feeding system using a diet containing 9 or 10 % protein on a dry weight basis and adequate in other essential nutrients.

Although the PER method has achieved wide spread acceptance, it may be criticized from several aspects. It has been pointed out by Hegsted and Worcester (1947) that PER is a function of weight gain. The method makes no allowance for body tissue maintenance but assumes that all protein consumed is used for growth. The other draw back is that, there is not a proportional relationship between one PER value and another, ie, a PER of 2.0 is not twice as good as a PER value of 1.0. Again, maximum PER values are not obtained at the same dietery protein level for different proteins. Thus, the method underestimates the quality of the best proteins. Finally, the use of casein as a standard and the attempt to correct values to a standard PER to account for variability in rats such as environmental conditions does not decrease the interlaboratory variability (Derse 1962).

In spite of these shortcomings, the PER method, until recently, has been considered simple, highly reproducible and generally the most applicable. Furthermore, it has the advantage of requiring a minimal work input (Mclaughlan and Campbell 1969). It is considered to be a good method because it detects effects of processing and deterioration of protein quality, and it also detects improvements brought about by amino acid supplementation (Osborne and Mendel 1919; Mauron 1973). According to Mauron (1973) since most methods appear to rank proteins in the same general order, preference should be given to simpler procedures. According to Campbell and Mclaughlan (1970) the PER given at 8-10 % dietary

protein level is the most appropriate for rat assays since it appears to give an accurate estimate of protein value for man.

2.5.2.2 Apparent Protein Digestibility) (APD)

Measurements of feed intake are made, and all faecal matter collected. This is dried, weighed and analysed for nitrogen. Apparent Protein Digestibility is calculated by the following formula:-

$$\% \text{ APD} = \frac{(\text{N intake} - \text{faecal nitrogen})}{(\text{N intake})} \quad 100$$

The digestibility of proteins are influenced by several factors, but only some that directly affect the pre-digestibility of legumes will be discussed. The low digestibility is caused mainly by the trypsin inhibitors and hemagglutinin compounds (Liener 1977). The result of the destruction of their inhibitory activity by heat is an increase in protein digestibility. The improvement of the legumes APD depends on the heat treatment in terms of time and temperature, that destroys the action of the inhibitors.

There is also evidence to show that poor storage conditions, colour of the seed coat suggesting the precence of phenolic compounds, phytic acid, sugars, saponins, and possibly other compounds, may inhibit protein digestibility and therefore, amino acid availability, reducing the efficiency of protein utilization (Bressani 1973; Elias et al. 1977; and Bressani and Elias 1977).

2.6 The Feeding Value of Grain Legumes for Rats and Pigs

2.6.1 Beans (P. vulgaris)

As early as 1920, dry raw beans were shown to have a marked toxic effect on rats which was only improved with heating (Johns and Finks 1920 a,b). Since then numerous reports have appeared in the literature on these marked improvement in the feeding values of dry beans following some form of heat treatment (Everson and Heckert 1944; Bandemer 1967). It appears that the poor growth rate in rats fed raw beans is not entirely due to trypsin inhibitors since Jaffe' and Vegalette (1968) observed that even those bean varieties with relatively low levels of trypsin inhibitor activities still produced poor growth-promoting properties for rats unless autoclaved. Pancreatic hypertrophy accompanies the poor growth in rats fed raw beans (Kakade et al. 1967).

Rat bioassay studies conducted by Kelly and Bliss (1975) indicated that although all the four different types of beans studied were nutritionally inferior to a diet based on protein supplied by casein, differences in the feeding value existed within the different bean lines studied. According to Patwardhan (1962) and Bressani (1973) such differences appear to be related at least in part to the availability of methionine within the different bean lines. The feeding value of cooked beans for rats assayed by PER and nutrient digestibility is improved by methionine

supplementation. However, such improvement is still inferior to the performance obtained on a casein diet, (Bressani 1973).

2.6.2 Cow peas

2.6.2.1 Rats

Recent data presented by Maner and Pond (1971) indicate that the nutritive value of cow peas can be greatly improved by cooking and by supplementing with methionine. Rats fed cow pea diets as the only source of protein grew very poorly when the cow peas were offered raw. Cooking improved gains by 70 % and efficiency of feed conversion by about 17 %. Methionine supplementation was found to be beneficial to both raw and cooked cow peas.

Owusu-Domfeh et al. (1970) noted that mice fed raw cow peas lost weight while cooking the cow peas resulted in a positive weight gain by mice. According to Onayemi et al. (1976), methionine addition to drum-dried cow peas and to raw soaked cow peas results in an increase in weight gain, protein efficiency ratio, feed efficiency and to liver and pancreas weights. Similar results in the improvement of the feeding value of raw and cooked cow peas supplemented with methionine were earlier reported (Sherwood et al. 1954).

2.6.2.2 Pigs

Pigs fed 16% protein diet, in which raw or cooked cow peas provided the only protein source in the cow pea sucrose diet, performed very poorly on the raw cow pea diet (Maner and Pond 1971). Cooking of the cow peas markedly improved gains, feed consumption and efficiency of feed conversion. Methionine supplementation was without effect when added to either raw or the cooked cow pea diets.

Studies by Heitman and Norwarth (1960) involving the substitution of raw ground cow peas for barley at 20 and 50% levels in the diets of growing pigs indicated that as the level of cow pea was increased in the diet, body weight, feed consumption and feed efficiency were decreased even though no evidence of toxicity was observed.

2.6.3 Pigeon peas

Dako (1966) reported that cooking of pigeon peas improved their nutritional value by destroying the substances inhibiting trypsin activity. Raw peas caused hyperactivity of the pancreas, diarrhea and apathy. Addition of tryptophan or methionine, each alone, to raw or cooked peas had no effect on the protein efficiency ratio, but the combination of the two amino acids improved the protein efficiency ratio by about 100%. Similar improvements in the feeding value of pigeon peas or closely related red gram (C. indicus) have been reported by Pakistani workers (Akbar et al. 1973; Ahmad and Shah, 1975).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Origin of feedstuffs

Rose coco beans (kidney beans), Mwezi Moja beans (kidney beans), Canadian Wonder beans (kidney beans) Mexican 142 beans (navy beans), Cow peas and the pigeon peas were all obtained from the Maize and Produce Board, Nairobi. All the beans, with the exception of Mexican 142, were not pure strain.

3.1.2 Heat treatments

Each legume sample was divided into two portions. One half sample was weighed and to this, five parts of water were added to one part of the legume. After soaking for 18 hours, the water was poured off and the legumes dried in an oven at 65^{o}C for 24 hours. The sample was then ground, spread at the bottom of an enamel pan and autoclaved for 30 minutes in a preheated Barnstead sterilizer at 121^{o}C. It was then dried in an oven at a temperature of 65^{o}C. This was referred to as autoclaved sample. The other half, designated as raw was grounded and left untreated.

3.2 Chemical Analyses

All analyses were done on raw samples except for Phytohemagglutinins and trypsin inhibitors where both the raw and autoclaved samples were analysed. Samples were analysed for proximate composition according to the standard methods of the Association of Official Agricultural Chemists (AOAC, 1975). Gross energy of the feedstuff was determined in an adiabatic oxygen-parr bomb calorimeter according to the Gallen Kamp Method (1961). The minerals were determined using atomic absorption spectroscopy (Atomic Absorption Spectrophotometer, Perkin-Elmer 303) according to Perkin-Elmer (1976). Phosphorus was determined on Beckman-model 24 spectrophotometer according to AOAC (1975).

For amino acid analysis, the procedure of Niece (1975) was followed using a Technicon TSM amino acid analyser.

A partially purified trypsin inhibitor concentrate was prepared and determined from the raw legume samples according to the procedure of Kakade et al. (1969 b). For the autoclaved legume samples the procedure of Kakade et al. (1974) was followed.

Phytohemagglutinins were extracted from legumes flours according to Huprikar and Sohonie (1965) and Bassir and Ikegwuonu (1975) and the procedure of Hankins and Shannon (1978) was used to assay the phytohemagglutinins activity.

The protein content of the phytohemagglutinin was estimated by the method of Lowry et al. (1951) using Bovine Serum albumin as the standard.

Protein quality was determined using essential amino acid index (Oser 1951), chemical score and limiting amino acid (Block and Mitchell (1946). They were calculated from the amino acid data using the FAO/WHO (1973) reference pattern and without tryptophan values.

3.3 Experimental Design:

3.3.1 Rats

This was a completely randomised design using a total of 126 male rats. Six rats were individually allotted to either each of the raw, autoclaved or autoclaved plus methionine supplemented diets. Only the pigeon pea diet was supplemented with tryptophan. The casein diet served as the control.

3.3.2 Pigs

A total of 30 pigs, two and half to three months old and weighing 23 kg were randomly allocated to five groups. The essay groups were assembled so that each pen consisted of 3 barrows (castrates) and 3 females (gilts) which were equalized as nearly as possible with respect to age, weight, and litter. All the experimental animals were dewormed, and after an adaptation period of 3 days, they were introduced to their respective diets when the average live weight of a group was 23 kg.

3.4 Animals, Management and necropsy procedures

3.4.1 Rats

Weanling albino wistar male rats weighing between 60 and 65 gm and about four weeks old were used. Six rats individually housed in wire-bottomed-cages were allotted to each dietary treatment. The environmental temperature was maintained at $22^{o}C$ and feed and water were provided ad libitum during the 28 days test. The weights of feed consumed was recorded daily, and the spillings collected and weighed. After 28 days, all rats were subjected to euthanasia with ether. Livers and pancreas were weighed, and their specimens together with those of the kidneys from four rats per group were fixed in 10% neutral formalin. These were processed in the usual manner, sectioned at 5 micra (μ) and stained with Hematoxylin and Eosin according to Luna (1968).

3.4.2 Pigs

Throughout the experimental period, feed and water were provided ad libitum. Individual weight gains were determined on a weekly basis in order to record their growth rate data. The animals were removed from test when they reached 45 kg and when the fifth animal was removed from the pen, the last animal was removed as well.

3.5 Formulation of diets

3.5.1 Rats

The composition of the test diets for the rats is given in Table 1. The test grain legume was incorporated

in the diets to supply all the 10% protein. The experimental diets were also isocaloric 4000 Kcal/kg dry matter. Methionine supplementation at 0.3% of the diet containing autoclaved legume seeds was done at the expense of corn starch. Tryptophan was supplemented at a level of 0.1% in the Pigeon pea diets.

3.5.2 Pigs

In the feeding studies conducted, comparisons were made between the animals fed raw and autoclaved cow peas with or without methionine supplementation. The cow pea diets were supplemented with maize to supply 16% protein, methionine was included at a constant level of 0.1% to the specified diets. The composition of the test diets for the pigs is given in Table 2. All the diets were blended in a food mixer for 1 hour. The control diet (sow and weaner) was prepared by Unga Limited, Kenya.

3.6 Digestibility determinations

3.6.1 Rats

Digestibility determination was done from fourteenth day of the experiment and was conducted over a period of four days. The faeces from six rats on each diet were collected twice daily. The samples were pooled, dried, weighed and ground for nitrogen analysis. Feed intake was also recorded. Apparent protein digestibility was calculated using the following formula:

: apparent protein digestibility = $\frac{(N \text{ intake} - \text{Faecal } N)\ 100}{N \text{ intake}}$

TABLE 2: COMPOSITION OF THE COW PEA DIETS FOR GROWING PIGS

Ingredients %	Raw Cow pea meal	Raw Cow pea meal plus	Autoclaved Cow pea meal	Autoclaved Cow pea meal plus Methionine	Control *
Cow peas	51.4	51.35	51.4	51.35	
Maize-meal	45.6	45.55	45.6	45.6	
Nutrafos**	2.0	2.0	2.0	2.0	
Dicalcium Phosphate	1.0	1.0	1.0	1.0	
DL-Methionine	-	0.1	-	0.1	

*Unga Limited (Sow and Weaner) Ration.

**Nutrafos "the Commercial mineral-vitamin antibiotic premix was purchased from Hoescht Limited Nairobi.

**Nutrafos contained the following ingredients: Phosphorus (P) 10.5%; Calcium (Ca) 16.2%; Magnesium (Mg) 3.0%; Sodium (Na) 7.2%; Ca:P ratio 1:5:1; Zinc (Zn) 5,000 mg; Copper (Cu) 1,250 mg; Manganese (Mn) 3,400 mg; Iodine (I) 20 mg; Cobalt (Co) 25 mg; Iron (Fe) 3,750 mg; Vitamin A 500,000 I.U.; Vitamin D 100,000 I.U.; Vitamin E 300 mg; Vitamin B_1 67 mg; Vitamin B_2 162 mg; Vitamin B_6 50 mg; Vitamin B_{12} 660 mcg; Nicotinic acid 416 mg; Pantothenic acid 133 mg; Folic acid 20 mg; Choline 2,000 mg; Vitamin C 100 mg; Flavomycin 300 mg.

Protein Efficiency ratio calculated from growth rate of the animals related to the protein consumed was used as the biological evaluation of the legumes.

3.6.2 Pigs.

After 3 weeks of the experimental period, chromic oxide was mixed at 0.5 % level in the feed. This ration was offered for twelve days comprising seven days of adjustment followed by collection of fresh feacal sample twice daily, for five days. The faecal samples were preserved in boric acid-mercuric chloride solution and stored in a deep freezer. The faecal samples were thawed and subsampled on the basis of the pens. The samples were dried, and analysed for nitrogen, and dry matter content. . The method of Kimura and Miller (1957) for chromic oxide determination was employed. Apparent digestibility was calculated using the formula of Crampton and Harris (1969).

3.7 Statistical Analyses

Apparent protein digestibility (%) and body weight changes (gm) values were subjected to a square root transformation for reasons given by Steel and Torie (1960). Tukey's Multiple Comparison test was thought most appropriate in comparing means from treatments with significant F values.

4. RESULTS AND DISCUSSION

4.1 Chemical composition

4.1.1 Beans

Nutrient levels (Tables 3 and 4), while generally similar to those reported elsewhere (Watt and Merrill 1963; FAO 1970), differed in several notable respects.

Moisture levels for the beans were generally similar and typical of those reported in literature (Watt and Merril 1963; de Moraes and Angelucci 1971; Kadwe et al. 1974; and Ritchey et al. 1976). The similarity in the moisture contents may be due to the fact that the samples were stored under the same conditions at the Produce and Marketing Board stores, Nairobi. According to Ritchey et al. (1976) the moisture content of grain legumes is greatly influenced not only by harvesting conditions, but also by the relative humidity in the storage rooms.

For the protein contents, differences ($P < 0.01$) in Rose coco with 19.80 % and Mexican 142 with 22.8 % were observed. The variability in the protein content of the beans observed in this study supports the observations reported elsewhere (FAO/CCTA 1959; Purseglove 1968; Bressani et al. 1973; Kelly 1973). These variations in the levels of protein may be due to differences in species varieties, environment and crop husbandry practices (Bressani et al. 1973).

TABLE 3: CHEMICAL COMPOSITION OF BEANS

Legume Grains

Nutrient	Mwezi Moja beans	Rose Coco beans	Canadian Wonder beans	Mexican 142 beans	S.E.
Crude Protein %	21.00^{ab*}	19.80^{b}	20.20^{ab}	22.20^{a}	± 0.56
Nitrogen %	3.07^{ab}	3.11^{b}	3.04^{ab}	3.58^{a}	± 0.56
Moisture %	12.12^{a}	12.06^{a}	12.05^{a}	11.29^{b}	± 0.03
Fiber %	8.39^{ab}	8.37^{b}	6.66^{c}	9.07^{a}	± 0.13
Carbohydrate %	63.42	63.35	62.40	60.75	-
Nitrogen free extract %	55.03	54.98	55.74	51.68	-
Ether extract %	1.59^{a}	1.87^{b}	1.72^{ab}	1.53^{a}	± 0.05
Ash %	3.34^{a}	3.47^{ab}	3.76^{b}	4.05^{c}	± 0.07
Gross energy (kcal/g)	4.43^{a}	4.33^{b}	4.47^{a}	4.40^{ab}	± 0.10
Calcium (mg/100 g)	184.00^{a}	185.30^{a}	149.00^{b}	217.00^{c}	± 0.19
Phosphorus (mg/100 g)	397.02^{a}	400.00^{a}	375.00^{a}	402.01^{a}	± 0.38
Magnesium (mg/100 g)	326.00^{a}	307.01^{b}	333.00^{c}	370.00^{d}	± 0.01
Iron (mg/100 g)	10.00^{a}	10.50^{ab}	7.80^{c}	11.00^{b}	± 0.20

*Means without similar superscripts within each row are different ($P < 0.01$).

TABLE 4: AMINO ACID COMPOSITION (g/16 gN) OF BEANS

Amino acid	Grain Legume			
	Mwezi Moja	Rose Coco	Canadian Wonder	Mexican 142
Aspartic acid	12.58	12.19	12.47	11.47
Threonine	4.42	4.41	4.48	4.29
Serine	5.54	5.60	5.67	5.50
Glutamic acid	14.41	14.12	14.22	12.53
Proline	4.72	4.70	4.81	4.62
Glycine	3.62	3.58	3.60	3.44
Alanine	4.20	4.10	4.39	4.06
Valine	4.89	4.80	4.98	4.57
Cystine	0.98	0.84	1.02	0.99
Methionine	1.98	1.88	2.04	1.90
Isoleucine	4.08	4.01	4.17	3.90
Leucine	7.77	7.73	7.91	7.51
Tyrosine	3.50	3.91	3.71	3.47
Phenylalanine	5.56	6.23	5.87	5.67
Lysine	7.06	6.70	7.01	6.48
Histidine	3.18	2.78	3.11	2.94
Arginine	5.31	5.09	6.34	6.57

The beans studied were typically low in fat content ranging from 1.53 % in Mexican 142 to 1.87% in Rose Coco. Rose Coco was higher ($P< 0.01$) in fat content than Mwezi Moja and Mexican 142. Purseglove (1968) and Ritchey et al. (1976) reported values ranging from 1.0 to 1.5 % in the different beans they studied which are similar to the values reported in this study.

The fiber values obtained in the bean varieties are higher than those reported in literature. The fiber values in this study, ranged from 6.7% in Canadian wonder to 9.07% in Mexican 142. Watt and Merrill (1963) and Aykroyd and Doughty (1964), however, reported values ranging from 4.2 to 4.3 % for Phaseolus vulgaris.

The carbohydrate values of the beans ranged from 60.75 % in Mexican 142 to 63.42% in Mwezi Moja. These results are generally similar to those of Watt and Merrill (1963), Aykroyd and Doughty (1964) and Purseglove (1968).

The ash values which ranged from 3.44 % in Mwezi Moja to 4.14 % in Mexican 142 concur with the reports of Aykroyd and Doughty (1964) and Watt and Merrill (1963), however, Mexican 142 differed ($P< 0.01$) from the other beans studied.

The energy levels were very similar in the beans studied except for the Rose coco (4.33 kcal/g) which was significantly ($P< 0.01$) different from Canadian wonder (4.47 kcal/g) and Mwezi Moja (4.44 kcal/g). The calorific

contents of these beans appeared to be much higher than those reported in literature (FAO/CCTA 1959; Watt and Merrill 1963).

There were variations in the mineral content of the legumes. Similar variations have also been reported in legumes studied by Aykroyd and Doughty (1964) and appear to be due to differences in species, and to such factors as variety, climate, cultural methods and mineral content of the soil. It was of significance to note that Mexican 142 contained the highest amounts of calcium, phosphorus, magnesium and iron. Calcium levels in the beans were different ($P< 0.01$) except in Rose coco and Mweri Moja which did not differ. The calcium levels ranged from 149.0 mg/100 g in Canadian wonder to 217.0 mg/100 g in Mexican 142. These values are lower than those of Beeson (1941). Aykroyd and Doughty (1964) and Orraca Tetteh (1976) have however, shown variation in calcium content from 50 mg/100 g to 300 mg/100 g.

There were no significant differences among the phosphorus levels in beans, but the values were lower than those reported by Beeson, (1941). The magnesium contents of beans ranged from 307.01 mg/100 g in Rose coco to 370 mg/100 g in Mexican 142. These values concur with those of Beeson (1941).

Iron levels ranged from 7.80 mg/100 g in Canadian wonder to 11 mg/100 g in Mexican 142. Iron contents in legumes has been reported to vary between 2-10 mg/100 g

(Singh and Banerjee 1955; Aykroyd and Doughty 1964). However, according to the studies carried out by McCance and Weddowson (1960) Aykroyd and Doughty (1964) and Doughty et al. (1966), if all the iron in the beans would be available, the beans would make useful contributions to dietary iron needs.

The amino acids pattern both essential and non essential of the beans is presented in table 4. It is worth noting that the cystine and methionine values of all the beans were very low. Apart from the valine figures, these results compared favourably with those of Aykroyd et al. (1951) and FAO (1970). Unfortunately, it is not possible to compare the amino acid values in this study with many others because of the differences in the units used to express the amino acids. However, the lack of agreement in reported amino acid composition values for a variety arises because of different origins of materials, varying conditions of hydrolysis and varying methods of analysis (Boulter et al. 1973).

The essential amino acid pattern of the beans is presented in table 5 together with FAO/WHO (1973) reference pattern of essential amino acid requirements for comparison. All the beans were higher in lysine, threonine, leucine, and phenylalanine and tryrosine compared to FAO/WHO (1973) pattern. If all these amino acids were available, the beans should be excellent sources of these amino acids. According to this pattern, the beans were

TABLE 5: COMPARISON OF ESSENTIAL AMINO ACIDS IN THE BEANS WITH FAO/WHO REQUIREMENT PATTERN

Amino Acid	World Health Organization (1973) Requirement Pattern (mg/g Protein)	Mwezi Moja Amino Acid Content (mg/g Protein	Rose Coco Amino Acid Content (mg/g Protein)	Canadian Wonder Amino Acid Content (mg/g Protein)	Mexican 142 Amino Acid Content (mg/g Protein)
Lysine	55.0	70.8	66.8	69.9	64.8
Threonine	40.0	44.3	44.2	44.7	42.9
Methionine plus Cystine	35.0	29.7	27.2	30.5	28.6
Leucine	70.0	77.6	77.1	78.9	75.1
Isoleucine	40.0	40.6	40.1	41.5	38.9
Valine	50.0	48.9	47.8	49.9	45.6
Phenylalanine plus Tyrosine	60.0	90.6	101.2	95.7	91.5

borderline with regard toisoleucine and they were slightly deficient in valine. These results are in agreement with those of Evans and Bandemer (1967).

The essential amino acid index, chemical scores, and resultant observations respecting, limiting amino acids are summarized in table 6. The chemical socre of the beans studied here ranged from 77.8 in Rose coco to 87.1 in Canadian wonder. The chemical scores of these beans were much higher than those reported by FAO (1970). It might be noted, however, that direct comparison of chemical scores of the beans in this experiment is not possible because most values in literature were compared to the ideal egg of Block and Mitchell (1946).

In table 6, the first two limiting amino acids are presented. These values do not take into account tryptophan since it was not analysed for. It can be seen that in all the beans, the sulphur containing amino acids (methionine and cystine) were first limiting, followed by valine. These results concur with those of FAO (1970); Owusu-Demfeh et al. (1970) and Burr (1975) who have indicated sulphur amino acids to be first limiting in most legumes.

Table 6 also outlines the essential amino acids index (EAAI) of the different beans studied, based on FAO/WHO (1973) provisional pattern. The values for EAAI ranged from 88.7 in Canadian wonder to 94.4 in Mexican 142.

TABLE 6: ESSENTIAL AMINO ACID INDEX (EAAI), CHEMICAL SCORE (CS) AND LIMITING AMINO ACIDS OF BEANS

Protein quality Evaluation		Protein Source			
	FAO/WHO 1973 Amino Acid Reference Pattern	Mexican 142 beans	Rose Coco beans	Mwezi Moja beans	Canadian Wonder beans
E.A.A.I.	100	94.4	91.5	91.1	88.7
C.S.	100	81.7	77.8	84.8	87.1
1st Limiting Amino Acid	-	Methionine plus Cystine	Methionine plus Cystine	Methionine plus Cystine	Methionine plus Cystine
2nd Limiting Amino Acid		Valine	Valine	Valine	Valine

4.1.2 Cow Peas

Table 7 outlines the proximate composition, gross energy and mineral content of cow peas. The results in this study are in general similar to those reported by Johnson and Raymond (1964), Purseglove (1968); and Owusu-Demfeh et al. (1970). However, there was notable difference in the level of fiber content which was twice that reported by Watt and Merrill (1963); Aykroyd and Doughty (1964) and FAO (1970).

The fat value of 1.6 % reported by Owusu-Domfeh et al. (1970) using cow peas produced in Ghana is similar to that reported on cow peas in this study. Very low fat values (0.69 %) have, however, been reported (Hermano 1930 and Ranganathan et al. 1937 a,b). The value of 23.4 % protein reported on cow peas in this study concurs with that reported by Mtenga and Sugiyama (1974) using cow peas grown in Tanzania.

The carbohydrate value is very similar to that reported by Ranganathan et al. (1937 a,b) and Purseglove (1968).

The energy value appeared to be much higher than those reported by FAO/CCTA (1959) and Watt and Merrill (1963) but the value reported here is similar to that of Owusu-Domfeh et al. (1970).

Magnesium and Calcium levels in cow peas concur with those of Beeson (1941) and Aykroyd and Doughty (1964).

TABLE 7: CHEMICAL COMPOSITION OF COW PEA

Nutrient	Legume Grain Cow Peas
Crude protein %	23.4
Nitrogen %	3.36
Moisture %	13.07
Fiber %	8.19
Carbohydrate %	57.13
Nitrogen free extract %	48.94
Ether extract %	1.55
Ash %	3.29
Gross energy (kcal/g)	4.49
Calcium (mg/100 g)	134.00
Phosphorus (mg/100 g)	337.02
Magnesium (mg/100 g)	406.12
Iron (mg/100 g)	11.00

The iron value is similar to those of Aykroyd and Doughty (1964) and Doughty et al. (1966). The phosphorus content is much lower than those reported in literature (Beeson 1941; Owusu-Domfeh et al. 1970).

The amino acid composition of cow peas is presented in Table 8. With the exception of high methionine and low glutamic acid contents, the amino acid contents of cow peas reported in this study were similar to those reported elsewhere (Owusu Domfeh et al. 1970; Boulter et al. 1973; Mtenga and Sugiyama 1974; and Evans and Boulter 1974).

The essential amino acid levels in cow peas are compared with FAO/WHO (1973) provisional pattern in Table 9. Cow peas were higher only in lysine, leucine and phenylalanine plus tyrosine compared to FAO/WHO (1973) pattern. The cow peas were border line with regard to threonine and isoleucine but deficient in methionine and cystine and valine. These results concur with those of Owusu-Domfeh et al. (1970).

The results of chemical score and limiting amino acids of cow peas are shown in Table 10. The chemical score of 77.5 was similar to that of Godfrey-Sam-Aggrey et al. (1976) but was higher than the chemical score value of Owusu-Domfeh et al. (1970) and Boulter et al. (1973). This may be due to the fact that they used the ideal egg of Mitchell and Block (1946); FAO (1970) for comparison.

TABLE 8: AMINO ACID COMPOSITION (g/16 gN) OF COW PEA

(Vigna Unguiculata)

Amino acid	Grain legume Cow pea
Aspartic acid	11.33
Threonine	3.92
Serine	4.85
Glutamic acid	14.71
Proline	4.84
Glycine	3.92
Alanine	4.18
Valine	4.38
Cystine	0.74
Methionine	1.96
Isoleucine	3.74
Leucine	7.17
Tyrosine	3.43
Phenylalanine	5.45
Lysine	7.35
Histidine	3.85
Arginine	6.15

TABLE 9: COMPARISON OF ESSENTIAL AMINO ACIDS IN THE COW PEA WITH FAO/WHO (1973) REQUIREMENT PATTERN

Amino acid	World Health Organization (1973) Requirement Pattern (mg/g Protein	Cow Pea Amino Acid content (mg/g Protein)
Lysine	55.0	73.3
Threonine	40.0	39.0
Methionine plus Cystine	35.0	27.1
Leucine	70.0	71.8
Isoleucine	40.0	37.6
Valine	50.0	43.8
Phenylalanine plus Tyrosine	60.0	89.0

TABLE 10: ESSENTIAL AMINO ACID INDEX (EAAI), CHEMICAL SCORE (CS) AND LIMITING AMINO ACIDS OF COW PEAS

Protein quality evaluation	FAO/WHO 1973 Amino Acid Reference Pattern	Cow peas
Essential Amino Acid Index	100	96.80
Chemical Score	100	77.5
1st Limiting Amino Acid	-	Methionine plus Cystine
2nd Limiting Amino Acid	-	Valine

The limiting amino acids of cow peas are presented in Table 10 without the values of tryptophan. It is shown that the derived limiting amino acids were methionine plus cystine and valine. These results are in agreement with those of other workers; (Jaffe' 1949; Borchers and Ackerson 1950; Orr and Watt 1957; Bressani et al. 1961; Elias et al. 1964; FAO 1970; Owusu-Domfeh 1970; Ntenga and Suguyama 1974; Godfrey-Sam-Aggrey 1976).

In Table 10, the essential amino acid index (EAAI) is also shown. Concepcion and Cruz (1961) and Owusu-Domfeh et al. (1970) reported the EAAI values of 90 and 72.9 respectively for cow peas. These values did not agree with the values obtained for cow peas in the present experiment. Again, direct comparisons may not be possible because the provisional amino acid scoring pattern they used was different from the one used in this experiment.

4.1.3 Pigeon peas

Table 11 outlines the proximate composition, gross energy and mineral content of pigeon peas (C. cajan). The results are in general similar to those reported by Watt and Merrill (1963) Jonhson and Raymond (1964) and Purseglove (1968).

The protein value of pigeon peas in this study concurs with that reported by Aykroyd et al. (1951), FAO (1970) and Swaminathan and Jain (1973).

TABLE 11: CHEMICAL COMPOSITION OF PIGEON PEAS

Nutrient	Legume grain Pigeon peas
Crude protein %	22.8
Nitrogen %	3.01
Moisture %	11.25
Fiber %	8.70
Carbohydrate %	62.38
Nitrogen free extract %	53.68
Ether extract %	2.12
Ash %	4.08
Gross energy (Kcal/g)	4.41
Calcium (mg/100 g)	111.00
Phosphorus (mg/100 g)	278.003
Magnesium (mg/100 g)	252.00
Iron (mg/100 g)	20.00

The moisture content of 11.3 % of pigeon peas studied here is similar to that reported by Ayrkroyd et al. (1951); Johnson and Raymond (1964) and FAO (1970).

Ramiah and Satyanarayana (1938) studied five different varieties of Indian pigeon peas reported values ranging from 1.6-2.5 percent fat which are generally similar to the one reported in this experiment.

The fiber content although much higher than those of Aykroyd et al. (1951), concurs with those reported by FAO/CCTA (1959), Watt and Merrill (1963) and Purseglove (1968).

Carbohydrates, ash and energy values of pigeon peas in this study are much higher than those reported elsewhere (Aykroyd et al. 1951, FAO/CCTA 1959, Watt and Merrill 1963; Aykroyd and Doughty 1964; Purseglove 1968).

With the exception of the lower values of iron and magnesium, the mineral values are in agreement with those reported by Aykroyd et al. (1951). Magnesium content of pigeon peas is similar to that reported by Beeson (1941).

The amino acid composition of pigeon peas is presented in Table 12. The amino acid content of pigeon peas reported in this study concurs with those reported elsewhere (Aykroyd et al. 1951; Van Etten et al. 1967; Hanumantha and Subramanian 1970).

TABLE 12: AMINO ACID COMPOSITION (g/16 gN) OF PIGEON PEAS

Amino Acid	Pigeon peas
Aspartic acid	10.86
Threonine	3.70
Serine	4.95
Glutamic acid	17.59
Proline	7.10
Glycine	3.67
Alanine	4.42
Valine	4.30
Cystine	1.20
Methionine	1.84
Isoleucine	3.52
Leucine	7.57
Tyrosine	3.16
Phenylalanine	8.51
Lysine	6.98
Histidine	4.09
Arginine	6.61

The essential amino acid levels of pigeon peas are presented in Table 13. The contents of lysine, leucine, and phenylalanine plus Tyrosine in pigeon peas reported here are higher than the values of the FAO/WHO (1973) requirement pattern. All other amino acids were below the minimum level of nutritional requirement. The same observations were made by Bannerjee (1960); Van Etten et al. (1967); and Hanumantha and Subramanian (1970).

The calculated amino acid score, as well as the first and second limiting amino acids for pigeon peas are presented in Table 14. Orr and Watt (1957); Swaminathan (1967); Hanumantha and Subramanian (1970); Kaul (1973) and PAG (1973) reported tryptophan to be first limiting followed by methionine plus cystine. According to the FAO (1970) and Mtega and Sugiyama (1974) methionine plus cystine are more limiting than tryptophan in pigeon peas. Tryptophan was not analysed for in this study. The results obtained in this study indicate, however, that valine and isoleucine can also be considered as first and second limiting amino acids respectively. Such differences may be due to differences between varieties, (Krober 1956; Laximan et al. 1973; Hulse 1977).

In Table 14, the essential amino acid index (EAAI) is presented. Unfortunately, the EAAI is compared to FAO/WHO (1973) amino acid reference pattern and hence cannot be compared with the essential amino acid index presented

TABLE 13: COMPARISON OF ESSENTIAL AMINO ACIDS IN PIGEON PEAS WITH FAO/WHO (1973) REQUIREMENT PATTERN

Amino acid	World Health Organisation (1973) Requirement Pattern (mg/g Protein)	Pigeon pea Amino Acid Content (mg/g Protein)
Lysine	55.0	70.1
Threonine	40.0	37.2
Methionine plus Cystine	35.0	30.8
Leucine	70.0	75.9
Isoleucine	40.0	35.0
Valine	50.0	43.0
Phenylalanine plus Tyrosine	60.0	116.2

TABLE 14: ESSENTIAL AMINO ACID INDEX (EAAI) CHEMICAL SCORE (CS) AND LIMITING AMINO ACIDS OF PIGEON PEAS

Protein quality Evaluation	FAO/WHO 1973 Amino Acid Reference Pattern	Pigeon pea
E.A.A.I.	100	93.1
C.S.	100	86.0
1st Limiting Amino Acid	-	Valine
2nd Limiting Amino Acid	-	Isoleucine

in other tables. However, the EAAI for pigeon peas in this study is very near to the FAO/WHO (1973) essential amino acid index of 100.

4.2 Effect of Autoclaving on Trypsin Inhibitor Activity (TIA) on Beans, Cow peas and Pigeon peas

4.2.1 Beans

A summary of the trypsin inhibitor activity (TIA) of the raw beans is presented in table 15. All the beans studied contained trypsin inhibitor. Rose coco had the highest trypsin inhibitor activity of 45.1 TUI/ml and Mwezi Moja had the lowest activity, 24.3 TUI/ml. Bowman (1944); Kakade et al. (1965 b); Pusztai (1967); and Bressani and Elias (1977) have also reported a wide spread distribution of trypsin inhibitor in almost all legumes.

Autoclaving reduced the trypsin inhibitor activities of the beans (Table 15). Bowman (1944) when working with navy beans, Kakade et al. (1972) and Liener (1973), 1975) with Soy bean flour showed the presence of a partially heat-labile trypsin inhibitor in those legumes they studied. The incomplete destruction of TIA may be explained by the recent findings of Elias et al. (1977) suggesting that two types of trypsin inhibitor exist, one which is heat labile and the other being heat resistant. They also suggest that this heat resistance is probably due to the presence of tannins and polyphenols.

Mwezi Moja had the least amounts of trypsin inhibitor activity of 7.0 TUI/ml and Rose coco had the highest levels of residual TIA of 14.8 TUI/ml. However, percentage

TABLE 15: ANTITRYPTIC ACTIVITIES OF CRUDE EXTRACTS OF RAW AND AUTOCLAVED BEANS, COW PEAS AND PIGEON PEAS

Legume	Trypsin inhibitor activity		
	Raw extract TUI/ml	Autoclaved extract TUI/ml	Destruction %
Rose coco	45.1	14.8	67
Mexican 142	34.6	8.3	76
Canadian Wonder	24.8	11.4	54
Mwezi Moja	24.3	7.0	71
Cow peas	21.7	9.7	55
Pigeon peas	12.8	11.0	14

reduction in TIA which occurred in Mexican 142, Mwezi Moja Rose coco, and Canadian wonder were 76, 71, 67 and 54 respectively.

The extent to which the TIA in legumes is destroyed by heat is a function of the heating temperature, duration of heating, particle size and moisture conditions (Liener 1973). Patwardhan (1962) and Hanovar et al. (1962) found that autoclaving in an atmosphere of steam at 121°C for 15-30 minutes almost completely inactivated the TIA in most legumes. However, Kakade and Evans (1965 a) investigated the effect of heating at 121°C for different time lengths on TIA in raw navy beans (P. vulgaris) and noted that autoclaving raw beans for 5 minutes destroyed 80 % of the TIA. Autoclaving for periods longer than 30 minutes was required for complete destruction of TIA. On the other hand, the absence of inhibitory activity may not always be a quarantee for a product having optimal nutritional quality (Lang 1960; Elias and Bressani 1977).

4.2.2 Cow peas

Table 15 also gives a summary of the trypsin inhibitor activities (TIA) of cow peas. Raw cow peas contained measureable amounts of TIA.

Autoclaving cow peas at 121°C for 30 minutes destroyed only 55 % of the original trypsin inhibitor activity. These results concur with those of Owusu-Domfeh (1972).

4.2.3 Pigeon peas

A summary of the trypsin inhibitor activity (TIA) of both the raw and autoclaved pigeon peas can also be seen in Table 15. The presence of trypsin inhibitor activity in pigeon pea reported here concurs with the report of Sohonie and Bhandarkar (1951), Tawde (1961), and Liener (1973).

It is worth noting that the trypsin inhibitor activity found in pigeon peas was almost resistant to heat denaturization. A similar observation was made by Tawde (1961). Although little is known about the polyphenol content of pigeon peas (Hulse 1977), it is possible that the heat resistance of the Pigeon peas trypsin inhibitor is due to the presence of polyphenols as suggested by Elias et al. (1977) and Hulse (1977).

4.3 Effect of Autoclaving on Phytohemagglutinating Activity (PHA) of Beans, Cow peas and Pigeon peas

4.3.1 Beans

The results of the PHA obtained from beans are summarised in table 16. The crude Saline Extracts of all the raw bean seeds were found to contain PHA when assayed with trypsin treated erythrocytes. This confirms the observations of Honavar *et al.* (1962); Jaffe' (1973 a,b); Jayne-Williams and Burgess (1974); Pusztai and Palmer (1977) and Pusztai and Stewart (1978). The different values reported may be due to the differences in the species used, or the blood types used to detect the PHA. Bruchers *et al.* (1969), Jaffe' and Bruchers (1972) and Jaffe' *et al.* (1972) using different varieties of (*P. vulgaris*) found that the hemagglutinins in the seeds exhibited different degrees of specificity depending on the species of the animal from which the red blood cells were obtained and whether or not the cells had been pretreated with proteolytic enzyme such as trypsin. Red cells treated with trypsin contribute additional sensitivity over the use of ordinary erythrocytes (Kabat and Mayor 1961). Figure 2 shows that where agglutination is maximal, a thin film covers the entire bottom of the well (E) as if the clumps adhered to the bottom of the well at the point of settling. In the wells where no agglutination occurs (C) the cells settle as a central, sharply demarcated round disc.

TABLE 16: HEMAGGLUTINATING ACTIVITY OF CRUDE EXTRACTS OF RAW AND AUTOCLAVED BEANS

Legumes	Extracts Purification step	Hemagglutinating activity/ml
Rose Coco	Raw crude saline extract	218.5
	Amonium sulphate precipitate $(NH_4)_2SO_4$	17066.7
	Autoclaved crude saline extract	0.0
Mwezi Moja	Raw crude saline extract	862.3
	$(NH_4)_2SO_4$	952.6
	Autoclaved crude saline extract	0.0
Canadian Wonder	Raw crude saline extract	84.5
	$(NH_4)_2SO_4$	114.0
	Autoclaved crude saline extract	0.0
Mexican 142	Raw crude saline extract	13.50
	$(NH_4)_2SO_4$	213.30
	Autoclaved crude saline extract	0.0

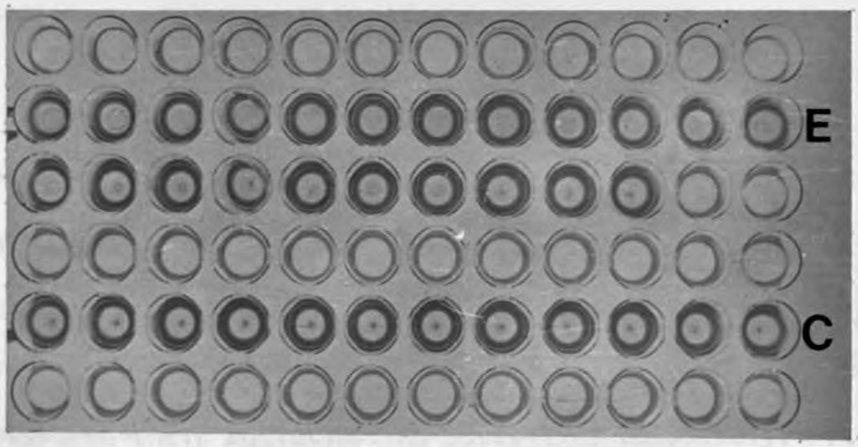

Fig. 2: The control cells (C) show no agglutination.

Note the agglutination of the red blood cells in all the wells (E).

The legume proteins obtained by precipitation with 50 % saturated ammonium sulphate solution is also presented in Table 16. There was a lot of variability in the PHAs of the beans, with the lowest activity obtained in Canadian Wonder and the highest activity in Rose coco. It is worth noting that in all cases, the precipitated proteins from saline extract of beans by saturation with ammonium sulphate had higher PHAs than the corresponding values in the crude extracts.

PHAs were completely destroyed by the autoclaving process (Table 16). These results concur with those of Jaffe' (1949) Honavar et al. (1962); Liener (1962); de Muelenaere (1964); Kakade et al. (1972) and Gallardo et al. (1974), who reported that autoclaving presoaked bean seeds destroyed their agglutinating activities completely. It appears therefore, that PHA was possibly leached out of the beans as a result of the soaking process and/or were destroyed by autoclaving.

4.3.2 Cow Peas

The results of the phytohemagglutinating activities (PHA) of the cow peas are presented in Table 17. Both the crude saline extracts and the ammonium sulphate precipitation of cow peas were weakly active against trypsinated cow erythrocytes. These results concur with those of Ikegwuonu and Bassir (1976) who extracted PHA from cow peas grown in Nigeria.

TABLE 17: HEMAGGLUTINATING ACTIVITY OF CRUDE EXTRACT OF RAW AND AUTOCLAVED COW PEAS AND PIGEON PEAS

Legume	Extracts	Purification step	Hemagglutinating activity/ml
Cow peas	Raw	Crude Saline Extract	1.3
		$(NH_4)_2SO_4$	2.5
	Autoclaved	Crude Saline Extract	0.0
Pigeon peas	Raw	Crude Saline Extract	7.8
		$(NH_4)_2SO_4$	9.8
	Autoclaved		0.0

Since PHA appears to be comparatively thermolabile, it is doubtful if they are of any great importance in human diets. Certainly, they appear to be of little consequence in cooked cow peas.

4.3.3 Pigeon Peas

The results of pigeon pea agglutinating activity against trypsinated erythrocytes are also shown in Table 17. The crude saline extracts of pigeon peas and the activity of the ammonium sulphate precipitation against trypsinated blood cells seems to be quite low. Although Ikegwuonu and Bassir (1976) found measureable amounts of PHA in pigeon peas, Honavar et al. (1962); Liener (1973); and Liener (1975) did not observe any PHA in pigeon peas. This could be due to the differences in the species of the animal from which the red blood cells were obtained and whether or not the cells were pretreated with proteolytic enzyme.

When the pigeon peas were autoclaved, their agglutinating activities were destroyed. It has been reported that the nutritionally deleterious factors like (PHA) in most legumes can be completely eliminated by soaking and heat treatment (Evans and Butts 1949; Liener 1962; Honavar et al. 1962; Kakade and Evans 1965 b; Pusztai 1967; Kakade et al. 1970; Jaffe' and Bruchers 1972; and Gallardo et al. 1974).

4.4 Feeding experiments

4.4.1 Beans

4.4.1.1 Performance of the rats fed the bean diets

Raw beans when offered as the sole source of protein caused rapid loss of body weight and death within 4-10 days. Before death, rats offered raw bean diets exhibited signs of weakness, shivering, prostration, dyspnoea, anorexia, marked abdominal distension, growth inhibition, debilitation with eyes closed all the time, emaciation, had rough scarce hair coats, very fiece and biting when touched and were always wet around the genitalia due to excessive floorof urine. In addition to causing diarrhea, the raw bean diets caused the rat faeces to adhere tenaciously to the cage floor and to the anal area and tail of the rat.

The rats fed diets containing autoclaved beans survived the 28 day test period, although there were reductions ($P< 0.01$) in feed intake and body weight when compared to those fed the casein control diet (Table 18).

There was a significant increase in body weight gain of rats fed on supplemented diet over those which were not supplemented although they were still inferior to those fed the casein diet (Table 18).

Casein diet stimulated significantly greater gain responses although there was no difference between the average daily feed intakes of Mexican 142 and casein.

TABLE 18: EFFECT OF AUTOCLAVED AND SUPPLEMENTATION OF BEANS ON THE GROWTH OF RATS

Protein source	Average feed intake (gm)	Average weight change (gm)	Protein efficiency ratio	Feed efficiency ratio
Casein	13.2[a]+	4.7[a]	2.5[a]	2.9[a]
Raw Mexican 142*	-	-	-	-
Auto Mexican 142	6.3[b]	- 0.23[b]	-	-
Auto Mexican 142 plus Methionine	11.3[ac]	2.5[c]	1.6[b]	4.6[b]
Raw Mwezi Moja*	-	-	-	-
Auto Mwezi Moja	7.0[bd]	- 0.19[b]	-	-
Auto Mwezi Moja plus Methionine	10.1[ce]	2.0[cd]	1.4[b]	5.3[bc]
Raw Canadian Wonder*	-	-	-	-
Auto Canadian Wonder	6.2[b]	- 0.6[b]	-	-
Auto Canadian Wonder plus Methionine	8.5[de]	1.4[d]	1.3[b]	7.8[d]
Raw Rose coco*	-	-	-	-
Auto Rose coco	6.0[b]	- 0.5[b]	-	-
Auto Rose coco plus Methionine	8.0[bd]	1.3[d]	1.2[b]	6.3[cd]
SEM	± 0.5	± 0.53	±0.11	± 0.44

*Animals died within 4-10 days.

+Means without similar supercripts within each column are different ($P < 0.01$).

casein diet stimulated significantly greater gain responses. The feed intake on Rose coco was not significantly increased by methionine supplementation. The small insignificant response to methionine addition in Rose coco may be explained on the basis that methionine was not the only limiting amino acid, but also it has the highest amounts of residual trypsin inhibitor activity compared to the other three bean types studied.

The PER values, obtained with 10 % protein of Rose coco, Canadian Wonder, Mwezi Moja and Mexican 142 supplemented with 0.3% of methionine supported 48 %, 52 %, 56 % and 64 % respectively, as good growth of rats as did casein. The PER for the beans were low and not significantly different for each other. This observation may have some practical implications in developing countries, like Kenya where there is a shortage of animal protein food.

The results obtained with the feeding of raw beans for rats concur with those, of Jaffe' (1960); Honavar et al. (1962); Kakade and Evans (1965 b); Jaffe' (1973 a) and Ikegwuonu and Basir (1977). The mode of action of the toxic factor(s) in beans has already been described by (Jaffe 1960; Kakade and Evans 1966; Jaffe' and Vegalette 1968; Pusztai et al. 1975 and Liener 1977).

Rats fed autoclaved beans did not die but only lost weight and had reduced feed intake. This observation is in agreement with those of Riley (1961) Honaver et al. (1962) Salman and McGinnis (1968); Cappella (1974); Goatcher and McGinnis (1972) and McGinnis and Cappella (1976).

The beneficial effect of autoclaving the raw beans in decreasing the mortality or inhibiting the death of the animals could be due to the destruction of the trypsin inhibitors and the toxic phytohemagglutinins found in the raw beans (Everson and Heckert 1944; Liener 1958; Kakade and Evans 1963; 1965b; and Liener 1977). Comparison of the amino acid composition of raw beans with that of FAO/WHO (1973) reference pattern showed bean proteins to be deficient primarily in methionine and cystine. The poor growth of rats fed on beans could also have been due in part to the critical limiting sulphur containing amino acids. The ingestion of such an imbalanced diet must be responsible for the low appetite of the rats. Rose (1938) Sanahuja and Harper (1962) and Swisher and Kendal (1968) observed that the omission of a particular amino acid led to a marked reduction in feed intake and concurently to a severe loss of weight.

Heating the beans at 121^{o}C for 30 minutes may have served to accentuate more deficiency of other amino acids in the beans. Kakade and Evans (1965 b) found that rats fed navy beans which had been autoclaved for five minutes gained weight. Rats fed beans autoclaved

for longer than five minutes showed some adverse effects of heat treatment on the nutritive value of the beans. It is likely that the retardation in growth in their study and in this study may have been caused by the destruction or inactivation of essential amino acids (Evans and Butts 1949).

The marked improvement in rat performance following autoclaving and methionine supplementation of the bean diets concurs with the resports of (Jaffe' 1949; Kakade and Evans 1965 b; Bressani and Elias 1968; PAG 1973; Jansen 1973; and Kon et al. 1974). This suggests that poor performance of rats fed autoclaved bean diets was due to at least in part to the inherent methionine deficiency in the beans (Patwardhan 1962; Aykroyd and Doughty 1964; Jaffe' 1973 a; Kelly 1973; Siegel and Fawcett 1976; and Bressani and Elias 1977).

4.4.1.2 Apparent protein digestibility of beans

Apparent protein digestibility of various beans is shown in Table 19. The casein had superior ($P < 0.01$) protein digestibility. There were however, differences between the beans. Canadian Wonder and Rose coco were poorly digested with values as low as 56.2 and 58.0 % respectively compared to the protein digestibility of Mexican 142 and Mwezi Moja of 71.0 and 82.0 % respectively. Although all these values were obtained from feeding autoclaved samples, the low apparent protein digestibility

TABLE 19: APPARENT PROTEIN DIGESTIBILITY OF BEANS FED TO RATS

Protein source	Apparent Protein Digestibility %	
	Autoclaved	Autoclaved plus Methionine
Casein	94.00*	94.00
Mwezi Moja Beans	82.00	83.00
Mexican 142 Beans	71.00	74.30
Rose Coco Beans	58.00	60.00
Canadian Wonder Beans	56.2	69.00
SEM	0.08	0.08

*Casein was not autoclaved.

of Canadian Wonder and Rose coco might have been due to at least in part to high residual levels of resistant trypsin inhibitors, which are known to decrease protein digestibility (Seidl et al. 1969 and Elias et al. 1977). These results concur with those of Bressani (1973) who found that significant losses of nitrogen occurred in the faeces of children fed beans. Other investigators Jaffe' (1950); De Muelenaere (1964); Kakade and Evans (1966); Jaffe' (1973 a); Jaffe' (1975) and Elias and Bressani (1977), have also shown that raw beans (P. vulgaris) are poorly digested with values, ranging from as low as 15.6-56% when fed raw, and 68.1-91% when cooked. On the whole there was a noticeable improvement due to methionine supplementation in all the beans.

4.4.1.3 Effect of feeding bean diets on weight and appearances of different organs of rats

The effects of the various bean diets on the weights of pancreas and livers of the rats are given in Table 20. The data indicate that the weight of pancreas of rats fed raw bean diets were not significantly increased ($P > 0.01$) as compared to the weight of pancreas in rats fed the casein diet. This could be due to the presence of more than one trypsin inhibitor with different biological activity (Liener 1969; Seidl et al 1969).

TABLE 20: EFFECT OF FEEDING BEANS ON PANCREAS AND LIVER WEIGHTS OF RATS

	Liver weight in g/100 body weight	Pancreas weight in g/100 gm body weight
Casein	6.20[a+]	0.23
Raw Mexican 142	4.50[b]	0.23
Autoclaved Mexican 142	6.00[a]	0.27
Autoclaved plus Methionine Mexican 142	6.10[a]	0.44
Raw Mwezi Moja	4.20[b]	0.27
Autoclaved Mwezi Moja	5.20[ab]	0.33
Autoclaved Mwezi Moja plus Methionine	5.90	0.34
Raw Canadian Wonder	4.40[b]	0.16
Auto Canadian Wonder	4.90[ab]	0.29
Auto Canadian Wonder plus Methionine	5.10[ab]	0.37
Raw Rose Coco	4.1[b]	0.26
Auto Rose Coco	4.90[ab]	0.20
Auto Rose Coco plus Methionine	4.90[ab]	0.42
SEM	± 0.37	± 0.04

[+]Means without common superscripts within each column are different ($P < 0.01$).

The liver weights of the livers of the rats fed raw bean diets were lighter ($P < 0.01$) than those of rats fed autoclaved Mexican 142, autoclaved and methionine supplemented Mexican 142 and Mwezi Moja and Casein diets. However, the liver weights of the rats fed methionine supplemtned diets and the Casein control diet were not different ($P > 0.01$) from those fed autoclaved bean diets. The reduction of weight of the livers of rats fed raw diets might have been due to the toxins present in the raw beans together with a deficiency of the critical essential amino acids (Kakade et al. 1965 b; Jaffe' and Vegalette 1968; Onayemi et al. 1976).

At necropsy it was observed that there was distinct paleness of the mucous membranes and the livers were very small and yellowish in colour. There was accumulation of yellowish fluid in the abdominal cavity which was more marked in those animals that stayed for 10 days on the experiment than those that stayed for shorter periods. The rats fed autoclaved bean diets also had very small livers, while the control animals remained normal throughout the duration of the investigations. These results concur with those of Kakade et al. (1965 b); Kakade and Borchers (1967) and Ikegwuonu and Bassir (1976).

Liener (1969) outlined a possible mechanism by which the consumption of certain foods (beans in particular) could give rise to gases in the intestine. Oligosaccharides such as raffinose and stachyose, which occur in beans

may not be digested by intestinal enzymes but may accumulate in the lower gut (Subba Rao and Desikachar, 1964) where they would be broken down by bacteria with the formation of gas. Kakade and Borchers (1967) observed a reduction (up to a half) in the amount of gas found in the rat intestine when a raw navy bean diet was replaced by one containing autoclaved beans. As the content of oligosaccharides in beans affects the gas forming properties of the gut microflora it seems possible that the amount of these oligosaccharides reduced during autoclaving.

4.4.1.4 Histopathological changes of some organs of the rats fed various bean diets.

Livers from rats fed raw beans were characterized by focal areas of coagulative necrosis in the regions of the portal triads. Early stages of Karyorrhexis and Karyolysis were evident. Pyknotic nuclei were distinct in certain areas of the liver. The nuclei were in places more densely packed, indicating a relative degree of regeneration. Some nuclei contained enlarged nucleoli. Other hepatic cells were binucleated. Mitotic figures were observed in some areas of the liver (Fig. 3B). In addition was severe congestion and many small blood vessels as well as sinusoids were dilated with blood (Fig. 4). Some of the big vessels were ruptured thus causing marked haemorrhage. The increased evidence of

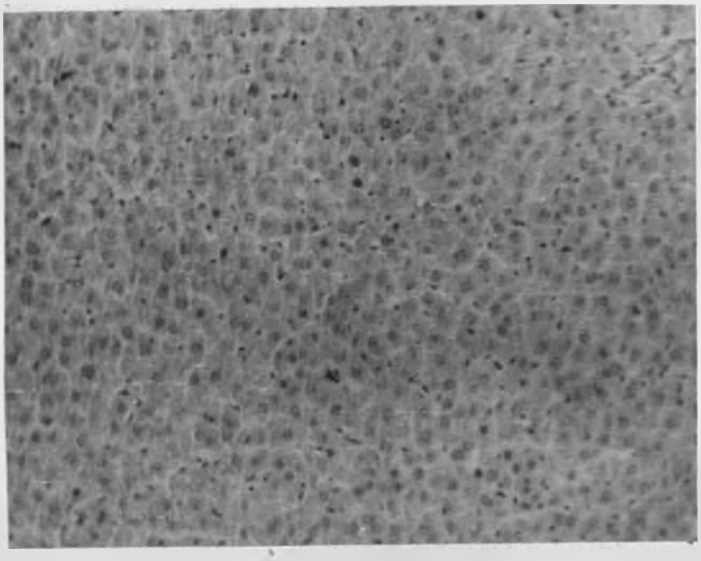

Fig. 3A: Liver from a Control rat fed a casein diet. x 125.

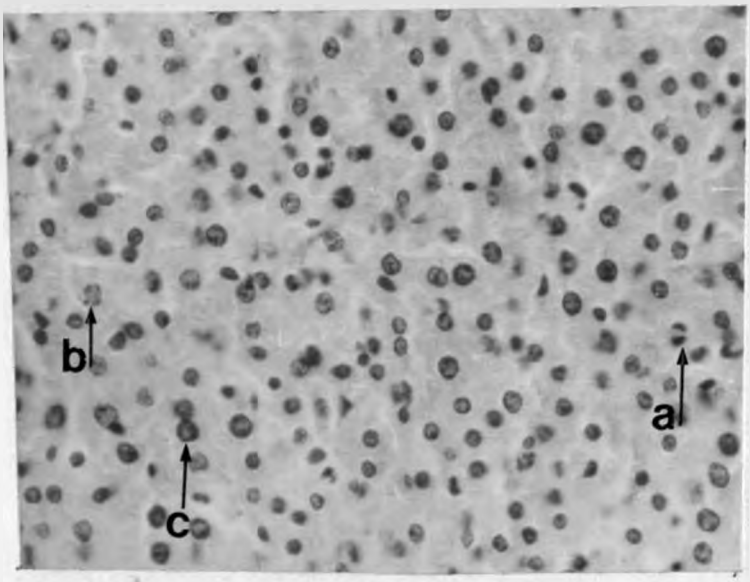

Fig. 3B: Liver from a rat fed a raw bean diet.

Note: (a) Mitotic figure (b) Karyolysis
(c) Binucleated cells. x 125. (——→)

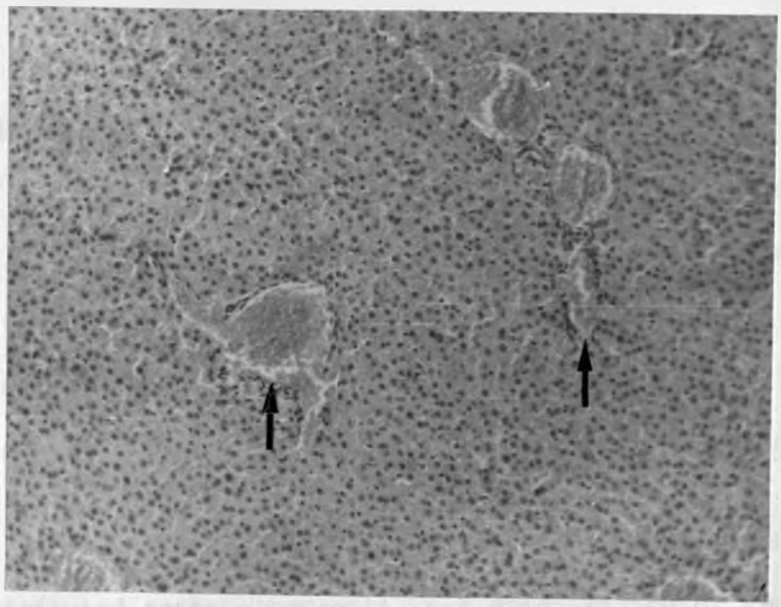

Fig. 4: Liver from a rat fed a raw bean diet.

Note the severe congestion (⟶)

liver changes in rats fed raw bean diets and the association of the lethality of different bean samples with their PHA content lends support to the suggestion of Jayne-Williams and Hewitt (1972, 1973) that PHA toxicity may cause impairement of body defence mechanism. The results in this study are also in accord with those of Jaffe' (1960); Robins (1964); Liener (1969); Olsnes and Pihl (1976), King et al. (1979).

Livers from rats fed autoclaved bean diets had a number of binucleated hepatic cells distributed in most parts of the organ. Many of the hepatic cells had enlarged nuclei and nucleoli. Evidence for cloud swelling was observed in the liver (Fig. 5B). These reports are similar to those reported in rats fed the diets deficient in essential amino acids (Pomeranze et al. 1959; Sidransky and Baba 1960).

Pancreas from rats fed raw bean diets had unevenly distributed caseous necrosis, and degeneration of the parenchyma was apparent. The cytoplasm was almost devoid of the secretory granules and occupied less area. Acinar arrangement and cell outline were indistinct and some individual cells were very small in size, isolated and not forming acini (Fig. 6B). This pancreatic acinar atrophy has been described by Liener (1969) as an indication of TIA effects. The whole organ was congested with extended capillaries and some blood vessels were ruptured.

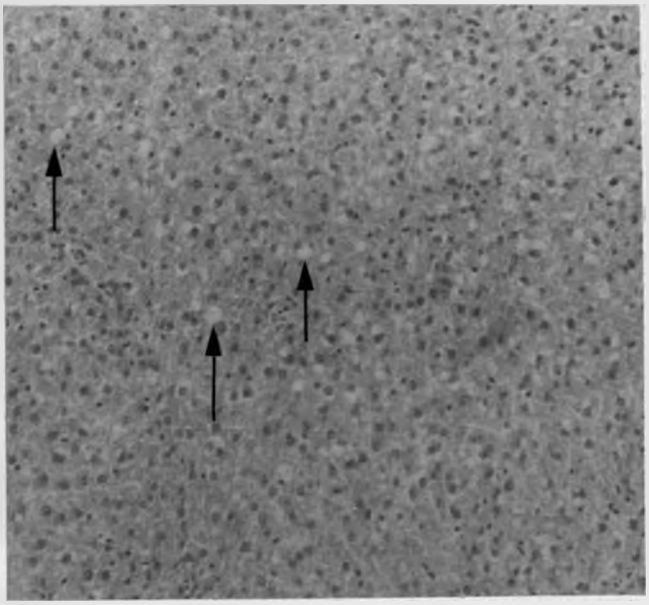

Fig. 5A: Liver from a rat fed an autoclaved bean diet. Note the marked hydropic degeneration (⟶) x 125

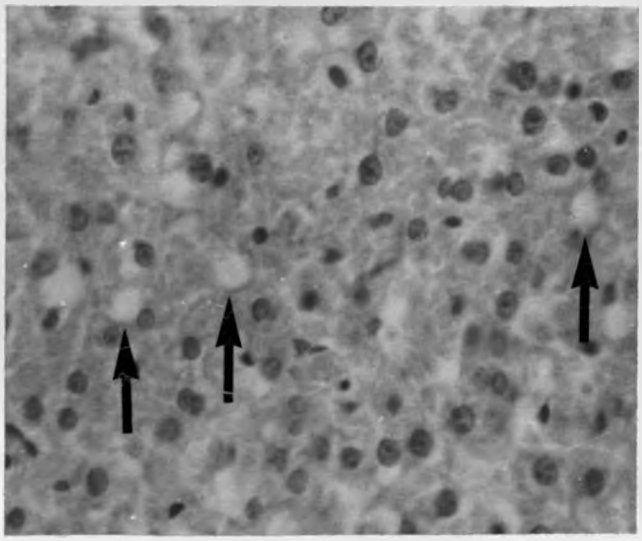

Fig. 5B: Liver from a rat fed an autoclaved bean diet. Note hydropic degeneration (⟶) x 400.

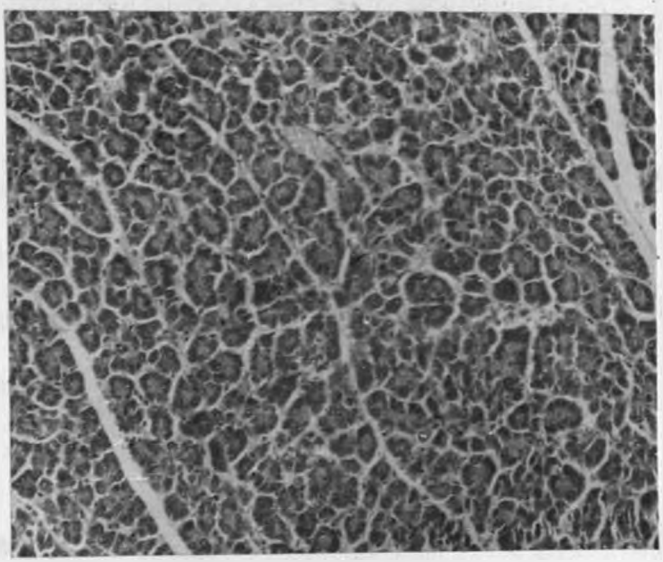

Fig. 6A: Pancreas from a Control rat fed Casein diet. x 125.

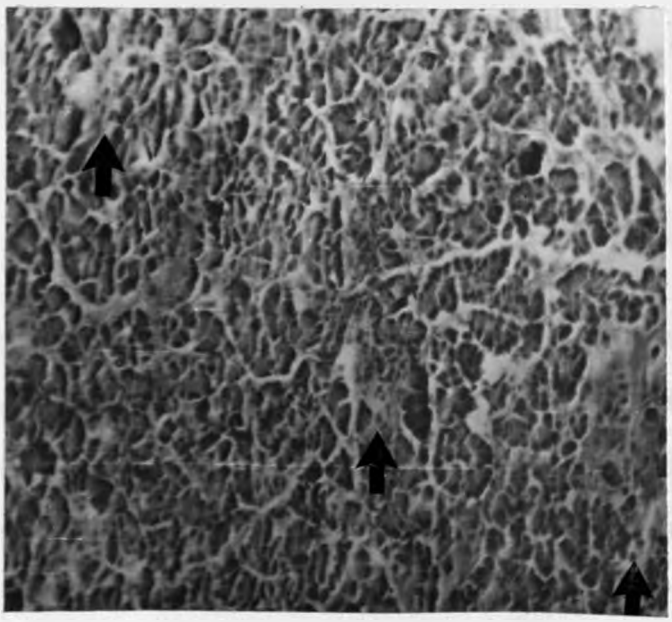

Fig. 6B: Pancreas from a rat fed raw bean diet. Note Atrophic acini. x 125.

In pancreas from rats fed autoclaved bean diets, some cells on the edge of the pancreatic lobe were not forming acini.

Kidneys from rats fed raw beans had many extended blood vessels within the tubular epithelium. In some areas there was marked haemorrhage. There were degenerative changes and necrosis with evidenced Karyolysis and slight Pyknotic nuclei.

Kidneys from rats fed autoclaved bean diets showed evidence of cells sloughing off from the tubules. Significant microscopic lesions were not observed in the tissues of rats fed the autoclaved bean diets plus methionine as well as the casein diet.

These observations are similar to those reported on rats given phytohemagglutinin (Szperl-Seyfriedowa 1951; Phadke and Sohonie 1962; Salgarkar and Sohonie 1965; Kakade et al. 1965a,b; Liener 1969, Ikegwuonu and Bassir, 1976).

It appears that the differences in the histo-pathological alteration observed in the tissues of rats fed the various bean diets are due mainly to a deficiency and unavailability of critical amino acids such as methionine and low food intake of the animals consuming the raw bean diets. This observation is similar to the works of Kakade et al.(1965a,b). Bowman (1944) has shown the presence of heat labile TIA in navy beans, the action of which would be

expected to accentuate the deficiency of critical amino acids in inhibiting the action of trypsin. The beneficial effect of autoclaving the raw beans in inhibiting the histopathological changes in rate could be due to the destruction of TIA and PHA and thereby increase the availability of critical amino acids. It has already been pointed out in (Tables 15 and 16) that raw beans were poorly digested in vitro by trypsin as compared to autoclaved beans, and contained high levels of heat labile PHA.

4.4.2 Cow Peas

4.4.2.1 Performance of Rats and Pigs fed Cow pea diets

In general, feed intake, weight gains, PER and feed utilization responses (Table 21) of rats that fed raw cow pea at 10 % protein level were significantly ($P < 0.01$) lower than those on casein diet. Although raw cow peas had no lethal action, it permitted very slow growth.

The growth promoting effects of the protein of cow pea was not significantly ($P > 0.01$) enhanced by cooking (Table 21).

The methionine supplemented cow pea diets stimulated significantly ($P < 0.01$) faster body weight gain, higher feed consumption and better feed efficiency ratio and

TABLE 21: EFFECT OF AUTOCLAVING AND METHIONINE SUPPLEMENTATION OF COWPEAS ON RAT PERFORMANCE

Protein source	Average daily feed intake (gm)	Average daily weight change (gm)	Protein efficiency ratio	Feed efficiency ratio
Casein	13.27[a]+	4.69[a]	2.50[a]	2.87[a]
Raw Cow pea	7.48[b]	0.26[b]	0.30[b]	26.31[b]
Auto Cow pea	7.62[b]	0.33[b]	0.35[b]	28.17[b]
Auto Cow pea plus Methionine	11.42[a]	3.15[a]	2.43[a]	3.70[a]
SEM	± 0.51	± 0.60	± 0.08	± 0.35

+Means without a common superscript within each column are different ($P < 0.01$)

protein efficiency ratio than the unsupplemented cooked cow pea diets (Table 21). The rats fed methionine supplemented cow pea diets grew as well as those fed the casein control diet.

The results obtained with raw cow peas do not agree with the results reported by Owusu-Domfeh et al. (1970) and Owusu-Domfeh (1972) that rats lost weight when fed raw cow peas. However, the results in the experiment reported here concur with those of Sherwood et al. (1954).

The results of Pusztai (1967); Owusu-Domfeh et al. (1970) and Owusu-Domfeh (1972) suggest that the unheated cow peas did not support growth adequately probably due to the trypsin inhibitors and traces of PHA contained in them.

The lack of improvement when cow pea was autoclaved could have been due to the residual trypsin inhibitor activity together with the limiting amino acids especially methionine and cystine. Reports in literature concerning the effect of heat on the cow pea species are contradictory. Thus a slight improvement in the case of cow pea as a result of heat treatment has been reported by Finks et al. (1922); Sherman (1941); Owusu-Domfeh et al. (1970) and Owusu-Domfeh (1972). Onayemi et al. (1976) and Elias et al. (1977) reported a detrimental effect while Richardson (1948) and Elias and Bressani (1977) reported no effect on rat performance following

autoclaving cow peas. Some of the apparent discrepancies among the results of these investigators may have been due to differences in experimental techniques. There are indications, however, that the quality of cow pea proteins differs between samples of the same variety as well as between varieties cultivated in different parts of a country or in different countries (Patwardhan 1962). Thus Richardson (1948) reported the quality of the protein in Jackson Purple-Hall to be inferior to that in long pod cream or dwarf California Blackeye No.5, Borchers and Ackerson (1950) reported differences between two samples of the Blackeye variety. A careful study of such variations and their nutritional significance in relation to their genetic and cultural characteristic does not seem to have been made on any appreciable scale (Patwardhan 1962).

It is very clear that most of the added methionine was used to combat a deficiency of cystein, hence the great increase in PER of cow peas. The improvement in rat performance following autoclaving and methionine supplementation indicates this amino acid to be critically limiting in cow peas and probably no amino acid other than methionine is seriously deficient in the cow pea protein. The cow peas also contain nothing that seriously depressed rat growth or appetite, and the heat stable antimetabolites appeared to have been of no

significance. These findings concur with those of other workers (Finks et al. 1922; Borchers and Ackerson 1950; Sherwood and Weldon 1953; Sherwood et al. 1954; PAG 1973; and Onayemi et al. 1976).

Comparisons among the cow pea meal diets with respect to feed intake, growth rate, and efficiency of food conversion of the growing pigs are shown in Table 22. There were no significant ($P > 0.01$) difference between the treatment groups in respect to any of these parameters. For any difference to be shown in growth rate and feed utilisation between treatment groups, substitution levels of maize have to be higher than 50 % in the diet (Braude et al. 1950 and Vanschoubroek et al. 1964). Another reason could be that since there was no heat effect on growth performance of pigs, it could be accounted for by the fact that cow peas do not have much antinutritional factors which requires heat treatment as no evidence of toxicity was observed.

Methionine supplementation was also without effect when added to either the raw or cooked cow pea diets. The same observation was made by Maner and Pond (1971) but their suggestion that cooking of cow peas, markedly improved gains, feed consumption and efficiency of feed conversion is not in agreement with the results in this study.

TABLE 22: PERFORMANCE OF GROWING PIGS FED VARIOUS COW PEA MEALS FROM 23-45 kg LIVE WEIGHT

	Raw Cow pea meal	Raw Cow pea meal plus Methionine	Autoclaved Cow pea meal	Autoclaved Cow pea meal plus Methionine	Control	S.E.M.
Average Daily Weight gain (kg)	0.42	0.35	0.40	0.36	0.43	± 0.82
Feed Conversion Ratio	3.50	3.60	3.60	3.70	3.30	± 0.29
Mean Daily Feed Intake (kg.)	1.50	1.20	1.40	1.30	1.40	± 0.13

According to Tunksley (1976) maize has been found to contain adequate levels of the essential amino acid for the growing pigs, but deficient mostly in tryptophan, threonine, sulphur bearing amino acids and isoleucine which were also found limiting in cow peas in this study. Since methionine supplementation had no effect on the growth performance of pigs, this may be a reflection of an inadequate tryptophan intake (Lawrence 1971 and 1972). Orraca-Tetteh (1976) who also gave a mixture of cow peas and maize to infants found that it gave growth performance of 75% compared to that of the proprietary milk mixture. Pond and Maner (1974) in a review on the feeding value of peas to pigs indicated that peas could be used to supply a portion or all of the supplemental protein requirement in a practical cereal based diet for all ages of pigs except the early weaned pigs.

4.4.2.2 Protein digestibility of cow pea diets fed to Rats and Pigs.

The apparent protein digestibility of various cow pea diets is shown in Table 23. The apparent protein digestibility of raw cow pea diet was not different ($P > 0.01$) from that of autoclaved cow peas. These results also show that added methionine did not significantly change the apparent protein digestibility of autoclaved cow peas. However, protein digestibility was significantly higher in rats fed autoclaved plus methionine

TABLE 23: APPARENT PROTEIN DIGESTIBILITY OF COW PEAS FED TO RATS

Protein Source	Apparent Protein Digestibility %
Casein	94^{a+}
Raw Cow pea	78^{b}
Autoclaved Cow pea	79.4^{bc}
Autoclaved Cow pea plus Methionine	84.4^{c}
SEM	$\pm$ 0.07

+Means without a common superscript within a column are different ($P < 0.01$)

supplemented diets compared to those fed raw cow peas. Although cow peas did not show low apparent protein digestibility values when fed raw; cooked and supplemented with methionine, the values were still lower ($P< 0.01$) than those obtained with the casein control diet.

Finks et al. (1922) attributed the increase in the nutritive value of cow pea protein following cooking to an increase in digestibility. The results of Fraps (1945); Jaffe' (1950 a); Owusu-Domfeh et al. (1970); Owusu-Domfeh (1972) and Elias and Bressani (1977) are similar to those reported here and indicate no significant improvement in the digestibility of the protein of cooked cow peas over that of the raw seed.

Digestibility coefficients of various cow pea meals fed to growing pigs are presented in Table 24. The value for protein digestibility seem to be low in all the treatments and this may be due to the presence of proteolytic enzyme inhibitors (Swaminathan 1974).

TABLE 24: DIGESTIBILITY COEFFICIENTS OF VARIOUS COW PEA MEALS FED TO GROWING PIGS

Dietary treatments	Digestibility coefficients	
	Protein digestibility	Apparent dry matter digestibility
Raw Cow pea meal	64	80
Raw Cow pea meal plus Methionine	65	82
Autoclaved Cow pea meal	65	86
Autoclaved Cow pea meal plus Methionine	70	84
Control	61	85

4.4.2.3 Effect of Processing of Cow peas on Liver and Pancreas weights of Rat

The effects of feeding raw, autoclaved and autoclaved plus methionine supplemented cow peas on the weights of pancreas and livers of the rats are given in Table 25. The weights of the pancreas of the rats fed the various cow pea diets were not significantly different from each other. However, pancreas from rats fed cow pea diets were all significantly heavier ($P < 0.01$) than the control group. This may be due to the presence of TIA in the various cow pea diets. Trypsin inhibitors have been reported to cause pancreatic hypertrophy, (Booth et al. 1960; Kyayambashi and Lyman 1966; Jaffe' and Vegalatte 1968 and Liener 1969).

All the weights of the livers of the rats fed casein and the various cow pea diets were not significantly different from each other. Onayemi et al. (1976) however, reported different results from those presented in this experiment and this may be due to the different methods used in processing the cow pea diets.

4.4.2.4 Histopathological changes in rats fed the various cow pea diets.

Livers from rats fed diets containing raw Cow peas showed slight congestion. There were also small foci of hepatocytes undergoing degenarative changes.

TABLE 25: EFFECT OF PROCESSING OF COW PEAS ON PANCREAS AND LIVER WEIGHTS OF RATS

Protein source	Liver weight/ 100 gm body weight	Pancreas weight/ 100 gm body weight
Casein	6.2	0.23^{a}
Raw Cow pea	5.3	0.41^{b}
Autoclaved Cow pea	5.54	$\pm$ 0.34^{b}
Autoclaved Cow pea plus Methionine	5.67	0.42^{b}
SEM	$\pm$ 0.24	$\pm$ 0.03

[+]Means without similar superscripts within each column are different ($P < 0.01$).

Livers from rats fed autoclaved Cow pea diets had a few small foci of mononuclear inflamatory cells, scattered throughout the parenchyma.

While the Pancreas from rats fed raw Cow peas was characterized by the unevenly distributed caseous nucrosis throughout the whole pancreas, there was a small focus of acinar atrophy in the pancreas from rats fed autoclaved Cow pea diet.

In kidneys from rats fed raw Cow peas there was generalized and rather mild disintegration of some tubules as evidenced by pyknosis and concommittant dislodgement of the epithelium from the basement membrane.

In the kidneys from rats fed autoclaved Cow peas, there were acidophilic proteinaceous casts within the renal tubules.

Significant microscopic lesions were not observed in the tissues of rats fed the autoclaved Cow pea diet plus methionine and the control casein diet. These results are in accord with those of Ikegwuonu and Bassir (1976, 1977).

4.4.3 Pigeon peas

4.4.3.1 Performance of rats fed pigeon pea diet

The performance of rats is outlined in Table 26. The feed intake of rats fed raw pigeon pea was significantly ($P < 0.01$) lower than that of the control group fed casein diet. While the control group grew normally, the rats on the raw pigeon pea continued to lose weight and two out of six died on the twenty fifth day of the experiment.

All rats that fed on the autoclaved pigeon peas diet survived the 28 days of the experiment but grew at a slower ($P < 0.01$) rate than those fed the casein diets.

The addition of tryptophan to autoclaved pigeon pea did not significantly improve the feed intake, body weight gain, PER and feed efficiency ratio. The protein of heated pigeon pea when supplemented with tryptophan supplied only 12% as good growth of rats as did casein.

The results obtained on raw pigeon peas are similar to those of Ochse (1931) who claims that raw seeds of pigeon pea contained an unidentified narcotic that if eaten in quantity induces sleepness. This may also be due to the simultaneous occurence of PHA and TIA in the raw pigeon pea, and probably the severe deficiency of the critical amino acids.

TABLE 26: EFFECT OF FEEDING RAW, AUTOCLAVED OR AUTOCLAVED AND TRYPTOPHAN SUPPLEMENTED PIGEON PEAS ON RAT PERFORMANCE

Protein source	Average Daily Feed Intake	Average Daily Weight Change (gm)	Protein Efficiency Ratio	Feed Efficiency Ratio
Casein	13.20[a]*	4.70[a]	2.50[a]	2.90[a]
Raw Pigeon pea	5.80[b]	- 0.90[b]	-	-
Auto Pigeon pea	7.40[c]	0.14[c]	0.10[b]	45.43[b]
Auto Pigeon pea plus Tryphtophan	8.0[c]	0.30[c]	0.30[b]	32.5[b]
SEM	± 0.47	± 0.42	± 0.04	± 0.53

*Means without similar superscripts within each column are different ($P < 0.01$)

These results obtained with autoclaved pigeon peas concur with those of Hirwe and Nagar (1951) and Liener (1973). The comparatively low value of PER in this study reflects the lack of balance in the amino acid content of pigeon pea. Very low PERs in pigeon peas have also been indicated by Patwardhan (1961).

Results obtained with autoclaved plus tryptophan supplementation suggest that in pigeon pea diets, there is little advantage in raising the tryptophan content . The results of Braham et al. (1965) and Dako (1966) show that methionine addition had no effect on improving protein quality of pigeon pea. However, when both methionine and tryptophan were added, the protein quality increased which indicates that in pigeon pea, both amino acids are about equally limiting. In the experiment reported here, valine and isoleucine were more limiting than methionine plus cystine.

4.4.3.2 Apparent protein digestibility of Pigeon peas fed to Rats

The apparent protein digestibility of various pigeon pea diets is shown in Table 27. Raw pigeon peas were poorly digested 44%. However, the apparent protein digestibility of pigeon peas significantly ($P< 0.01$) improved after heating. This may be due to the destruction of PHA and TIA and possibly other unrecognized anti-

TABLE 27: APPARENT PROTEIN DIGESTIBILITY OF PIGEON PEA (Cajanus Cajan) FED TO RATS

Protein Source	Apparent Protein Digestibility %
Casein	94.00[a] *
Raw Pigeon pea	44.00[b]
Autoclaved Pigeon pea	55.33[c]
Autoclaved Pigeon pea plus Tryptophan	60.33[c]
SEM	± 1.48

*Means without similar superscripts within each column are different ($P < 0.01$).

nutritional factors which could be partially responsible for the low digestibility of the protein in pigeon peas (Liener 1962).

Even though these factors were destroyed by heat, it was of interest that when pigeon peas were fed cooked, the protein digestibility was still inferior ($P< 0.01$) to casein. The heat resistant trypsin inhibitors might have contributed to the low protein digestibility of cooked pigeon peas.

Autoclaving followed with tryptophan supplementation did not significantly ($P> 0.05$) improve the protein digestibility of pigeon peas. Jaffe' (1950 a,b) and Viteri and Bressani (1972) have also indicated low protein digestibility in pigeon peas.

4.4.3.3 Effect of processing of Pigeon peas on Liver and Pancreas weights of Rats.

The weights of pancreas and livers of rats fed the various pigeon pea diets are shown in Table 28. There was a trend towards increased pancreas weight with pigeon pea feeding compared to the casein control diet. This indicates the presence of hypertrophy of the pancreas. This was probaly due to the residual TIA in the pigeon peas.

TABLE 28: EFFECT OF PROCESSING OF PIGEON PEAS ON PANCREAS AND LIVER WEIGHTS OF RATS

Diet description	Liver weight/ 100 gm body wt.	Pancreas weight/ 100 gm body wt.
Casein	6.20[a+]	0.23[a]
Raw Pigeon pea	3.42[b]	0.27[a]
Autoclaved Pigeon pea	4.62[c]	0.38[b]
Autoclaved Pigeon pea plus Tryptophan	4.77[c]	0.43[b]
SEM	± 0.29	± 0.04

[+]Means without similar superscripts within each column are different ($P < 0.01$)

The livers of rats that fed raw pigeon peas was significantly ($P < 0.01$) lighter than those of rats fed either autoclaved pigeon peas, autoclaved pigeon peas plus tryptophan supplementation or casein control diet. Addition of tryptophan did not have any significant effect on the liver weights.

4.4.3.4 Histopathological changes of some organs of the rats fed the various pigeon pea diets.

In livers from rats fed raw pigeon peas, there was slight variation in the size of hepatocytes, some of which were bigger than the others. There was also slight haemorrhage, pyknosis, and congestion in the liver. Most of the livers from rats fed autoclaved Pigeon pea diet were normal and only focal congestion and karyolysis were observed.

There was pancreatic acinar atrophy as evidenced by the disorganization of acinar cells in pancreas of rats fed raw pigeon pea diet. There was also a mixture of both coagulative and liquifacture types of necrosis. Necrosis of some lobes of the pancreas is evident together with congestion.

In pancreas from rats fed autoclaved pigeon pea diets, there was only slight acinar atrophy of the cells. There was severe Karyolysis type of necrosis involving convoluted tubules in the kidneys from rats fed raw pigeon pea diets (Fig. 7B). Every one of the blood vessels and capilaries

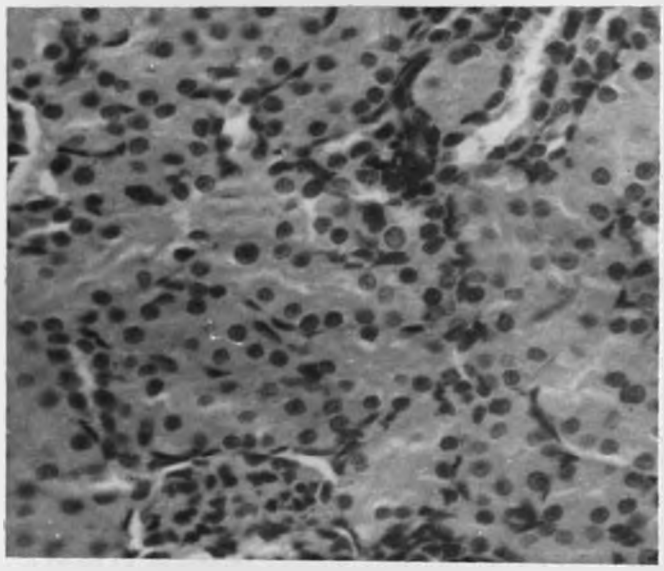

Fig. 7A: Kidney from a Control rat fed Casein diet.
x 125.

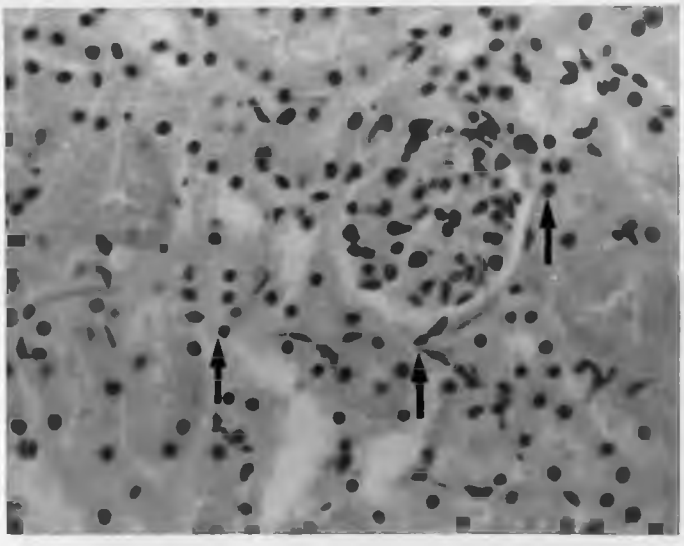

Fig. 7B: Kidney from a rat fed a raw Pigeon pea diet.
Note Pyknotic cells. x 125.

were extended with blood. Autoclaving Pigeon peas resulted in normal appearing kidneys.

Kidneys, Livers and Pancreas from rats fed autoclaved and tryptophan supplumented diets and casein group were normal. These results concur with those of Ikegwuonu and Bassir (1977).

5. GENERAL DISCUSSION

The adequacy of a protein for man or farm animals depends upon the content of essential amino acids in the protein, the availability of the amino acids and the presence of growth inhibitors in the protein or the concentrate that carries the protein. The nutritional value of a pulse may be impaired by the presence of naturally occuring factors, which interfere with digestibility and/or nutrient availability to human or other animals. The studies reported here were undertaken, to determine the chemical composition and the relative growth promoting value of the protein in the most commonly eaten bean and pea seeds in Kenya, using weanling rats and growing pigs.

The demonstration that raw beans can be toxic to experimental animals may have important implications, in the field of human nutrition. There have been a number of reports in the literature of cases of human intoxication as a result of the consumption of legumes known to contain PHA, such as insufficiently cooked kidney bean flour and raw soybean (Cartwright and Wintrobe 1946; Griebel 1950; Liener and Kakade 1969).

The feeding trials in this study confirmed that those varieties that exhibited very high agglutinating activity towards trypsinized cow cells were toxic and those that were not toxic, supported very poor growth when fed to rats. Jaffe' and Vegalette (1968) and Jaffe' and Bruchers (1972) have

reported similar results. Other workers whose contributions have been reviewed by Jaffe' (1969) have observed a correlation between high concentration of PHA in various raw legumes and the lethal and severely growth depressing effects noted when these legumes were fed to different animals. However, Kakade and Evans (1965a, b, 1966) have suggested that a variety may have an agglutinin which is not toxic. Therefore, a positive agglutination test may not necessarily distinguish between toxic and non toxic hemagglutinins.

If a comparison is made of the TIA contents (Table 15) and the toxic PHA in the legumes (Tables 16 and 17) it is significant to note that Rose coco beans had the highest amounts of both PHA and TIA. The two unheated legumes that had the lowest levels of TIA namely cow peas and pigeon peas likewise were the only two that displayed low levels of PHA. Bressani and Elias (1977) have also noted lower levels of antinutritional factors in cow peas than in *P vulgaris*.

The relation between the ingestion of trypsin inhibitor and excessive pancreas weight is accepted by most investigators in this field (Chernic *et al*. 1948; Booth *et al*. 1960; Mickelson and Yang 1966). It has not however, remained unquestioned (Saxana *et al*. 1963). In the present study, a hypertrophic pancreas was only observed in some animals although all the legumes samples had some trypsin inhibitor activities *in vitro*. The cow

pea and pigeon pea diets caused a moderate but significant increase in pancreas weights as compared to casein (Tables 25 and 28). They had little antitrypsin action in vitro. It is interesting as observed in this study and that of Booth et al.(1960) and Kyayambashi and Lyman (1966), that methionine supplementation to cow peas was found to effectively counteract most of the growth depression caused by the inhibitor despite the persistence of pancreatic hypertrophy. The results of the present study points out the great variation in biochemical activities, both in vitro and in vivo, existing between different legumes. This should be taken as a demonstration of the importance for a clear definition of the factors present in a given legume sample in the correct interpretation of growth effects on experimental animals. These results also point to the feasibility of selecting legume varieties for a low or high agglutinin or enzyme inhibitor content as suggested by Jaffe' and Vegalatte (1968).

Although there is little doubt that trypsin inhibitors can produce adverse physiological effects in animals, little is known about their effects in human beings. Feeney et al. (1969); Coan and Travis (1971); Whitaker and Feeney (1971) have demonstrated, that human trypsin is only weakly inhibited by the soy bean inhibitor. However, Liener (1972) points out that properly processed legumes in which the inhibitor has been destroyed are a very satisfactory source of protein for humans and most animals particularly if supplemented with the sulfur containing amino acids.

Growth of rats was increased when fed on supplemented beans and peas used in this study. However, with Rose coco beans, Canadian wonder beans and Pigeon peas, the increase was slight and very inferior to cow peas and casein. The reports of Salaman and McGinnis (1968) and Elias et al. (1977) have indicated that phenolic compounds in the seed coats of beans and other legumes may have growth depressing effects. However, considering that Mexican 142 and Mwezi Moja beans had lower residual TIA contents than cow peas, higher chemical scores, and relatively the same EAAI, one would expect even higher growth rates among the beans. The fact that the residual TIA did not interfere significantly with in vivo digestion in rats fed cow peas would rule out TIA as the major factor to explain the case of the inferior nutritive value of beans and pigeon peas. It was summarised that methionine and tryptophan were not the only limiting amino acids in the beans and Pigeon peas respectively. Other amino acids may also be as indispensable as the first limiting amino acids.

It was surprising to note that methionine supplementation did not significantly increase the growth performance of pigs fed raw and autoclaved cow peas. Yet as evidenced from the study with rats, it is indicated that the nutritional value of cow peas when properly processed and supplemented with methionine is much higher than the nutritional value of raw and autoclaved cow peas without

methionine supplementation. The same observations have been reported elsewhere (Riley 1961; Goatcher and McGinnis 1972; Cappella 1974; and McGinnis and Cappella 1976). Much could be achieved nutritionally if beans had the nutritional attributeds of cow peas because they are more popularly consumed in Kenya.

To encourage the use of legumes, intensive research on production of legume food mixtures within the purchasing power of the people in developing areas of the world is very urgently needed. Therefore, a combination of cereal protein and legume proteins may come very close to providing an ideal source of dietary proteins for human beings where animal protein is scarce and protein-energy malnutrition is prevalent.

Efforts also should be made towards the selection of appropriate cereal grain-legume combinations that blends each other and meet the nutrient requirement of man.

6. SUMMARY AND CONCLUSIONS

1. Beans (*P. vulgaris*) cow peas (*V. unguiculata*) and pigeon peas (*C. cajan*) were analysed for amino acids, proximate principles, gross energy, minerals, PHA and TIA. The legumes were soaked in water, dried, finely ground and heated in the autoclave at 121^{o}C for 30 minutes. Effects on rat performance of raw, or autoclaved and autoclaved supplemented with first limiting amino acid of legume. proteins were determined.

2. All the legumes contained TIA and PHA. Autoclaving partially destroyed the TIA in the beans and peas, but the PHA was completely destroyed.

3. Raw beans when offered to rats were found to be toxic. However, although autoclaving did not produce toxicity in rats, rats fed such beans still lost weight. It can be concluded that the growth inhibition of the rats fed beans could be partly due to the impairment in the availability of critical amino acids but more severe reductions may be attributed to methionine deficiency. Moreover, this condition can be further aggrevated by the low food intake resulting in a more severe limitation of the first limiting amino acid for the animals. The reason for the failure to obtain a positive growth response when the seeds were autoclaved and supplied at 10% level protein, is not very clear and deserves further study.

4. The rats fed methionine supplemented Mwezi Moja and Mexican 142 beans and cow pea diets grew normally suggesting that a lack of available methionine is the chief factor that limits the growth of young rats fed cooked cow peas and beans as the sole source of their protein.

5. The autoclaved pigeon pea diet plus tryptophan supplementation did not effectively counteract the growth depression of the rats. The low nutritive value of autoclaved pigeon pea diets, Rose coco, and Canadian Wonder beans appear to be due to a deficiency of the second limiting amino acids and probably the presence of the heat stable TIA and other unrecognized antinutritional factors.

6. Pancreatic hypertrophy was found to occur after the ingestion of cow peas and pigeon peas and persisted even after the supplementation of methionine and tryptophan respectively.

7. With regard to the nutritional efficiency of heat processing and methionine supplementation of cow peas for pigs, it can be concluded that it is safe to feed diets containing raw unsupplemented cow peas with no adverse effects.

7. SCOPE FOR FURTHER WORK

Additional research is needed to identify all the antinutritional factors present in legumes. The unavailability of many essential amino acids in legumes that have been cooked to the point at which all the known toxic factors have been destroyed suggests the presence of other antinutritional factors. As stated by the National Academy of Sciences (1974), primary concern should be the presence of factors that interfere with proteolysis, their identification and the development of processing methods that inactivate or destroy them. Therefore it is to be hoped that this area of research will attract more investigators in the future.

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