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NUTRITIONAL AND SENSORY EVALUATION OF MEAT PRODUCTS EXTENDED WITH TEXTURED PLANT PROTEINS

by

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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This thesis is dedicated to the memory

of my mother, the late

Hanna Sokolsky

who instilled in me the value

of knowledge

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A B S T R A C T

The nutritional and sensoric properties of meat products extended with texturized plant proteins (TPP), were studied by evaluating four sources of plant protein at various levels. Fababean protein concentrate (FBPC), soy protein concentrate (SPC) and pea protein concentrate (PPC) were texturized by extrusion to evaluate the effect of heat treatment on the biological value of the protein. Oats were used in the form of flakes (rolled oats). A portion of the FBPC was extracted with ethanol:water (60:40), to remove vicine and convicine which are believed to be anti-nutritional factors. Growth assays were conducted to obtain protein efficiency ratios (PER) for the plant proteins and mixtures of meat plus plant proteins incorporated into rat diets. Organoleptic characteristics of meat loaves, which contained three levels of texturized plant proteins (5, 15 and 25%), were evaluated by five trained panelists using the method of magnitude estimation.

Meat showed a better biological value than plant proteins when fed as the only source of protein. When

formulated meat loaves were the source of protein in the assay diet, meat, supplemented with other sources of proteins, demonstrated a superior biological value and better protein utilization than the pure meat product. Untreated fababean proved to be nutritionally a better source of protein, while ethanol extraction improved water absorption plus water and fat retention capacities. Overall performance of fababean was superior to the other sources of proteins used in the study. A decrease in the level of several amino acids (lysine, cystine, methionine, etc.) in the heat treated samples, indicated a negative effect of heat treatment on biological value of proteins. Supplementation of a protein system by other sources of proteins eliminated this effect.

There were no significant differences in flavor perception between samples, since the spices added to the product masked the beany (bitter) flavor of the protein concentrates. Firmness of the product was affected by the source of the plant protein and not by the level added to the meat loaf. A similar trend was found to be true for chewiness, with fababean and soy concentrates showing a high degree of chewiness. Grittiness was found

to be high in samples which contained 15 and 25% of plant proteins, with oats being rated very low in grittiness. Panelists have found the texturized fababean proteins to be acceptable meat extenders when used at levels between 5 and 25%. Generally, meat products extended with 5 and 15% plant proteins were preferred over samples which contained higher levels.

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I N T R O D U C T I O N

Changes are taking place continuously all over the world. Constant increase in populations and changes in life styles bring many countries to the verge of shortage of the most basic and essential foods. At the present time, North American food protein sources are in abundance, however, shortages may be a future food issue as it is presently in many other countries. From estimation of supply and demand data on a world basis, it has been projected that there will be a deficit of 30.2 million metric tons of animal protein sources by 1980 (Burrows, Greene, Korol, Melnychyn, Pearson and Sibbald, 1972). An increase in demand and a constant or even decrease in supply drives prices up. Ground meat, one of the cheapest and readily available source of animal protein, is reaching the $2.00 a pound mark (Anon, 1979). This sharp rise in price will eliminate one of the most essential food items from the tables of many North Americans. It has been postulated that in the next few decades, plant proteins will constitute up to two-thirds of the world's high grade protein (Bird, 1974). At the

present time, cereal grains account for the major portion of consumed plant proteins. Oilseed meals and legumes offer potential new sources of fairly high quality protein. An immediate increase in the use of soy and other available vegetable protein concentrates was clearly anticipated by the food industry as early as 1973 (McCleary, 1973). Because of the limited region adapted to growing soybeans in Canada, current interest is focused on the use in human foods of high protein crops adaptable to the prairie region. Fababeans (Vicia faba minor) and field peas (Pisum sativum) offer such a potential (Evans, Seitzer and Bushuk, 1972; Youngs, 1970).

The use of texturized soy product as an extender in ground beef was introduced to Canada in 1973 without prior notice (Vaisey, Tassos, McDonald and Youngs, 1975). Although work has been done by many scientists on the effects of added textured plant proteins to beef (Vaisey et al., 1975; Seideman et al., 1977) most of it has been done on beef patties. In spite of the work done on the meat extenders, the lack of an extensive picture still exists. Studies on the nutritional aspects of the meat-plant protein mixtures, on the effects of texturization on rat

growth performance, on chemical score, limiting amino acid, complementation, amino acid availability as well as functional properties and sensory attributes are needed. An inclusive study which will enable the industry to manufacture high-quality and palatable products and at the same time will educate the consumer to appreciate the benefits of the new products, will prevent only partial acceptance or repulsion by consumers because of lack of product knowledge (Woolcott, Vaisey and McDaniel, 1974).

It has been found that meat products containing high plant protein concentrate in the texturized form are more acceptable than when they were added in the form of flour (Vaisey et al., 1975). During texturization of high protein concentrates and subsequent processing of the product, heat is introduced which will affect the amino acid composition of the protein, plus availability and retention (Sarwar et al., 1975).

One of the new protein sources on the prairies for human consumption, is the fababean. As a legume high in protein, it has attracted the attention of many scientists but there are obstacles to wide utilization

of the fababean as food. Bitter flavor is one flavor parameter frequently ascribed to plant proteins and the fababean is no exception (Donaldson, 1978). Another drawback to the more widespread use of the fababeans stems from its toxicity which, in certain circumstances, can give rise to the disease favism (Jamalian et al., 1976), an acute heamolytic anaemia. Whether or not texturization of high protein concentrates and chemical removal of toxins or anti-nutritional factors from the protein source have adverse effects on its nutritional value, has not yet been made clear.

The objectives of the present study were as follows:

1. To observe the performance of plant protein concentrates as meat extenders by means of bioassay, and sensory evaluation.
2. To observe the effects of heat treatment as applied during texturization on the individual amino acids and the protein as a whole.
3. To determine the changes that will occur in the level and availability of several amino acids as a result of vicine removal.

4. To evaluate the nutritional value and protein quality of several plant proteins (namely: fababean, oats, pea, soy) by a biological evaluation.
5. To study the effect of increasing protein level on the sensory perception of flavor and textural parameters.
6. To evaluate the method of predicting protein efficiency ratio as a potentially fast and reliable way for determination of the food quality.

LITERATURE REVIEW

Proteins and Amino Acid Functions

Proteins are peptides of high molecular weight. They are either made of amino acids alone ('holoproteins'), or they may contain, in addition to their peptide moiety, a so-called prosthetic group of various chemical compositions ('heteroproteins', also called 'conjugated proteins'). In the field of food and nutrition, protein and amino acid functions are limited to those directly concerned with (a) digestion processes in the gastro-intestinal tract (b) with anabolic, and (c) with catabolic processes and considered both locally in various body tissues, and more generally, in the living body as a whole in its metabolic exchanges with the environment (Bigwood, 1972). There are several nutritional functions of proteins and amino acids; nitrogen balance of the body, nitrogen and sulfur supply to the body and metabolic processes. A major nutritional aspect of amino acid metabolism concerns the method of evaluation of protein quality of foods and diets. The distribution of the amino acid supply to the body tissues varies with the

amino acid pattern and the nutritive quality of the protein material available. The closer this pattern happens to be to the amino acid pattern essential to satisfy the requirements of the body, the greater the biological value of the protein source happens to be. This very essential problem of determining biologically the quality of a dietary protein or of a diet has led to very extensive research.

Biological Evaluation of Dietary Proteins

Protein quality relates to the efficiency with which various food proteins are used for synthesis and maintenance of tissue protein. Chemical methods, such as various amino acid scoring procedures, can give a fairly good indication of the protein value of a food (Jansen, 1978). Unfortunately, such methods are of somewhat limited value because they do not take into account digestibility and availability of amino acids. There are several methods of biological evaluation of protein sources (Hartog and Pol, 1972), but since rat growth evaluation is the standard used for regulatory purposes by both the United States and Canadian governments, a great

deal of work on protein evaluation was done applying this method. Evaluation of protein quality by utilizing the Protein Efficiency Ratio (PER) method was performed on various sources of proteins such as meat (Wilding, 1974, Brinkman and MacNeil, 1976, MacNeil, Mast and Leach, 1978), nutritional values of plant proteins (Sarwar, Sosulski and Bell, 1977, Womack, Bodwell and Vaugham, 1974), and mixtures of meat and plant proteins (Olson, Sosulski and Christensen, 1978). In spite of the fact that the failure of the PER assay to properly credit protein used for maintenance is a serious flaw (Jansen, 1978), it has been used, as was shown, by many scientists. Results obtained from growth rate experiments give a clear indication of the biological value of proteins from various sources.

Time is another limiting factor in the PER method for evaluation of protein quality. Feeding trial lasts twenty-eight days plus five days of acclimation period, makes the growth trial both lengthy and expensive. Several food standard proposals by the United States Department of Agriculture and Canada Department of Agriculture, require a demonstration of adequate protein

quality, but PER is the only method that has been accepted by the Association of Official Analytical Chemists (AOAC, 1975, Jansen, 1978). Therefore, a less expensive and more rapid technique is desirable. Alsmeyer, Cunningham and Happich (1974) proposed an equation which will assist in prediction of PER from amino acid analysis. In-vitro methods to predict PER by using enzymatic digestibility of the proteins, had a significantly high correlation with observed PER values for forty-five different sources of proteins (Satterlee, Kendrick and Miller, 1977, Hsu, Sutton, Banjo, Satterlee and Kendrick, 1978).

Supplementary Effect on Biological Value

Limiting amino acid is a cause for only partial availability and utilization of the other amino acids in the protein. The result of partial utilization is an inefficient exploitation of available proteins. Minimizing this effect can be done by supplementing the diet with other diets rich in the specific amino acid or with the limiting amino acid itself. Supplementation of pea protein concentrate with 0.5% and 1% DL-methionine or

methionine hydroxy analog (MHA), proved to be a successful method in improving biological value of the protein. The PER values were higher with 1% of amino acid added to the diet than with 0.5% (Keith, Youngs and McLaughlan, 1977). Methionine supplementation at the level of 0.3%, significantly increased the PER values of soy protein fed to rats (Kapoor and Gupta, 1975). It has also improved the values of net protein utilization and retention. Marquardt and Campbell (1975), reported that chicks fed a ration containing 90% fababeans, grew significantly better when 0.24% methionine was added to the diet. On the other hand, it was established that free methionine will enhance bitterness (Donaldson, 1978) and will adversely affect product palatability (Kies and Fox, 1971). Sarwar et al., (1977) investigated the availability of amino acids in soybean, field pea or fababean and their blends with methionine or wheat. Availability of the sulfur amino acids improved when diets were supplemented with either the methionine or the wheat. It is a well established fact that methionine and cystine are present in low levels in many legumes and oilseeds (soybean, fababean, field pea, etc.) with methionine the first

limiting amino acid (Bigwood, 1972, Kakade, 1974 and Blair, 1977). It is also known that proteins from animal sources (meat, egg) contain higher levels of sulfur amino acids (Bigwood, 1972). The utilization of plant proteins as meat extender should lessen the negative affect of the methionine deficiency in plant materials.

Quality of a protein may be estimated from its amino acid composition as compared with the reference pattern of amino acids. Block and Mitchell (1946) suggested that since all amino acids must be present at the site of protein synthesis in adequate amounts for protein synthesis to proceed, an equal percentage deficit of any essential amino acid would limit protein synthesis to a comparable degree. Also, they investigated the correlation of chemical structure and nutritive value of food proteins on rats fed seventeen proteins or food protein mixtures with egg as the reference protein since it was known to have a biological value closely approaching 100. They concluded that, if the comparison of an "ideal protein", i.e., one containing all the essential amino acids in sufficient amounts to meet requirements without any excess, were known, then it should be possible to compute the nutritive quality of a protein by calculating the deficit of each

essential amino acid from the amount in the "ideal protein" with the "most limiting amino acid" determining the nutritive value. The World Health Organization technical committee for energy and proteins, adopted a new provisional amino acid scoring pattern based on the level of individual amino acids needed to guarantee optimal growth and maintenance (World Health Organization, 1973).

Effect of Processing Conditions on Nutritive Quality of Plant Protein

The increase in the demand and consumption of plant proteins, forced researchers and industry to study the effects of protein concentration and texturization on nutritive value. The heat treatment involved in autoclaving of a diet or extrusion of the protein source (Marquardt et al., 1975), and oil extraction is a controversial subject. Marquardt et al., (1975) fed to chicks, heat treated fababean and observed an improvement of 16% in growth rates and 19% in feed/gain ratios when fababean was extruded or steam autoclaved. Nitsan (1971) on the other hand, observed a decrease in growth rate among rats fed autoclaved fababean. Bressani, Elias and Gomez

Brenes (1972), claim on the basis of extensive work done on oil extraction from cottonseed and soybean that heat processing causes a decrease in available amino acids in the majority of cases, because of destruction or inactivation. Both effects cause a deficiency of one or more amino acids and a decrease in digestibility. The presence or absence of reducing sugars in the diet can affect the degree of nutritional damage. In the presence of reducing sugars even mild heating results in reactions with free amino acid groups, primarily those of lysine. When reducing sugars are absent, however, severe conditions are necessary to cause nutritional damage, which may result from internal amide formation. Isolation of proteins from soybean meal by 0.2% sodium hydroxide, results in a decrease in availability of lysine by about 18% (Sarwar et al., 1975). In the same study, it was also shown that rapeseed meal autoclaved for four hours reduced the availability by 30%. Womack et al., (1974) have demonstrated that lysine, histidine, methionine, threonine and tryptophan, were affected by autoclaving at 120 C for thirty minutes. Similar work was done on chick peas (Cicer arietinum) which are a rich source of lysine

(6.5 to 6.7%). The lysine content is reduced about 10% when the seeds are heated in the autoclave to 121 C for thirty and sixty minutes (Gonzalez del Cueto, Martinez and Frampton, 1960). This study was initiated to compare the effects of heat on chick peas with previously noted effects on typical oleaginous seeds. The results of this study show that the level of oil in the source of protein has no effect on reduction of several amino acids.

Home cooking or industrial processing of foods causes a multitude of changes in appearance, taste and texture, related to chemical modification and interaction of food components. One of the detrimental products of protein processing is the lysinoalanine (LAL), a new amino acid formed from lysyl and cystinyl or glycosidically bound seryl residues (Sternberg and Kim, 1975). Work by Woodard and Short (1973) described the occurrence of a unique histologic lesion called nephrocytomegalia, in the kidney of rats fed an alkali treated industrial grade soy protein. They showed that there is a lower level of lysine and cystine and approximately 0.6% lysinoalanine in the protein which contain a known toxic compound. In contrast to Woodard and Short (1973), DeGroot, Slump and Feron (1976) and Chu, Pellett and Nawar (1976) who claim that LAL forms

only under alkali conditions; Sternberg and Kim (1977) conclude that LAL in the structure of heated proteins not necessarily subjected to alkali treatment, may be a factor to be considered in explaining reduction of nutritional value by heating. The formation of LAL in proteins by heating at pH values considerably lower than those obtained during alkali treatment, suggests the presence of LAL in cooked foods. Although there are significant findings of LAL in heat and alkali treated proteins, it has been shown by DeGroot, Slump, Feron and VanBeek (1976), that kidney lesions found in rats may be peculiar to that species, since other species of animal, including the dog, mouse and monkey, do not develop such lesions even with free LAL. Although it cannot be absolutely excluded, it appears only a remote possibility that LAL is a problem for man (Anon, 1976).

Functional Properties of Plant Proteins as Meat Extenders

Soybean proteins are available to the food industry in the form of flours, grits, concentrates and isolates with respective protein contents of 40 - 50%, 70% and 90% or more (Wolf, 1970). Much research was done on the

soybean proteins and their functional properties as a system by itself or in a mixture with other systems such as meat. The relatively low price of texturized soy protein plus the ruling by United States Department of Agriculture (Food and Nutrition Service Notice 219, 1971), that hydrated texturized vegetable protein could be substituted for not more than 30% of the weight of uncooked meat in type A School Lunch, gave great stimulus to the use of soy as a meat extender.

Very few studies were initiated on the functional properties of other sources of plant proteins such as fababean. It was well established that soy protein concentrates have a high degree of moisture absorbance, and hence a great hydration capacity. Usual hydration levels are ca. 2.5:1 (Rakosky, 1974, Carlin, Ziprin, Zabic, Kragt, Polsiri, Bowers, Rainey, Van Duyne and Perry, 1978). The dual effect of high absorption capacity and gelation tendency of plant protein explains the role added protein has in minimizing drip loss in meat products.

In a study of heat treated systems of meat and meat-plant proteins, properties measuring gelation were

shown to be highly negatively correlated with moisture loss (Hermansson and Akesson, 1975). In their study, they investigated the effect of various temperatures and plant protein levels added to a meat system on the water binding properties of the system. The effect of temperature on moisture loss was noticeable in the pure meat system at 90 C and a decrease occurred when temperatures reached 100 - 110 C. When various levels of plant proteins were added, an optimal temperature of 80 C was found to reduce moisture loss because of the gelation properties of the plant proteins. Ziprin and Carlin (1976) compared the quality of all-beef loaves with those containing 15% soy concentrates. They reported that volatile losses for all loaves were 7% but that drip losses were 12 and 6%, respectively. Conditions that may vary during thermo-plastic extrusion, especially temperature, profoundly affected the nutritive value as well as flavor, solubility and other functional properties of the textured soy product (Carlin et al., 1978). The latter group concluded that the level of texturized soy protein had a significant effect on total cooking and drip losses plus moisture and fat retention in the cooked loaves.

Other sources of protein concentrates as meat extenders were also investigated (Vaisey, Tassos, McDonald and Youngs, 1975). Inclusion of texturized fababean and field pea protein concentrates as meat extenders in beef patties is associated with increases in fat and moisture retention and concomitant decreases in drip loss. Lower fat retention in texturized plant protein - extended vs flour - extended patties, indicates physical changes within the protein structure during texturization.

It would appear from this study (Vaisey et al., 1975) that texturizing fababean and pea protein concentrates improves their performance as meat extenders, and their part in improving sensory characteristics of legume-beef blends such as: flavor, raw and cooked firmness, and apparent juiciness. Texturizing appears to bring the legume concentrates closer to the performance of beef proteins under cooking conditions. An improvement in the beef flavor, soy flavor and soy aroma, is also affected significantly by the level of soy in the loaves (Carlin et al., 1978).

Sensory Evaluation

Texture has been defined as a composite of those properties which arise from the structural elements of a food and the manner in which these register with the psychological senses (Sherman, 1970). There are three essential elements in texture; (1) texture is a sensory quality (2) texture stems from the structural parameters of the food; and (3) texture is a composite of several properties (Szczesniak, 1977). Since texture is so complex, sensory evaluation is probably the only method of obtaining reliable information on the texture of a food. The work of Szczesniak (1963), in which a proposed system for classification of the textural characteristics of food is the basis for most studies done recently in this area. Textural characteristics were classified into mechanical and geometric qualities as those related to moisture and fat content of food product. Standard rating scales for the sensory evaluation of the textured parameters were developed (Szczesniak, Brandt and Friedman, 1963a). This work was found to have several limitations (Moskowitz and Sidel, 1971), such as biaseness of judgement as a result of the reluctance of judges to use extreme categories

at both ends of the scale. A method that has been found to compensate for differences among panelists and allows each panelist to judge a sample on his own sensory perception, is known as Magnitude Estimation (Moskowitz et al., 1971). Each panelist judges the intensity of an attribute in relation to a reference sample that illustrates this attribute. The magnitude estimation is considered a simple technique to use, it requires little training and has been shown to give reproducible results (Malcolmson, 1978).

There is a growing interest in plant proteins as meat extenders, and especially new protein sources. In light of the various methods used for manufacturing and utilization of meat extenders, an indepth investigation on the effects of processing and levels of these materials used in meat systems must be carried out. Also, a study which will determine the nutritional value as well as sensory attributes of the processed food is very much needed.

METHODOLOGY

Plant protein concentrates from several plant sources were evaluated for their properties as meat extenders. Nutritional attributes, as well as sensory and functional properties of the various extruded and non-extruded plant proteins and their effects on meat systems were studied.

EXPERIMENTAL DESIGN

Three phases were planned for the study. In the first phase, texturized plant protein concentrates from various plant sources were added to freeze dried raw meat to compare the effect of the plant protein source on the Protein Efficiency Ratio (PER). Arbitrary levels of 0% and 20% and 40% of texturized soy protein concentrate (TSPC), texturized, untreated fababean protein concentrate (TUFPC), texturized treated fababean protein concentrate (TTFPC) and texturized pea protein concentrate (TPPC) were used in diet preparation.

In the second phase of the study, the plant protein concentrates were the only source of protein in preparation of rat diets (Table 1). Texturized plant

Table 1

Diets Prepared for the Study of Amino Acid Retention

Retention

Source of Protein	Form of Source
Nitrogen free diet	
Fababean protein concentrate - untreated	Powder form
Fababean protein concentrate - treated [1]	Powder form
Pea protein concentrate	Powder form
Meat - raw	Ground
Fababean protein concentrate - untreated	Texturized
Fababean protein concentrate - treated [1]	Texturized
Pea protein concentrate	Texturized
Soy protein concentrate	Texturized
Casein	

[1] Protein concentrate was extracted with ethanol-water (60:40) for vicine removal.

proteins (method of texturization in preparation of materials) were evaluated for their nutritional properties and the effect of severe thermal application on their performance. In phase two, the fecal samples were collected and used to determine the nutrient protein and individual amino acids, digestibility and retention by the rat.

In phase three, an evaluation of the effect of the protein concentrates on a finished product was done. Meat loaves were prepared with 15% and 25% of the meat substituted by equal weights of texturized protein concentrates. In the first part of this phase, the meat loaves containing the various protein concentrates were fed to rats to determine PER and nutrient digestibility (retention). In the second part of this phase, protein concentrates from five different sources were evaluated to determine their effect on sensory attributes. In addition, two methods of meat loaf processing were also assessed for the most desirable product.

DESCRIPTION AND PREPARATION OF SAMPLES

All plant protein concentrates were obtained in a powder form. Fababeans (Variety, Diana) from the Glenlea

Research Station, University of Manitoba, were dehulled, pinmilled and air classified. The high protein concentrate (FBPC) was divided into two parts. One-half of the sample was subjected to ethanol (ETOH) and water extraction. As mentioned in the literature review, Vicine and Convicine, although not considered as toxicants, are treated as anti-nutritional factors. For the purpose of evaluating any nutritional effect on the protein efficiency ratio (PER), ethanol extraction was done to remove the Vicine and Convicine from the FBPC sample.

To 4.0 kg of fababean protein concentrate, 9.6 l of distilled water and 14.4 l of ETOH were added to reach a 40:60 liquid ratio. The mixture was homogenized for five minutes to ensure complete dispersion. The well homogenized slurry was centrifuged at 15,000 x g for ten minutes in a Sorval Superspeed RC 2-B Automatic refrigerated centrifuge. The precipitate was mixed with 8 l of distilled water and 12 l of ETOH for a second extraction following the same procedure used in the first step. Wet sample was placed in stainless steel trays in a blast freezer (-30 C) for twelve hours. Ethanol was evaporated from supernatant for futher investigations. Frozen protein concentrate

was freeze-dried in a VIRTIS 10-145 MR-BA freeze drier. Duration of drying depended on the quantity of sample being dried at any one time. The dried FBC was milled in FITZMILL Model M comminuting machine. Soy protein concentrate (Nutri-soy) and pea protein concentrate were obtained from Griffith Laboratories in Toronto. Pea protein concentrate was steam treated to partially remove the bitter flavor which is characteristic to field peas.

Texturization (Extrusion) of Protein Concentrates

Texturization of treated (vicine removed) fababean concentrate, untreated fababean concentrate, pea protein concentrate and soybean protein concentrate was done in the Griffith Laboratories research section. A given sample (5 kg) with predetermined moisture content was placed in a stainless steel bowl. Water was added to bring moisture level to 23%. Mixing of ingredients was done in a Hobart blender on speed 1 for five minutes to ensure complete absorption and distribution of moisture. Extrusion was done in C.W. Brabender Type 2503 G8R extruder. Since the speed of the feeding screws and the temperature at each of the four stages are critical, the

speed and temperatures were adjusted according to the individual protein concentrate fed into the machine (Tables 2a, 2b). Extruded material was collected in polyethylene bags for storage and future use.

Meat

Meat was purchased at a wholesale outlet. Chuck and shoulder cuts from A graded steers were deboned and all visible fat removed. Meat was ground in a Hobart grinder through 1/2" plate and frozen in aluminum trays at a temperature of -30 C. Frozen meat was freeze dried in the VIRTIS 10-145 MR-BA freeze-mobile. Freeze dried meat was ground twice through a 3/16" plate to ensure complete distribution of fat which was not removed and then stored in polyethylene bags for future use.

Preparation of Samples

Diets for each phase of the study were prepared separately, prior to initiation of the phase. As previously noted, there were three rat feeding trials to evaluate nutritionally the various plant proteins. The second part of the third phase was a sensory evaluation of the plant protein extended meat loaf. All rat diets had a basal

Table 2a

Feeding Rate Setting for the Individual Protein Concentrate

Sample	RPM
Untreated fababean protein	150
Treated fababean protein	240
Pea protein	175
Soy protein	150

Table 2b

Temperature in the Four Zones for the Individual Protein Concentrate

Sample	Zone 1	Zone 2	Zone 3	Zone 4
Untreated fababean protein	90 C	150 C	160 C	180 C
Treated fababean protein	90 C	130 C	150 C	170 C
Pea protein	100 C	150 C	160 C	180 C
Soy protein	90 C	130 C	160 C	180 C

formulation (Table 3) with the variations of the plant protein source and level. Plant proteins were added to adjust protein level to ten percent (AOAC, 1975).

FEEDING TRIAL 1 - NUTRITIONAL EVALUATION OF RAW MEAT AND TEXTURIZED PLANT PROTEINS

Texturized plant protein was ground in a hammer-mill grinder fitted with a 50^{-3} inch sieve. Raw meat, texturized treated and untreated fababean protein, pea protein and soy protein were analyzed for N, ash, fat and moisture content. Protein (N x 6.25) was determined by the Kjeldahl method (AOAC, 1975). Ash, fat and moisture were determined by the final official methods (AOAC, 1975). For N analysis, 300 mg of sample were weighed and placed with the weighing paper in a Kjeldahl flask. To the sample, 10.5 g of titanium dioxide Kjeldahl mixture and 20 ml concentrated sulfuric acid were added. Samples were digested for one hour. After cooling for twelve minutes, 50 ml of tap water was added to dissolve salts and then an additional 240 ml of tap water was added. After adding 50 ml concentrated sodium hydroxide, samples were distilled for about forty minutes. Condensor tips were immersed in

Table 3

Basal Bioassay Diet (AOAC, 1975)

S =	$\frac{1.60 \times 100}{\%N \text{ of sample}}$
Oil [1] =	$8 - \frac{S \times \% \text{ ether extract}}{100}$
Salt mixture VSP =	$5 - \frac{S \times \% \text{ ash}}{100}$
Vitamin mix [2]	1%
Cellulose =	$1 - \frac{S \times \% \text{ crude fiber}}{100}$
Water =	$5 - \frac{S \times \% \text{ moisture}}{100}$

Corn starch to make 100%

Chromium oxide 0.4% (w/w)

1 Cottonseed oil was replaced by safflower oil which has similar unsaturation value.

2 Vitamin diet fortification mixture, ICN Laboratories, Cleveland, Ohio.

250 ml Erlenmeyer flasks containing 50 ml boric acid solution. When the level of the liquid in the Erlenmeyer flasks reached 200 ml, flasks were removed and titrated with dilute hydrochloric acid. Nitrogen and protein levels were calculated as follows:

$$\text{mg N} = \text{ml of Acid} \times \text{N} \times 14$$

$$\% \text{ N} = \frac{\text{mg N}}{\text{sample wt}} \times \frac{1}{10}$$

$$\% \text{ protein} = \% \text{ N} \times 6.25$$

The percent nitrogen, ash, fat and moisture were determined and diets were prepared. Ten diets were prepared for this feeding trial, utilizing four different protein sources at two levels, as well as casein and meat standard diets (Table 4). Based on the fact that a male rat consumes 18 - 21 grams of feed a day and that a trial lasts twenty-eight days, 6 kg of each diet were prepared. In each diet, a certain level of plant protein and meat complemented it to a level of ten percent. Oil, minerals, vitamins, water and starch were added as required (Table 3). All ingredients were mixed thoroughly in a Hobart mixer on speed 1 for five minutes. Chromium oxide was added at the level of 0.4% as a marker for the determination of digestibility and nutrient retention.

Table 4

Diets for Nutritional Evaluation - PER

and Nutrients

A - Casein
B - Meat
C - Meat + 20% Tex. fababean treated (Vicine removed)
D - Meat + 40% Tex. fababean treated
E - Meat + 20% Tex. fababean untreated (Vicine present)
F - Meat + 40% Tex. fababean untreated
G - Meat + 20% Tex. soy protein
H - Meat + 40% Tex. soy protein
I - Meat + 20% Tex. pea protein
J - Meat + 40% Tex. pea protein

Diets were placed in air-tight plastic containers in a cool room (4 C) for future use.

Sprague Dawley male rats were shipped to the laboratory at age twenty-one days and placed on an adaptation diet for seven days. During adaptation period, feed (rat chow pellets) and water were given ad libitum. Twenty-four hours after arrival, first signs of pneumonia were observed and anti-biotics were prescribed. Azratetra - 25 (at a level of 5 g/liter) was dissolved in tap water and replaced tap water in feeding bottles. Medication was given for a period of five days. During the adaptation periods, twelve rats died and hence, a decision was made to reduce the number of rats from twelve to eleven per group.

On the eighth day, rats were weighed and arranged in eleven groups according to weight. To randomize the rats, a rat was taken randomly from each of the eleven groups to form ten groups of eleven rats each. Average weight of rats in any one group on the day beginning the assay period, did not exceed by < 5 grams average weight of rats in any other group. Upon weighing the rats, each rat was placed in an individual stainless steel cage (24 x 17 x 17 cm) containing glass feeder (8 cm in diameter)

and a glass bottle for water. Throughout the assay period, each rat was provided with an appropriate assay diet and water ad libitum.

Rats were weighed every seven days and weights were recorded (Appendix A). Feed consumption was monitored by means of weighing and recording diets added to the individual feeders (Appendix A). Last weighing took place on the twenty-eighth day of the assay period. During the assay period, rats were under controlled temperature and duration of light periods. By automatic control, light was on for twelve hours and then off for a twelve hour period.

Fecal samples were collected from individual rats by means of nylon screens. Wooden frames with fly type nylon screen attached were divided into individual compartments, and each compartment on the screen fitted a cage. By placing the screens under the cages, the rat feces which dropped through the cage floor, fell into the screened compartment.

In the first rat assay, fecal samples were collected for the last two weeks of the period. Samples were cleaned of rat hair and feed which also fell through the cage

floor, ground to a very fine powder by the CRC Micro-mill and stored in a cool room in plastic bags.

FEEDING TRIAL 2 - AMINO ACID DIGESTIBILITY AND RETENTION

Effects of thermal application on the various plant protein concentrates were examined in phase two of the study. In four of the diets prepared for the study, the sole contribution to the protein content were; FBUTP, FBTP, PP and SP in the powder form. In the other four diets, the same sources of protein have been used but in texturized form. The remaining three diets were casein, N-free diet and freeze-dried raw meat as sources of protein (Table 1). Diets were prepared according to the official method (AOAC, 1975). All ingredients were mixed for five minutes in the Hobart mixer and stored in air-tight containers at 4 C. Rats used in the first trial, upon termination of experiment, were fed rat chow for five days. From the sixth day on, for a period of three weeks, each group of eleven rats was fed one of the diets to be evaluated. Feed and water were given ad libitum. Fecal samples were collected on the nylon screens from the fourth day of the trial for the duration of two weeks.

Feces were collected, cleaned from rat hair and feed, ground to very fine powder in CRC Micro-mill and stored at 4 C for further investigation.

FEEDING TRAIL 3 - NUTRITIONAL EVALUATION OF THE FINISHED PRODUCT

Meat loaves were prepared with four different sources of plant protein concentrate. Extruded fababean protein in the untreated and treated forms, extruded soy protein and extruded pea protein concentrates replaced 5%, 15% and 25% of the meat on a w/w basis. To evaluate the properties of oats as a meat extender, rolled oats were used to replace 15 and 25% of the meat. A comparison between the rolled oats as an elementary form of texturization vs the extrusion, which is a more elaborate method of processing and their effects on the texture of the finished product was done. As well, an evaluation of the nutritional value of the oats as a meat extender was done, in spite of the fact that it has a protein level lower than the fababean, soy and pea protein concentrates (15 - 19% protein vs 63 - 70%, 49 - 51% and 51 - 53%, respectively).

Frozen meat in the amount required to the preparations of one set of samples for the sensory evaluation panel was taken out of the freezer and thawed in the cool room at 4 C for thirty-six hours. Cubed meat was mixed well with the rehydrated texturized plant protein being sampled and ground. To ensure optimal distribution of the plant protein within the meat tissues, the meat was ground twice by passing it through a 7/16" plate and then through a 3/16" plate. Other ingredients (Table 5) were added to meat and mixed well for five minutes in a Hobart mixer. The mixture of meat, plant protein at various levels, binder and other ingredients were placed in a meat-loaf pan, 23 x 12 x 6 cm, and pressed by a ham-loaf mould to prevent air spaces within the loaves. The ham broiler, with its snug-fitting top, pressed the meat down and hence eliminated air entrapment in the loaf and manufactured a solid product. A meat thermometer was inserted in the center of each of the meat loaves to enable the measuring of the internal temperature. Meat loaf pans were placed in a pre-heated conventional electric oven, ten inches from the heating unit. The oven was pre-heated to 190 C (375 F) and meat loaves were baked to an internal

Table 5

Ingredients Used for the Preparation of Ground Meat Loaf

Ingredient	Quantity
Meat [2]	750 g
Fat	250 g
Binder [1]	85 g
Eggs	110 g (2 whole eggs)
Water	256 g
Ketchup	128 g

1 Binder used was Krispo Brand Sausage Binder, Griffith Laboratories, Toronto, containing skim milk, bread crumbs and spices.

2 When 5, 15 or 25% of meat was replaced by one of the various plant protein concentrates, quantity of meat used in the mixture was reduced by same levels respectively.

temperature of 71.6 C (161 F). Meat loaves were removed from the pans, cooled and samples were prepared for sensory evaluation.

Two processing methods were evaluated in preparation of the plant protein extended meat loaves. Since many plant protein concentrates have a distinct beany flavor, a processing method which will cover a large portion of this was sought. In the first method, the meat used for the meat loaves was mixed with the plant protein concentrates and was ground by the regular grinding method through a 3/16" plate. In the alternative method, the meat, fat, texturized plant proteins, binder and other ingredients (Table 6) were comminuted to fine particles by a Hobart model 84145 food cutter. The Hobart model 84145 food (silent) cutter has a stainless steel bowl of 35 cm diameter which rotates at a speed of 11 rpm and two blades on a shaft with rotation speed of 1750 rpm. Cubed meat, plant protein and half the quantity of the ice were placed in the bowl of the silent cutter and were comminuted for three minutes. The beef fat was added to the mixture and comminuting continued for another minute, after which the rest of the ingredients were added to the rotating bowl.

Table 6

Ingredients Used in the Preparation of Meat Loaf by the Food (Silent) Cutter

Ingredients	Quantity
Meat	3000 g
Fat	1000 g
Binder [1]	340 g
Ice	1024 g
Flavoring (ketchup)	512 g

[1] Krispo Brand Sausage Binder, Griffith Laboratories, Toronto.

Initial temperature of the mixture was -2 C to -1 C and it was measured on a regular basis until it reached a temperature of 10 C to 12 C. The emulsion was placed in the pans and baked to an internal temperature of 71.6 C.

Two methods of cooking the fine emulsion were tried and evaluated by a panel as to the total acceptance of the finished product. In the first method, the emulsion was baked in a conventional electric oven. In the second method, the fine emulsion was placed in the ham broiler, the snug-fitting top was pushed down and secured in place by ratchet catch at each end. The ham broilers were placed on a rack in steam kettles and set at 75 C for one hundred and thirty five minutes. Temperature was maintained constant by a Honeywell temperature controller and a thermocouple placed in the kettle.

Since the results of the preliminary sensory evaluation of the products indicated that the majority of the untrained panelists preferred the ground meat over the fine emulsioned product, attention was focused on the former. The meat loaves prepared by the silent cutter either boiled or baked, were preferred as cold cuts, while

the ground meat loaves were preferred hot.

SENSORY EVALUATION OF THE FINISHED PRODUCT

Fifteen untrained panelists participated in the initial stage of the sensory evaluation. The panelists, all of whom were students, graduate students and staff of the Department of Food Science, were evaluating samples of meat loaves processed by various methods (Table 7). Total acceptability of the products was determined by the nine point hedonic scale (Table 8). Each group of five panelists was evaluating four samples and each of the samples was evaluated both cold and hot. The cold cuts were kept in airtight containers for a period of twelve hours at 4 C before being tested. The hot samples were heated for forty-five seconds in a Panasonic microwave oven, prior to being served to the panelists. The samples were served in 2 mm thick slices. Distinct preference was shown toward the boiled product served over a cold cut and to the ground product being served hot (Table 8a).

Table 7

Description of Samples Used in Selection of Preferred Products

Method of Preparation	Method of Cooking	Level of Plant Protein (%)
S.C.[1] - F.E.[2]	H.B.[3]	0
S.C. - F.E.	H.B.	30
S.C. - F.E.	B.	0
S.C. - F.E.	B.	30
S.C. - F.E.	H.B.	0
S.C. - F.E.	H.B.	30
G.M.[4] - R.G.[5]	B.[6]	0
G.M. - R.G.	B.	30
S.C. - F.E.	H.B.	0
S.C. - F.E.	H.B.	30
G.M. - R.G.	B.	0
G.M. - R.G.	B.	30

1 S.C. - Silent Cutter

2 F.E. - Fine Emulsion

3 H.B. - Ham-Boiler

4 G.M. - Ground Meat

5 R.G. - Regular Grind

6 B. - Baking

Table 8

Questionnaire for Hedonic Scale

Taste the following samples and check how much you like or dislike each one.

	Code No.	Code No.	Code No.	Code No.
Like Extremely				
Like very much				
Like Moderately				
Like Slightly				
Neither Like nor Dislike				
Dislike Slightly				
Dislike Moderately				
Dislike Very Much				
Dislike Extremely				

Table 8a

Preference of Sample Being Processed in

Various Methods

Product (Method of Preparation)	No. of Panelists Preferred Cold	No. of Panelists Preferred Hot
S.C. - F.E. H.B.	11	4
S.C. - F.E. B.	13	2
G.M. - R.G. B.	4	11

Panel Training and Parameter Selection

A seven member panel consisting of graduate students and staff members of the Department of Food Science, was selected to participate in the study on the basis of their interest in the project and availability to perform as panelists. The first two sessions were conducted in a conference room at a large table to promote group discussion. The other two sessions were conducted in a sensory panel lab to familiarize the panelists with proper work habits during the sessions. Four training sessions were held to acquaint panelists with the method of magnitude estimation and to establish and clarify suitable parameters and definitions to be used in evaluating the samples.

During the first two training sessions, panelists were asked to rate a set of standards and definitions chosen from the literature for appropriateness (Table 9). Some of the definitions were adapted from Szczesniak (1963). Panelists were presented with a list of definitions (Table 9), a set of standards and a ballot. After completing the evaluation, the results were discussed as a group.

Table 91

Definitions Chosen to be Used for Evaluating Samples

Flavor (Hay - Beany): The sensation of a beany (bitter) taste in the mouth or throat during chewing and swallowing of the sample.

Juiciness: The amount of moisture in the mouth after seven chews between the molar teeth on a 1.2 cm cube of sample.

Firmness (Toughness): The force required to compress a 1.2 cm cube of sample between the molar teeth.

Chewiness: Chewing with a uniform force at a uniform rate and counting the number of chews to prepare the sample for swallowing.

Mouthcoat: Mouth or throat coating - degree of film perceived in the mouth or throat after swallowing.

Grittiness: Existence of particles relatively harder than the surrounding medium.

Spiciness: The sensation of spiciness in the mouth or throat during chewing and swallowing. List in order of intensity from low to high.

Total Acceptability: Rate the sample as to the degree of acceptability. (Would you like to consume this product frequently, not so often, or not at all?)

For the remaining two sessions, experimental samples were evaluated using the method of magnitude estimation. Panelists were presented with a revised ballot and with samples containing various levels of soybean and fababean protein concentrates. After each session, discussion of the results took place. As a result of the four sessions, two of the panelists showed inability to detect the beany (bitter) flavor in the samples and results of only five of the panelists participated were compiled and computed.

The number of parameters to be evaluated was increased from six to eight, since parameters given initially were not specific enough in regard to some attributes. Table 10 shows the initial six parameters and the final eight after some changes.

Preparation of Samples for Sensory Testing

There were five sources of plant protein used as meat extenders at three different levels (Table 11). Meat loaves containing the various levels of plant material were prepared several hours prior to each of the sessions and placed covered on a counter-top to cool. The crust of the meat loaves was removed to prevent variations

Table 10

Changes in Parameters During Training Sessions

Original Parameters	Final Parameters
Flavor (Hay - Beany)	Flavor (Hay - Beany)
	Spiciness
Juiciness	Juiciness
Firmness (Tenderness)	Firmness (Toughness)
Chewiness	Chewiness
Mouthfeel	Mouthcoat
	Grittiness
Total Acceptability	Total Acceptability

Table 11

The Sources of Plant Protein and Level of Application

Source of Protein	Level of Substitution (%)
Fababean - treated (Ethanol extracted) protein concentrate	5, 15, 25
Fababean - untreated protein concentrate	5, 15, 25
Soybean - protein concentrate	5, 15, 25
Oats - rolled flakes	5, 15, 25
Pea - protein concentrate	5, 15, 25
Meat	0

between pieces obtained from the same sample. The meat loaves were cut into 1.2 cm cubes on the Hobart meat slicer and five cubes of each sample were placed in coded 3.5 ounce water cups (Lily No. 450).

A standard procedure for preparing samples was established during the training session and was followed throughout the study. Since samples were cooled, each of the trays containing four coded cups was placed in a Panasonic microwave oven and samples were warmed for thirty-five seconds.

Sensory Testing

Eight texture and flavor parameters were assessed for a total of twenty samples, using the definitions developed during the training period. In each of the three replications, panelists received a total of twenty samples. For the evaluation of the eight parameters, each sample consisted of five cubes 1.2 cm^3 and each set of samples consisted of four samples presented to the panelists in a randomized order (Table 12).

All panel sessions were held in an air-conditioned, relatively sound-proof sensory panel room. Each panelist

Table 12

Randomized Ordering for Three Replications Used for the Sensory Evaluation

	Sample	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Panelist 5
Rep 1	Soy	3, 4, 2, 1	4, 2, 1, 3	1, 3, 2, 4	4, 3, 1, 2	3, 2, 4, 1
	Oat	4, 3, 2, 1	3, 2, 1, 4	3, 2, 4, 1	2, 1, 3, 4	4, 1, 2, 3
	Faba-T	1, 3, 4, 2	1, 4, 2, 3	4, 1, 3, 2	3, 4, 2, 1	2, 3, 1, 4
	Pea	2, 1, 4, 3	2, 1, 3, 4	2, 4, 1, 3	1, 2, 4, 3	3, 1, 4, 2
	Faba-U	3, 2, 1, 4	3, 4, 2, 1	4, 3, 2, 1	2, 4, 3, 1	1, 3, 2, 4
Rep 2	Faba-T	4, 3, 1, 2	4, 3, 2, 1	1, 4, 2, 3	4, 2, 1, 3	4, 2, 1, 3
	Pea	1, 3, 2, 4	1, 3, 2, 4	3, 2, 1, 4	3, 1, 2, 4	2, 4, 3, 1
	Soy	2, 1, 3, 4	2, 4, 1, 3	2, 1, 4, 3	1, 4, 3, 2	1, 2, 4, 3
	Faba-U	4, 1, 2, 3	1, 2, 4, 3	1, 3, 4, 2	4, 1, 2, 3	3, 4, 1, 2
	Oat	1, 2, 4, 3	4, 3, 2, 1	4, 2, 3, 1	3, 2, 1, 4	1, 4, 2, 3
Rep 3	Pea	3, 2, 4, 1	4, 2, 3, 1	1, 4, 3, 2	2, 3, 4, 1	4, 3, 1, 2
	Faba-U	2, 3, 1, 4	2, 1, 4, 3	3, 1, 2, 4	1, 3, 2, 4	2, 1, 3, 4
	Oat	2, 4, 3, 1	2, 3, 4, 1	2, 3, 4, 1	4, 2, 3, 1	4, 2, 1, 3
	Soy	3, 1, 4, 2	1, 2, 3, 4	4, 2, 1, 3	3, 4, 1, 2	3, 2, 1, 4
	Faba-T	1, 4, 2, 3	3, 2, 1, 4	3, 4, 1, 2	1, 2, 3, 4	2, 4, 3, 1

1 - 0% plant protein
2 - 5% plant protein
3 - 15% plant protein
4 - 25% plant protein

was seated at a well-lighted booth and was provided with a set of coded samples, water, plus unsalted crackers for rinsing, and a ballot (Table 13). Panelists were instructed to assign a value to the first sample and a corresponding ratio to each sample evaluated. If a parameter could not be perceived in the sample, panelists were asked to score "not present" (NP).

ANALYSTS OF DATA

Since panelists were unconstrained in their choice of scale units, the values they assigned to the various treatments contributed a large proportion of the variance among their judgements. These variations among panelists could interfere in determining the actual variability due to differences in treatment effects. In order to achieve a true ratio of differences between samples without affecting the information, the values assigned by the panelists must be reduced and brought to the same basis. Magnitude estimation data were normalized by calculating the geometric mean for each panelist's estimates and dividing each panelist's estimates by their geometric mean. Since samples with zero percent plant protein (100% meat) were used in each of the tests, the samples

Table 13

Questionnaire for Sensory Evaluation of Meat Loaves

Magnitude (Ratio) Estimation

Name_____ Date_____

Evaluate the following sensory dimensions of the product using magnitude estimation. In magnitude estimation, the first sample is given a value relative to the perceived strength of the particular attribute. Each of the following samples are evaluated in relation to the first (which is the reference). If the second sample seems three times as tender or juicy as the reference, give it a value three times larger. If it seems 1/7th as tender or juicy, give it a value seven times smaller. PLEASE EVALUATE THE SAMPLES IN THE ORDER IN WHICH THEY APPEAR ON THE BALLOT. SWALLOW ONLY THOSE SAMPLES AS STATED IN THE DIRECTIONS. RINSE BETWEEN EACH SAMPLE.

A. Flavor (Hay-Beany) - is the sensation of a beany (bitter) taste in the mouth or throat during chewing and swallowing of the sample.

1.	Code No.	Ratio Estimation Value
1.		
2.		
3.		
4.		
5.		
6.		

B. Spiciness - Estimate the sensation of spiciness in the mouth or throat during chewing and swallowing. List in order of intensity the spices you perceived.

Code No.	Ratio Estimation	Spice Perceived
1.		
2.		
3.		
4.		
5.		
6.		

C. Juiciness - is the amount of moisture in the mouth after seven chews between the molar teeth on a 1.2 cm cube of sample.

Code No.	Ratio Estimation Value
1.	
2.	
3.	
4.	
5.	
6.	

D. Firmness (Toughness) - is the force required to compress a 1.2 cm cube of sample between the molar teeth. (Assign LOWER values to MORE TENDER samples).

Code No.	Ratio Estimation Value
1.	
2.	
3.	
4.	
5.	
6.	

E. Chewiness - is chewing with a uniform force at a uniform rate and COUNTING the NUMBER OF CHEWS to prepare the sample for swallowing.

	Code No.	No. of Chews
1.		
2.		
3.		
4.		
5.		
6.		

F. Mouthcoat - mouth or throat coating - degree of film perceived in the mouth or throat after swallowing.

	Code No.	Ratio Estimation Value
1.		
2.		
3.		
4.		
5.		
6.		

G. Grittiness - existence of particles relatively harder than the surrounding medium.

	Code No.	Ratio Estimation Value
1.		
2.		
3.		
4.		
5.		
6.		

H. Total Acceptability - Rate the sample as to the degree of acceptability, considering all attributes required for an acceptable product. (Would you like to consume this produce frequently, not so often, not at all, etc.)

Code No.	Ratio Estimation Value
1.	
2.	
3.	
4.	
5.	
6.	

REMARKS: WRITE ANY CONSTRUCTIVE REMARKS OR IDEAS REGARDING THE SAMPLE.

containing no plant material were used as standards. In the analysis of the data, the samples containing three levels of various plant proteins were compared to the standard sample. A value, 1/8 the lowest value assigned for the same parameter by the panelist, replaced the notation "NP" for the purpose of computing the geometric mean. Analysis of covariance was used to assess differences among treatments.

Bioassay Number Three - Finished Products

Twelve diets were evaluated for their protein quality and nutrient availability, five different sources of plant protein were used at two levels as meat loaf extenders (Table 13a). Meat loaves were prepared with two levels of plant protein as meat extenders. Since replacement of 5% of the meat by plant protein will not affect the rat growth to any large extent, 15% and 25% were the levels of replacement which were chosen arbitrarily. The Food and Drug Administration of the United States, regulatestthe level of meat extender to be used in the United States in meat products not to exceed 30%. Since there are no studies available on the

Table 13a

Diets Evaluated for Protein Quality (PER) and Nutrient Availability

Source of Protein	Level of Plant Protein (%)
Casein - reference diet	
Meat -	0
Meat + Fababean - Treated (Ethanol extracted)	15%
Meat + Fababean - Treated	25%
Meat + Fababean - Untreated (Vicine present)	15%
Meat + Fababean - Untreated	25%
Meat + Soybean protein concentrate	15%
Meat + Soybean protein concentrate	25%
Meat + Pea protein concentrate	15%
Meat + pea protein concentrate	25%
Meat + Oat - rolled flakes	15%
Meat + Oat - rolled flakes	25%

utilization of the plant proteins under study as meat loaf extenders, the aim was not to exceed the present limit but to evaluate performance within the accepted levels.

Meat loaves were prepared following the procedures previously used to prepare the samples for sensory evaluation. Meat loaves were cubed, frozen in airtight containers and freeze-dried in the VIRTIS 10-145 MR-BA freeze-mobile. Dried samples were analyzed for nitrogen, fat, moisture, crude fiber and ash content. Nitrogen content of each of the samples dictated the quantity to be used in the preparation of the diets. All diets were brought to the isocaloric and isonitrogenous point with protein content of 10%. Since some of the diets contained fat levels above the suggested 8% level (AOAC, 1975), the fat contents of all diets were elevated to 10%. Six kilograms of each of the diet were prepared, mixing all the ingredients (Table 5) by the Hobart mixer for five minutes. Diets were stored in airtight containers at 4 C and small quantities were removed at frequent intervals, weighed and then fed to the rats.

Sprague Dawley male rats, twenty-one days old, were

placed three in a cage for seven days of adaptation period. During adaptation period, signs of pneumonia appeared and antibiotics were added to the drinking water for the twenty-eight davs of test period.

By randomization, the rats were divided into twelve groups with eleven rats in each one. Average weight of the rats in any one group on the first day of assay period did not exceed by $\geq$5 grams average weights of other groups. Rats were placed in individual 24 x 17 x 17 cm stainless steel cages and feed plus water were given ad libitum. Rats were weighed every seven days and weights were recorded for PER determinations (Appendix B). On the second week of the assay period, screens were placed under cages to collect fecal samples for chemical analysis. The experiment was terminated after twenty-eight days.

CHEMICAL ANALYSIS OF FEED AND EXCRETA

Determination of Chromic Oxide

Chromic oxide (Cr_2O_3) was used as a marker in the diets to determine digestibility (retention) of nutrients by the assay animals. Since chromic oxide will not be absorbed through the digestive tract and will be completely

excreted, it is widely used for availability and retention studies. Chromic oxide at a level of 0.4% was added to all diets (Table 1). Proper mixing ensured complete distribution and consumption by rats. Determination of chromic oxide was done on feed and fecal samples by Atomic Absorption Spectrophotometry (Williams et al., 1962).

Amino Acid Analyses

Diet and fecal samples were analyzed for their amino acid composition. Analysis was done on the Beckman model 119C amino acid analyzer. Samples (50 mg) were hydrolyzed with 6N hydrochloric acid (HCl) for sixteen hours at 121 C for all amino acids except cystine and methionine with a total run time of one hundred and forty minutes. Cystine and methionine were oxidized to cysteic acid and methionine sulfone, respectively, with performic acid for twenty hours and then hydrolyzed following the regular procedure. All results were recorded as percent amino acid. Tryptophan was determined by a spectrophotometric method (Messineo et al., 1972).

Vicine Determination

It has been established that vicine, a favism inducing compound, occurs naturally in seeds of certain legumes and in particular, in the fababean (Jamalian et al., 1976). Removal of the vicine was done by ethanol - water (60:40) extraction. Determination of the vicine content in the fababean protein concentrate before and after ethanol extraction, as well as in the extract, was done by the titanium tetrachloride ($TiCl_4$) method (Ismail, Eskin and Hoehn, 1979). The sample (10 g) was extracted with 100 ml trichloracetic acid 3% (w/v) in three phases, 50 ml, 25 ml and 25 ml, respectively, with centrifugation at 15,000 x g for ten minutes. The supernatant was then passed through 17 g of aluminum oxide in a glass funnel. Aliquots of 0.2 - 0.5 ml of supernatant plus 5 ml concentrated HCl were hydrolyzed at 80 C for 1.5 minutes, 0.8 ml of $TiCl_4$ concentrate was added to each sample and a reading was taken at 480 nm.

Functional Properties

One of the problems in meat processing on a large scale in industry, as well as in home preparation, is the drip loss. During the preparation of the finished product,

there is a weight reduction through a loss of fat and water. To evaluate the effect of texturized plant protein in retaining fat and water in the meat loaves, analysis of meat loaves for drip loss was done. Drip from meat loaves was collected and analyzed for fat and water content.

RESULTS AND DISCUSSION

Protein Efficiency Ratio (P.E.R.)

Ten diets (Table 4) were fed to rats, eleven rats in each test group for twenty-eight days, with casein as a reference diet. The PER value for the casein diet, which was obtained by the division of the mean weight gain by the mean protein intake, was corrected to a value of 2.5. The same correction factor was used in the other nine diets to obtain adjusted values. Figure 1 shows the PER values obtained for the ten diets. The diets containing 20% plant protein concentrates, showed a better performance than those containing 40%. The diet consisting of 100% meat (no replacement by any level of plant protein) showed a superior position among the ten diets in nutritional contribution to the rat growth. From the PER histogram (Figure 1) and the LSD (least Significant Difference) figures in Table 15, it can be seen that when meat and casein were the sole source of protein in the diet, there was a significant difference in their nutritional performance in rat bioassay. The PER value of the diet containing 20% texturized soybean protein,

Figure 1

Protein Efficiency Ratio Values - Bioassay No. 1

Legend

S - Soy protein

F.T. - Fababean protein, ethanol extracted

F.U. - Fababean protein, unextracted

O - Rolled oats

P - Pea protein

20 - 20% of meat was substituted by texturized plant protein

40 - 40% of meat was substituted by texturized plant protein

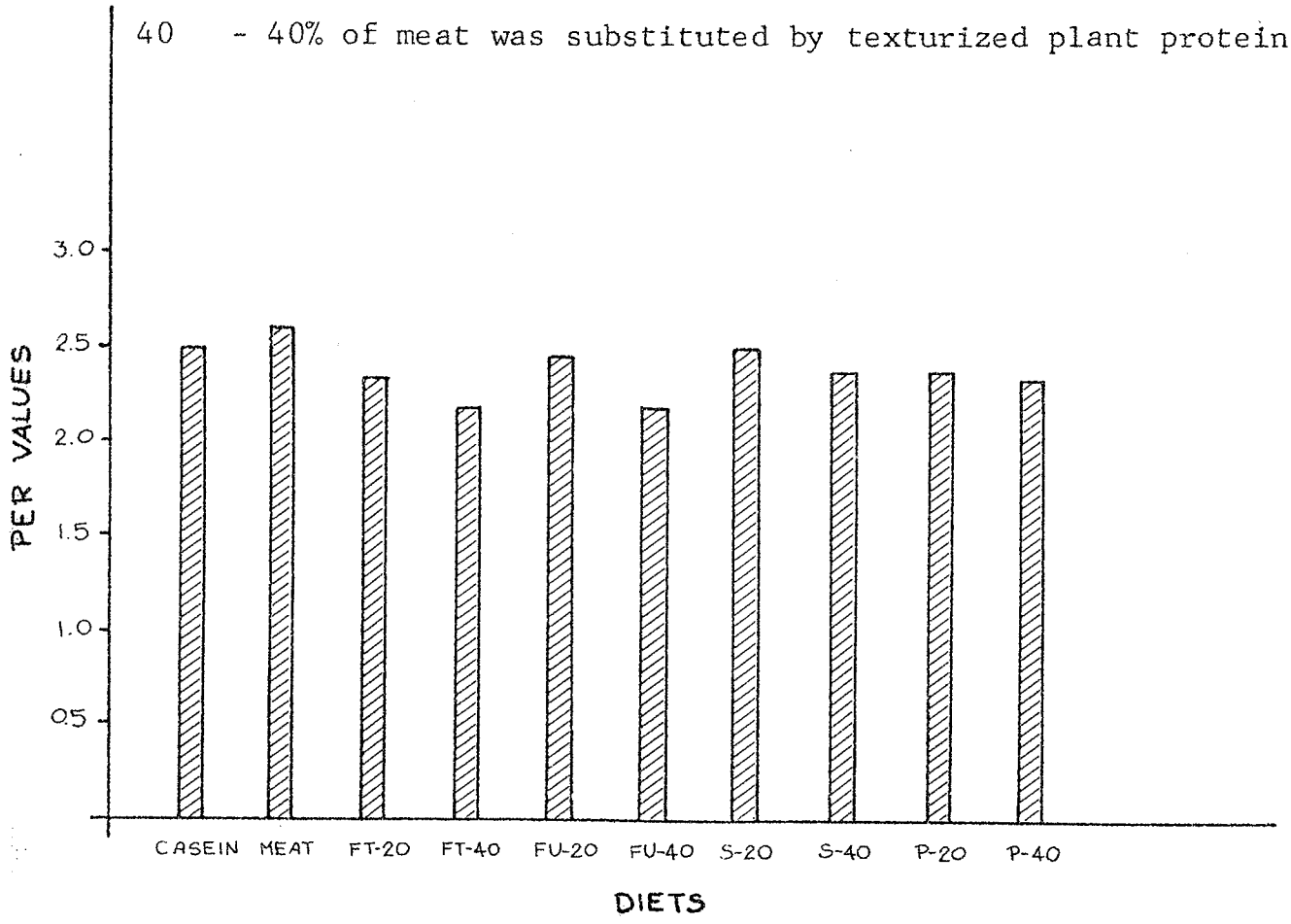


Table 14

Analysis of Variance for the Protein Efficiency Ratio - Raw Materials

Source	DF	SS	MS	F
Diet	9	2.881	0.320	7.926 *
Rats	10	0.808	0.081	2.000
Explained	19	3.689	0.194	4.807
Residual	90	3.635	0.040	
Total	109	7.324	0.667	

* Significantly different ($p < .05$)

Table 15

Significant Differences Between Diets - Raw Materials

Diet	Adjusted PER Value [1]
Meat	2.60 a
Casein	2.50 ab
Soy 20%	2.49 ab
F.B.U. 20%	2.46 b
Pea 20%	2.40 b
Soy 40%	2.40 b
F.B.T. 20%	2.36 bc
Pea 40%	2.34 bc
F.B.U. 40%	2.19 cd
F.B.T. 40%	2.17 d

[1] Treatments with the same letter are not significantly different ($p < .05$).

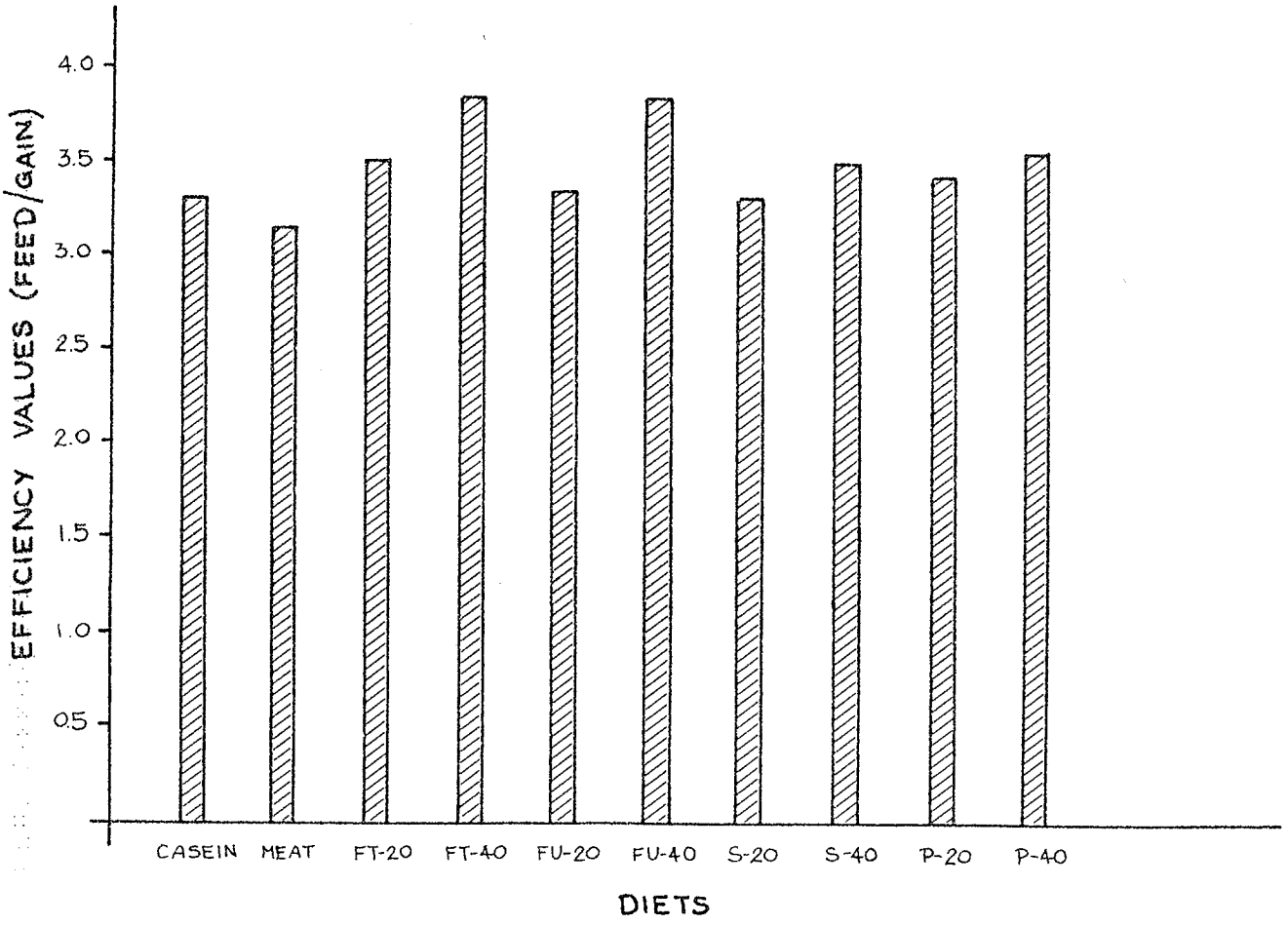
although significantly better than other diets, has lower values than those obtained for meat and casein. It is clear that the replacement of 40% of the meat by an equal amount of plant protein, is a limiting factor in the contribution of the diet to the body's requirement for maintenance and growth.

Since untreated fababean protein in both 20% and 40% level was found to enhance weight gain better than the treated supplement, reduction in biological value of the diet, as a result of ethanol extraction would be expected.

Mean feed intake value was divided by the mean weight gain value to determine the diet efficiency for each of the ten diets. Figure 2 demonstrates the efficiency of the ten diets with the lower values indicating higher efficiency. With the meat diet the most efficient, and the diets containing 20% plant protein significantly more efficient than those with 40% of the meat substituted, it was evident that greater the amount of meat replaced by plant protein in a diet, the lower its biological value. It was also evident from Figures 1 and 2, that the diet efficiencies were proportionally inverse to the protein efficiency ratios.

Figure 2

Diet Efficiency [1] - Bioassay No. 1



1 Diet efficiency values were obtained by feed intake over weight gain.

Since during the processing of meat products either on a commercial scale or a much smaller home preparation, there are other ingredients being added to the finished product which will alter their chemical composition. The PER and DE values obtained from the second bioassay (Table 13a) were more meaningful from the standpoint of human nutrition.

The twelve diets were fed to rats for twenty-eight days and diet intake and weight gain were recorded (Appendix B). Figure 3 indicates that in spite of the heat treatment of 190 C for approximately seventy-five minutes, the biological value of the diet increased. Supplementation of the diets with eggs and binder, largely eliminated the effect the limiting amino acids have on the digestibility and retention of the amino acids. All diets, except soy-15, performed better than the casein and unlike values in Figure 1, there were no significant differences between the two levels used from each plant protein source. The only exceptionswere the two diets containing 15% and 25% untreated fababean, which gave very high PER values. The significant difference between the two treatments (Table 17),

Figure 3

Protein Efficiency Ratio Values - Bioassay No. 2

Legend

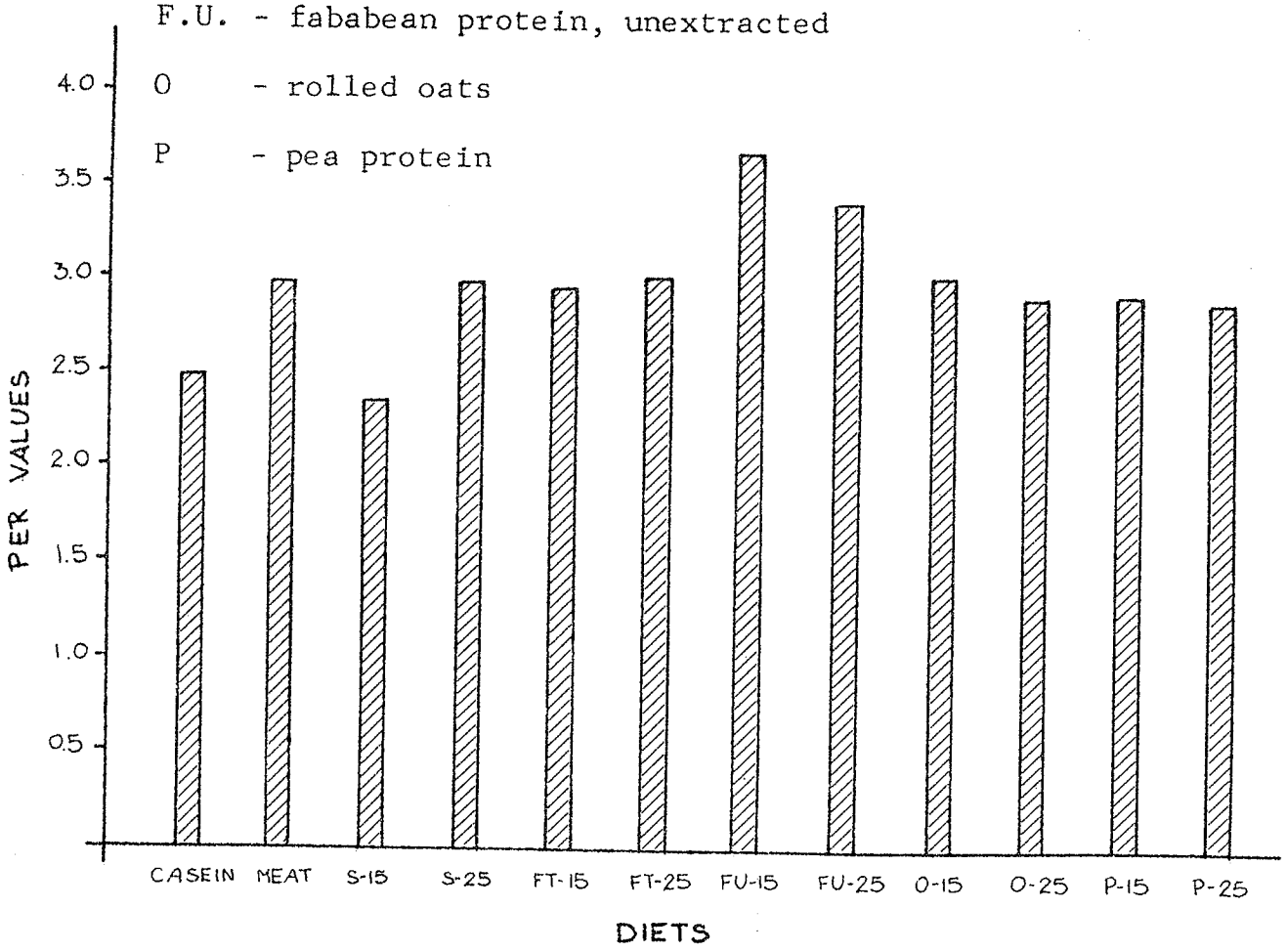
S - soy protein

F.T. - fababean protein, ethanol extracted

F.U. - fababean protein, unextracted

O - rolled oats

P - pea protein

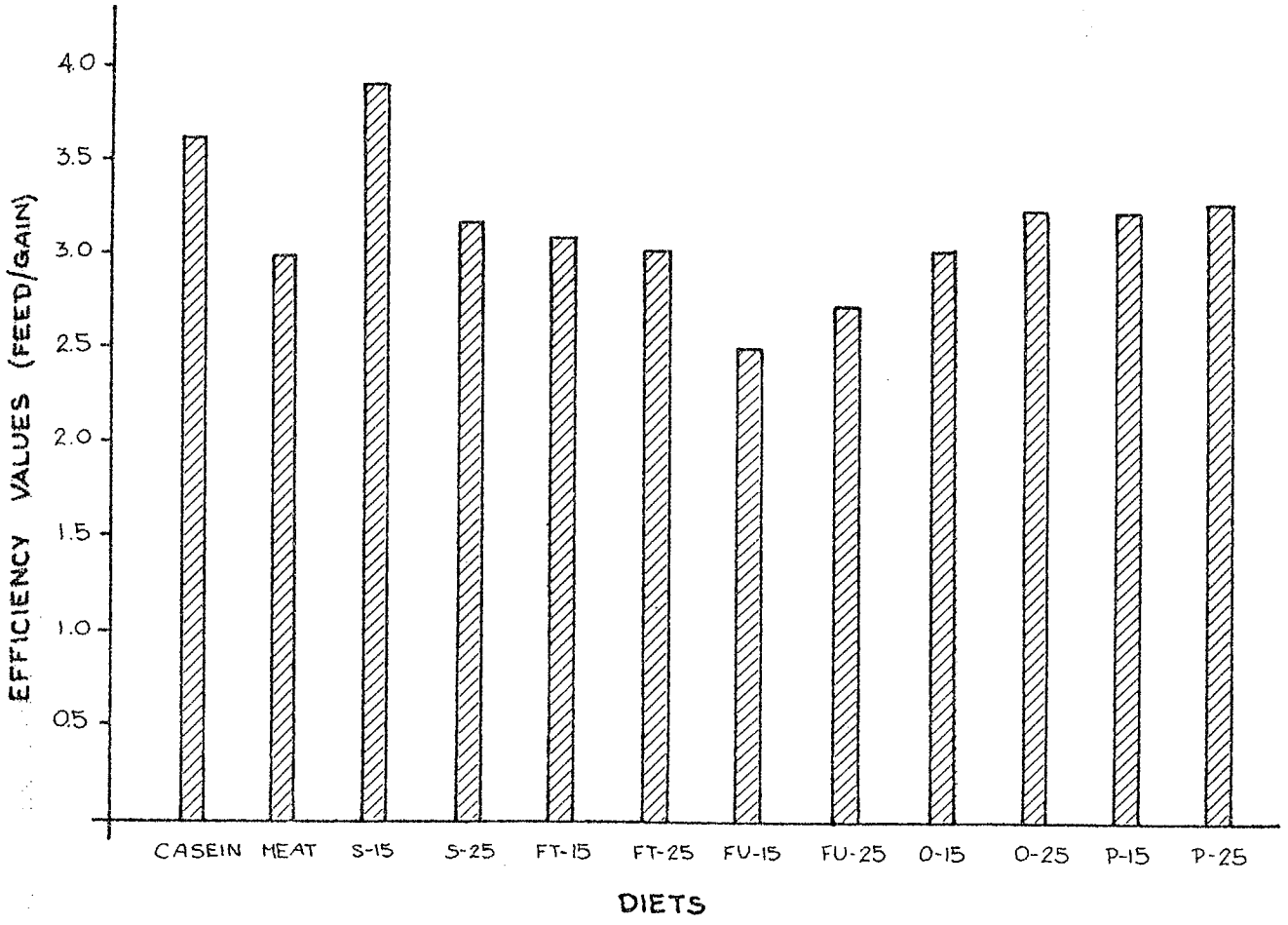


15 - 15% of meat has been substituted by texturized plant protein

25 - 25% of meat has been substituted by texturized plant protein

Figure 4

Diet Efficiency [1] - Bioassay No. 2



[1] Diet efficiency values were obtained by feed intake over weight gain.

Table 16

Analysis of Variance for the Protein Efficiency Ratio - Finished Products

Source	DF	SS	MS	F
Diets	11	18.570	1.688*	9.698*
Rats	10	1.287	0.129	0.739
Explained	21	19.857	0.946	5.432
Residual (Error Mean)	110	19.149	0.174	
Total	131	39.005	0.298	

* Significantly different ($p < .05$).

Table 17

Significant Differences Between Diets - Finished Products

Diet	Adjusted PER Value [1]
F.B.U. 15%	3.67 a
F.B.U. 25%	3.37 b
Meat	2.99 c
F.B.T. 25%	2.98 c
Oats 15%	2.95 c
Soy 25%	2.88 c
F.B.T. 15%	2.88 c
Pea 15%	2.81 cd
Oats 25%	2.81 cd
Pea 25%	2.77 cd
Soy 15%	2.74 cd
Casein	2.50 d

1 Treatments with the same letter are not significantly different ($p < .05$).

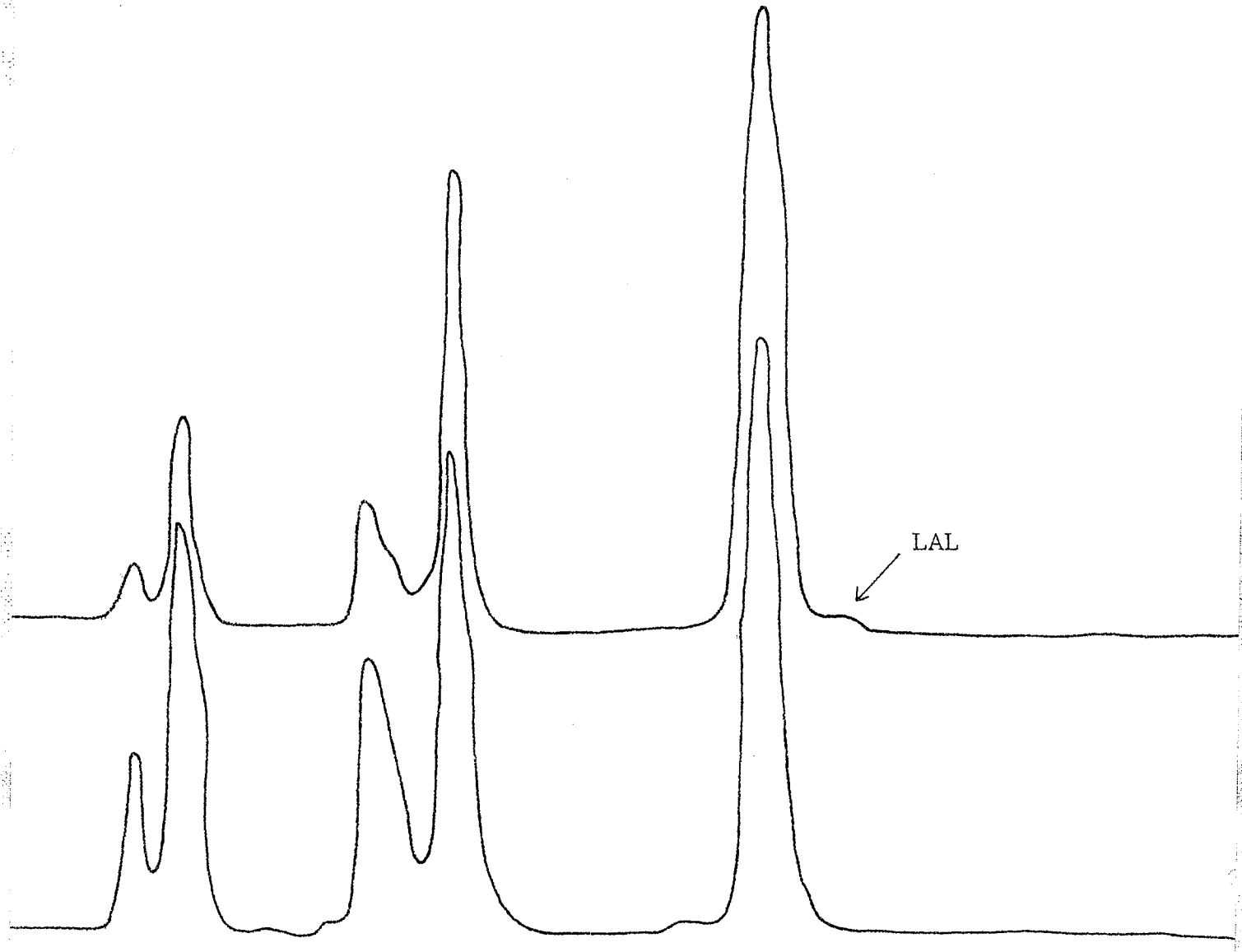
indicates that improvement in the biological value of the diet as a result of a reduction in plant protein level, could be expected. It is obvious that there is a correlation between level of plant protein in the diet and the biological value of same diet, but from Figure 3, it can be seen that all diets (with one exception) performed the same as the 100% meat. An extended meat product with up to 25% plant protein, will satisfy all the requirements for a positive nitrogen balance, as long as diets are consumed in the optimal quantities. The fababean protein concentrate from which Vicine and Convicine have been removed by ethanol extraction, show a lower protein efficiency ratio than the Vicine containing fababean in both bioassays.

In the third rat experiment, antibiotics were administered to the rats for the entire test period. Although during the first few days of the assay, feed consumption was low, rats showed signs of recovery. From the data accumulated, a conclusion cannot be drawn as to the effect the antibiotics had on feed consumption and rate of growth of test animals.

The fababean diets consisted of extracted and not extracted protein concentrates in powder and extruded forms. Ethanol extraction was done twice and extrusion was a high heat treatment. It is probable that by ethanol extraction, some free amino acids and low molecular weight protein were washed away. The heat treated fraction is lower in most essential amino acids and it is conceivable that chemical changes occurred with the formation of new compounds, not available for utilization. One of the compounds that might have evolved in the heat treated (extruded) fababean, is lysinoalanine (LAL). Figures 5 and 6 show an apparent increase in LAL with the formation of a new peak and its increase with the increase of plant protein level in the diet. In similar work with fababean protein being autoclaved before ethanol extraction, there was no evidence of amino acid loss by washing (Eskin and Atwall, 1979). The heat denatured (bound) the proteins and hence limited their washing off and loss. In the present study, heat treatment was applied to the fababean proteins after the extraction phase and it is possible that more nutrient loss occurred at that stage.

Figure 5

Lysinoalanine in Diets Containing Treated and Untreated Fababean at a Level of 15%

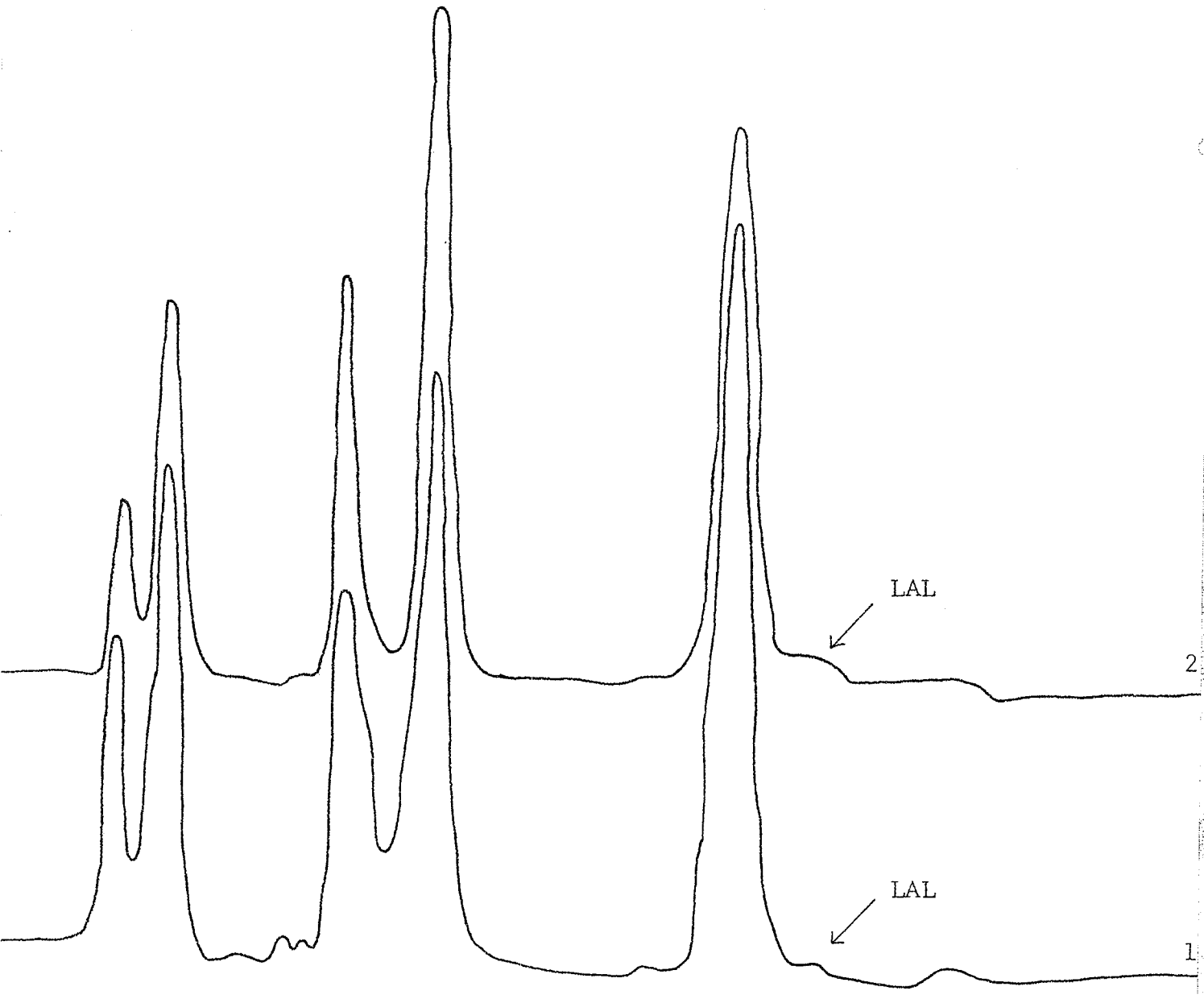


1 Untreated fababean protein concentrate.

2 Treated fababean protein concentrate.

Figure 6

Lysinoalanine (LAL) in Diets Containing Treated and Untreated Fababean at a Level of 25%



1 Untreated fababean protein concentrate.

2 Treated fababean protein concentrate.

A standard Protein Efficiency Ratio test continues for twenty-eight days, which is a long period of time if biological values of diets are to be evaluated on an ongoing process. A much more rapid method (Alsmeyer, Cunningham and Happich, 1974) was examined to determine the usefulness of such a method in further research. Table 18 compares the experimental PER values with the estimated ones. Prediction of the protein efficiency ratio from amino acid analysis, was done by using the following equation:

$$PER = 1.816 + 0.435\ (MET) + 0.780\ (LEU) + 0.211\ (HIS) - 0.944\ (TYR)$$

The predicted values were consistently lower than the experimental values and hence, it is conceivable that there was limited or no effect on the biological value of the diets by inhibitors or anti-nutritional factors. A method of predicting the biological value of a food which contains a single source of protein or a complex of sources complementing each other nutritionally, could be of much benefit to food research and the industry. By obtaining the chemical composition of the protein source, a manipulation in the level of supplementation in order

Table 18a

PER Values Obtained vs Predicted Bioassay No. 1

Diets	PER Observed	PER Predicted
Casein	2.5	2.5
Meat [1]	2.60	2.23
Meat + 40% F-T	2.17	2.10
Meat + 40% F-U	2.19	1.97
Meat + 40% Soy	2.40	2.11
Meat + 40% Pea	2.34	2.17

[1] Ground, freeze dried meat.

Table 18b

PER Values Obtained vs Predicted Bioassay No. 2

Diets	PER Observed	PER Predicted
Casein	2.5	2.50
Meat	2.99	2.53
Meat + 15% Soy	2.74	2.34
Meat + 25% Soy	2.88	2.40
Meat + 15% F-T	2.89	2.72
Meat + 25% F-T	2.98	2.57
Meat + 15% F-U	3.67	3.09
Meat + 25% F-U	3.37	3.13
Meat + 15% Oats	2.95	2.40
Meat + 25% Oats	2.81	2.07
Meat + 15% Pea	2.81	1.98
Meat + 25% Pea	2.77	2.00

to alter levels of some of the amino acids can be done to obtain the best combination which will result in the highest possible PER or the best biological value for the diet in question. Errors in the prediction of the PER could be attributed to differences in the digestibility of various tvpes of proteins and the fact that the amino acid profiles of the supplemented meat proteins may vary considerably from the pure meat diet. For instance, beans are high in tyrosine, an amino acid of great importance in the PER prediction and the levels of beans added to the meat influences the results of experimental vs predicted biological values.

Using the equation for predicted PER, a combination of meat and any one of the four sources of plant proteins was examined to obtain the optimal biological value. From the results obtained from mixtures containing only meat and plant proteins, it is apparent that PER values will not change significantly when plant proteins supplemented meat at the levels of 10 - 30%. When PER was predicted for processed meat loaves incorporated into rat diets, values obtained were slightly higher than those obtained from the raw materials. Although it has been

shown that heat treatment will have some effect on the level of labile amino acids, the supplementation, plus the interrelation effect of the amino acids as a complex diminish the negative effect of processing and will improve the biological value measured by rate of growth. The untreated fababean protein proved to be a superior source of protein among the four diets used in the study when PER values were predicted. This fact substantiates the results obtained from the feeding trials conducted throughout the present work.

Many nutritionists are concerned mainly with the limiting amino acids when the question of biological value arises. Legumes are known to be low in sulfur containing amino acids and attention is being paid to their effect on the wholesomeness of the food. From the equation for predicting PER, a new point arises, the negative relationship between tyrosine and the PER values. Higher levels of tyrosine will bring about a reduction in the nutritional value of the protein source. Legumes contain a high level of tyrosine and an elevation of plant protein content of a diet when used as meat extender, will initiate a decrease in protein utilization. When a mixture of pea

protein and meat were evaluated for the effect of tyrosine on the predicted PER values, various levels of tyrosine were used in the equation and from Table 18c, it is apparent that tyrosine has an adverse effect on the PER.

The fact that not only limiting amino acids, but other amino acids as well, will influence growth rate, should direct both nutritionists and plant breeders in their search for plants which will provide the optimal amino acid profile. Tyrosine should be one of the amino acids to which full attention must be given in future work,

Amino Acid Analysis

It has been well established that not all proteins are equally balanced in their amino acid composition, and hence not equally beneficial in meeting nutritional requirements. One of the major causes for differences in the efficiency of utilization of proteins, is the fact that some amino acids are present in lower levels than is required for balanced amino acid profile. The amino acid found to be present in the smallest proportion relative to that in the reference diet, is the limiting amino acid, and protein will be utilized to the level of its limiting amino acid. In most legumes,

Table 18c

The Effect of Tyrosine on Predicted PER Values

in Meat - Pea Protein (PP) Mixture

Level of Meat (%)	Level of PP (%)	PER Level of Tyrosine (g/16 gN) 1.627	0.827
100	0	2.347	2.347
90	10	2.256	2.286
80	20	2.165	2.225
70	30	2.074	2.165
60	40	1.983	2.104
50	50	1.892	2.043
40	60	1.801	1.982
30	70	1.710	1.922
20	80	1.619	1.861
10	90	1.528	1.800
0	100	1.460	1.755

the limiting amino acids are the sulphur containing acids (methionine and cystine) as shown in Table 19, while in meat, tyrosine and phenylalanine (Tables 19, 20) are limiting.

The level of limiting amino acid in the diets with plant proteins as the single source of nitrogen, was significantly lower than in diets which contained a mixture of meat and plant proteins (Tables 19, 20 and 21). Complementation of one source of protein system, which in the present study was meat, by a second source (various plant protein concentrates) will improve the biological value of the protein. Improvement of the availability and utilization of amino acids from any specific source of protein is probably the result of an elevation of the level of the limiting amino acid. Bigwood (1972) has shown that oats are deficient in lysine (3.4 g amino acid / 16 g N). In spite of this fact, chemical score and protein efficiency ratio were showing promising results and were equal in their level to other diets. Soy protein concentrates showed a much higher chemical score than other plant proteins when fed as the only source of protein. When a complementation of protein

Table 19

Chemical Scores and Limiting Amino Acids in Diets Containing Plant Proteins as Only Source of Proteins

Source of Protein	Form	Chemical Score (%)	Limiting Amino Acids
F.B. - U	Powder	45.5	Methionine + cystine
F.B. - T	Powder	39.1	Methionine + cystine
Pea	Powder	55.5	Methionine + cystine
Soy	Powder	74.1	Methionine + cystine
Meat	Freeze dried	85.3	Tyrosine + phenylalanine
F.B. - U	Texturized	49.1	Methionine + cystine
F.B. - T	Texturized	41.4	Methionine + cystine
Pea	Texturized	61.4	Methionine + cystine
Soy	Texturized	80.5	Methionine + cystine
Casein	Powder	>100	

Table 20

Chemical Scores and Limiting Amino Acids in Diets Containing Meat and Plant as Sources of Proteins

Source of Protein	Level of Plant Protein (%)	Chemical Score (%)	Limiting Amino Acids
Casein	0	>100	
Meat	0	83.7	Tyrosine + phenylalanine
Meat + F.B. - T	40	66.8	Methionine + cystine
Meat + F.B. - U	40	68.1	Methionine + cystine
Meat + Soy	40	82.0	Methionine + cystine
Meat + Pea	40	72.3	Methionine + cystine

Table 21

Chemical Score and Limiting Amino Acids in Diets Containing Finished Products

Source of Protein	Level of Plant Protein (%)	Chemical Score (%)	Limiting Amino Acids
Casein	0	>100	
Meat	0	76	Tyrosine + phenylalanine
Soy	15	70	Tyrosine + phenylalanine
Soy	25	87	Tyrosine + phenylalanine
F.B. - T	15	90	Tyrosine + phenylalanine
F.B. - T	25	83	Tyrosine + phenylalanine
F.B. - U	15	94	Tyrosine + phenylalanine
F.B. - U	25	86	Tyrosine + phenylalanine
Oats	15	85	Tyrosine + phenylalanine
Oats	25	70	Tyrosine + phenylalanine
Pea	15	72	Tyrosine + phenylalanine
Pea	25	69	Tyrosine + phenylalanine

sources took place, the gap between soy protein and the rest was eliminated (Table 21).

The relationship between the chemical score, protein utilization efficiency and the protein efficiency ratio were evaluated. In bioassay number one, ten diets were considered and the correlation coefficients generated are shown in Tables 22a, b. There is a high correlation between the protein utilization efficiency of the rats and the protein efficiency ratio of the diets or in other words, the rate of growth (weight gain) is highly correlated to the efficiency in which the proteins in the diets will be utilized. There is also a significant correlation between the chemical score and the PER, as well as between the chemical score and the efficiency of protein utilization ($p \quad 0.05$). It is likely that the chemical balance of the protein in the diet (chemical score) plus the metabolic system of the individual test animal and the digestibility of the protein, will determine the efficiency of utilization of the protein.

Efficiency of nutrient utilization controlled, to a large extent, the weight gain and growth of the test animals. This fact is supported by the values obtained from the feedigain of the diets (Figure 2, Appendix B) from which

Table 22a

Correlation Coefficients for PER, PUE and Chemical Score - Raw Materials

	PER	PUE	CS
PER	1.000	0.997	0.775
PUE	0.997	1.000	0.806
CS	0.775	0.806	1.000

Table 22b

Significance of Correlation Coefficients

(F - Values)

	PER	PUE	CS
PER	-	618.415 *	6.022 **
PUE	-	-	7.425 **

* $p < 0.01$

** $p < 0.05$

it can be seen that the higher the chemical score, the lesser the quantity of diet utilized to obtain optimal maintenance and growth.

A very high correlation between the protein efficiency ratio and protein utilization efficiency was also apparent in the finished products (Tables 23a, b). A significant correlation ($p < 0.05$) was observed in the same set of diets between the PER and the chemical score. The protein utilization efficiency and chemical score showed lower correlation coefficient.

Availability of the protein for utilization by the body depends on several factors; amino acid balance, (chemical score) presence of inhibitors in the diets, and the digestibility of the protein by the individual organism. There was a distinct variability between the digestibility among the test animals being fed the same diet. This variability can be attributed to the metabolism of the individual rat. The variabilities between the groups of rats (Table 24) are the result of the differences between the protein sources and the treatment each of the proteins received. It can be seen the effects ethanol extraction and heat treatment have on digestibility. The digestibility

Table 23a

Correlation Coefficients for PER, PUE and Chemical Scores- Finished Products

	PER	PUE	CS
PER	1.000	0.928	0.806
PUE	0.928	1.000	0.405
CS	0.806	0.405	1.000

Table 23b

Significance of Correlation Coefficients

(F - Values)

	PER	PUE	CS
PER	-	61.716 *	2.030 **
PUE	-	-	1.953 **

* $p < 0.01$

** $p < 0.05$

Table 24

Digestibility Variations Among Rats and Diets

Diet		Replicas 1	2	3	4	5	Mean Digestibility (%)
F.B. - U	Powder	87.1	90.0	89.6	89.4	87.5	88.8
F.B. - T	Powder	85.3	86.4	89.7	85.7	87.5	86.9
Pea	Powder	79.6	78.9	78.4	81.9	79.6	79.6
Soy	Powder	74.9	73.4	72.8	76.4	77.3	74.9
Meat	Freeze dried	89.3	91.0	90.6	90.8	89.3	90.2
F.B. - U	Texturized	80.3	81.6	82.0	83.3	81.0	81.6
F.B. - T	Texturized	83.3	84.0	84.2	82.8	82.1	83.2
Pea	Texturized	82.1	79.7	82.7	83.0	80.0	81.5
Soy	Texturized	81.6	80.2	80.9	80.4	82.7	81.1
Casein	Powder	92.1	92.2	92.5	91.8	92.2	92.2

was higher when rats were fed untreated fababean concentrate than with treated fababean in the powder form. Texturized fababean, both treated and untreated, had lower digestibility than when fed in the powder form. It is conceivable that the ethanol extraction and heat treatment bound or changed the composition of proteins and as a result, decreased digestibility. Digestibility of soy improved when fed in the texturized, as compared to the powdered form. Heat treatment is known to destroy the anti-nutritional factors in soy proteins and in the present study, substantiates this.

Although texturization showed an improvement in digestibility of soy proteins, it cannot be implied that availability and utilization will follow the same trend. Meat and casein show the highest digestibility value when used as the only source of protein. When mixtures of meat and plant proteins were fed to rats, an improvement in the digestibility has been shown (Tables 24, 25) with a slight decrease in digestibility with higher levels of plant protein. An overall improvement in protein efficiency ratio,ddigestibility, protein utilization and diet efficiency was observed when a complementation of meat

Table 25

Digestibility and Protein Utilization Efficiency (PUE) of Diets Containing Meat and Plant Proteins

Diet	Digestibility (%)	Protein Intake (g)	True Digestible Protein (g)	PUE
Casein	88	35.3	31.4	3.1
Meat	90	39.9	35.9	3.6
Soy - 15%	92	39.1	36.0	2.8
Soy - 25%	89	41.5	36.9	3.5
F.B. - T 15%	89	37.5	33.4	3.5
F.B. - T 25%	91	35.3	32.1	3.6
F.B. - U 15%	89	34.4	30.6	4.5
F.B. - U 25%	86	34.6	29.7	4.4
Oats 15%	87	39.9	34.7	3.5
Oats 25%	86	38.6	33.2	3.5
Pea 15%	90	40.2	36.2	3.4
Pea 25%	88	38.1	33.3	3.4

diets with plant protein concentrates were fed to test animals.

Extraction of vicine from fababean protein concentrate by ethanol, brought about a decline in the level of some of the essential amino acids. A heat treatment of the same fraction showed an even lower level of the amino acids (Table 26). Analysis of the amino acids was done on the diets in which plant proteins were the only source of nitrogen. From Table 26, a conclusion can be drawn that heat treatment of the FBPC after ethanol extraction will affect the labile amino acids. One of the major differences between the present study and similar work done on the effect of heat treatment and ethanol extraction on fababean (Marquardt et al., 1974, Marquardt et al., 1975, Eskin et al., 1979, Olaboro, 1979) is the initial autoclaving of the material. In the above mentioned studies, the FBPC went through autoclaving and there is no indication of loss of labile amino acids. Stability of the proteins as a result of denaturation by heat, will eliminate loss of free amino acids and low molecular weight proteins by washing.

Table 26

Labile Amino Acids in Treated and Untreated Fababean Protein Concentrates (gAA/16gN)

Amino Acid	Diets			
	Powder Form		Texturized Form	
	F.B. - U	F.B. - T	F.B. - U	F.B. - T
Lysine	5.947	4.822	4.595	4.269
Cystine	1.117	0.937	1.127	0.902
Methionine	0.482	0.446	0.506	0.465

Vicine Extraction and Determination

Fababean protein concentrate was extracted with six volumes of ethanol and water in the ratio of 60:40. Vicine levels in the untreated FBPC, treated FBPC and the dried extracts were determined by the $TiCl_4$ method. Pure vicine was used to establish a standard curve (Figure 7). From Table 27, it can be seen that vicine and convicine were not removed completely and 14.8% of total vicine was still present in the treated fraction. The effect of total vicine on biological value of the diets was not an objective of this study and hence, further work has not been done.

Sensory Evaluation

Five different sources of meat extenders added at three levels, were evaluated for their effects on sensory attributes. There was no indication of any significant difference in flavor among the various treatments (Table 28). The inability of the panelists to detect flavor variations can be attributed to two reasons: awareness of panelists of what was required of them influenced their judgement plus the masking effect of the spices incorporated into the binder. The Ketchup was another ingredient used in masking the bitter flavor of the beans.

Figure 7

Standard Curve for Vicine Determination

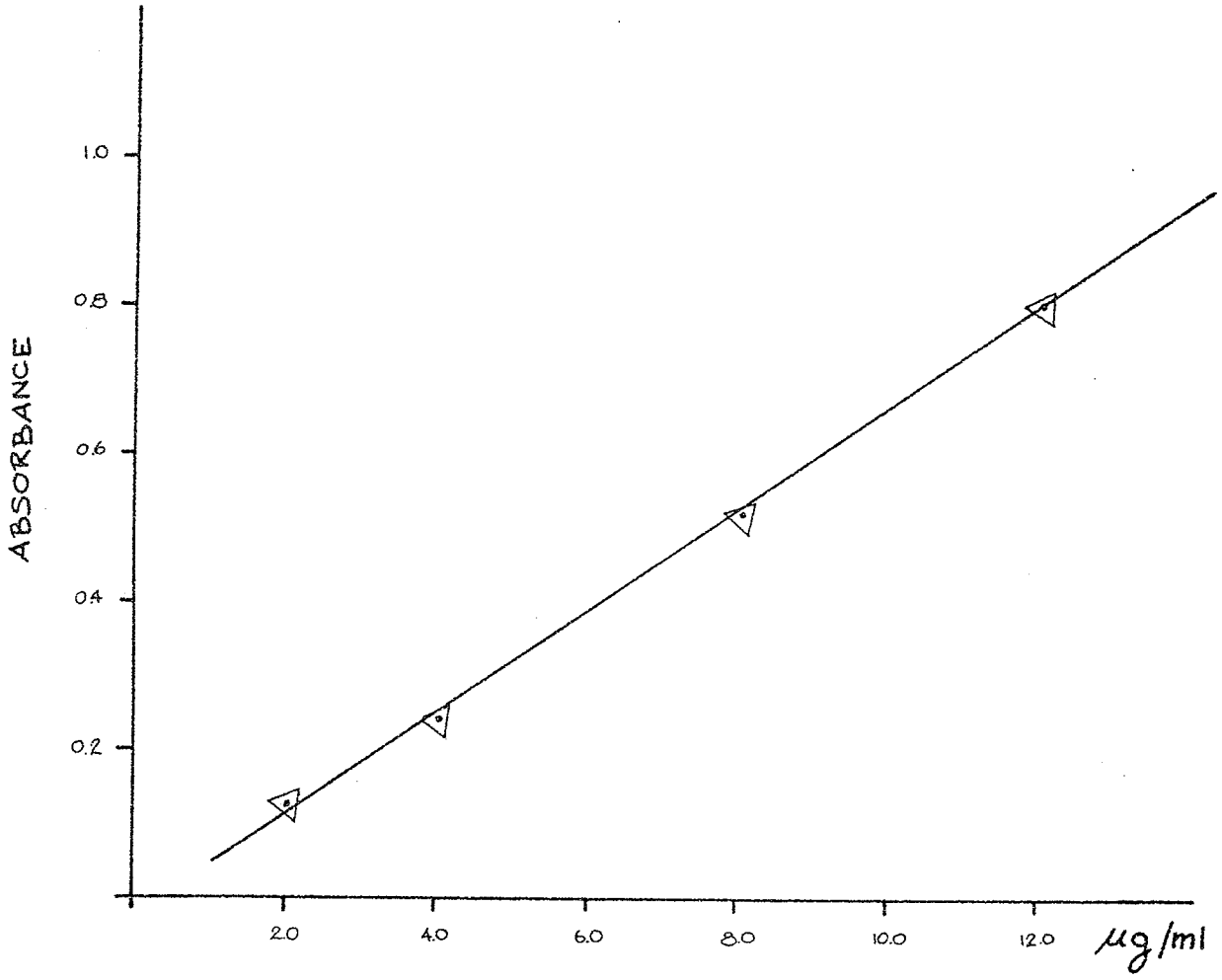


Table 27

Total Vicine Level in the Various Fractions Obtained from Ethanol Extraction *

Fraction	Total Vicine (%)
Untreated FBPC	2.425
Treated FBPC	0.360

* Extracting solution was assayed for vicine. Although mass balance was not calculated, considerable vicine was present in the soluble phase.

Figure 8

Texturized Plant Proteins

1. Extruded treated fababean.

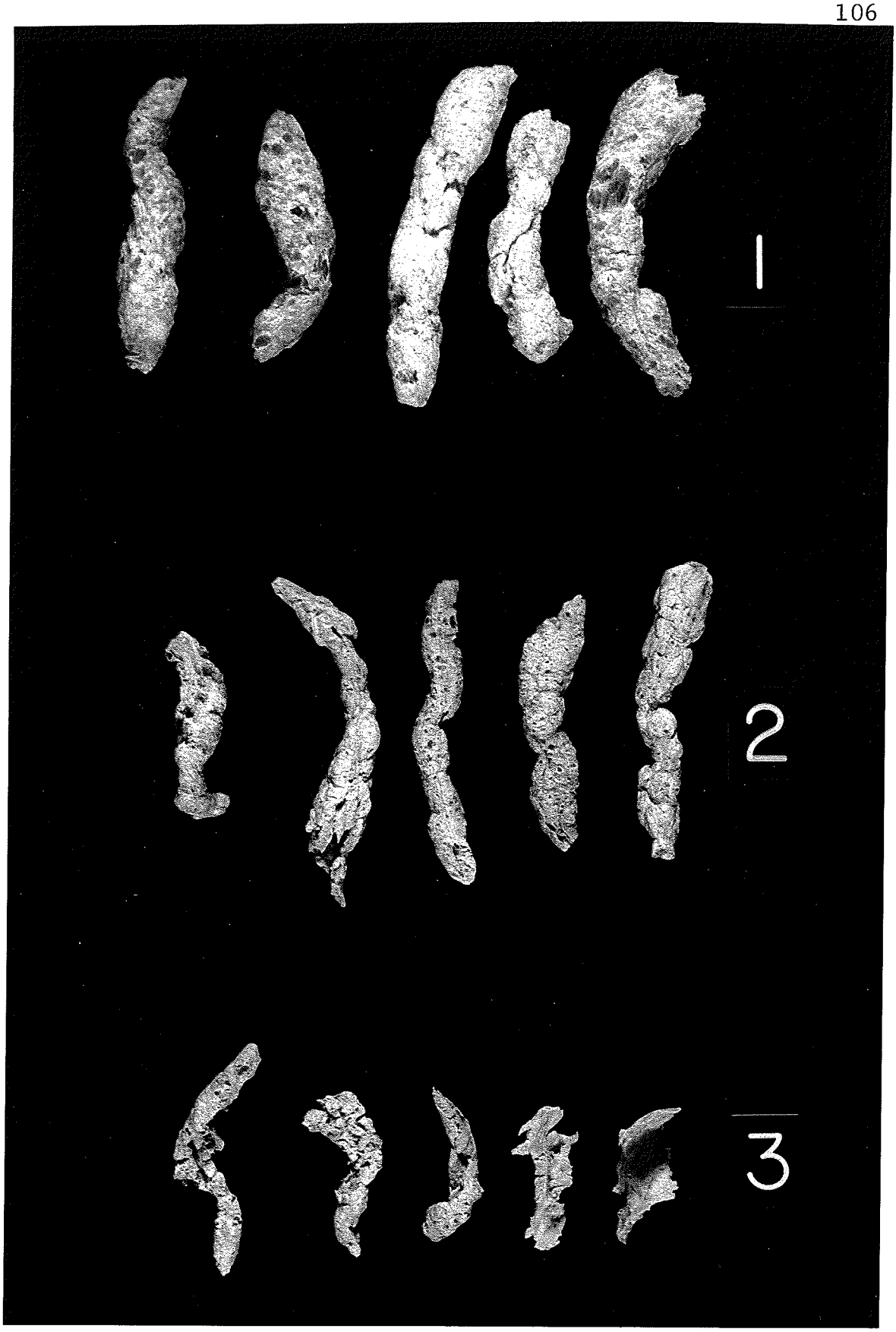
   Note the porosity of the texturized protein concentrate.

2. Extruded untreated fababean.

   Note the density of the protein concentrate and the hardened surface.

3. Extruded pea protein.

   Note the shape of the flat samples. Pea protein will not expand during extrusion and elongation is minimal.


1
2
3

Juiciness was significantly different in the various treatments with samples containing 15% and 5% of plant protein extenders showing higher level of moisture retention (Table 29). Higher juiciness values in the various treatments indicate lower drip loss during processing. The treatments which contained the 25% plant proteins, showed a general tendency to be somewhat lower in moisture retention. This fact implies that there is an optimal level of plant proteins which will retain highest moisture level in the extended meat loaf during processing. From the results obtained, this level of retention is between 5% and 15% of textured plant protein (TPP).

Although from the present study it can not be concluded which of the plant protein was superior to others in moisture retention, the soy and the treated fababean showed better moisture holding capacity than the untreated fababean protein and the pea proteins.

The variations among the texturized plant proteins can be attributed to the chemical constitution and the effect the texturization process had on the individual protein. From Figure 8, it can be seen that the

Figure 9

Meat Loaves from Three Different Methods of Processing

1. Baked meat loaf from ground meat.
   Note the rough texture of the particles and the air entrapments.

2. Baked meat loaf from fine meat emulsion.
   Note the fine texture of the particles with the increase of binding capacity.

3. Boiled meat loaf from fine meat emulsion.
   Note the fine texture and the binding capacity of the fine emulsion. Fat granules are less noticeable.

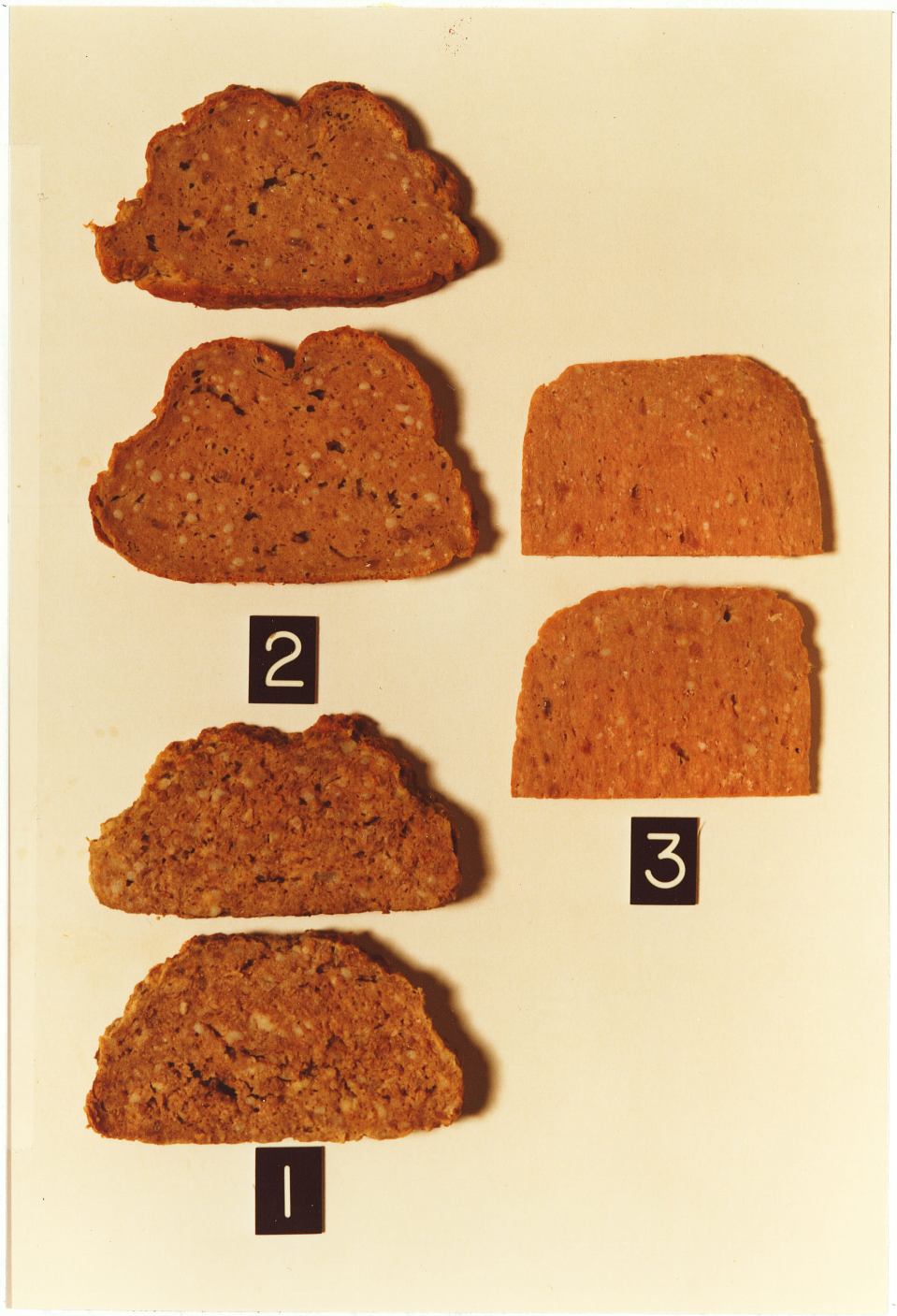

2
3
1

Figure 10

Meat Loaves with Treated and Untreated Fababean Proteins as Meat Extenders

1. Untreated fababean at a level of 15%.
   Note the density of the product. Texture finer than meat loaf from 100% meat (Fig. 9.1).

2. Untreated fababean at a level of 25%.
   Note lighter color and higher porosity than No. 1.

3. Treated fababean at a level of 15%.

4. Treated fababean at a level of 25%.
   Note that treated fababean enhances lighter color in the product and affects the binding capacity of the proteins.


2
4
1
3

Figure 11

Meat Loaves with Soy Protein and Oats as Meat Extenders

1. Soy protein at a level of 15%.

2. Soy protein at a level of 25%.
   Note the higher density of the product containing 25% soy. Meat lost its natural red color as a result of the soy being added.

3. Oats at a level of 15%.

4. Oats at a level of 25%.
   Note the coarse texture of the product with oats as the meat extender. Color of the meat loaf is unacceptable for a meat product.

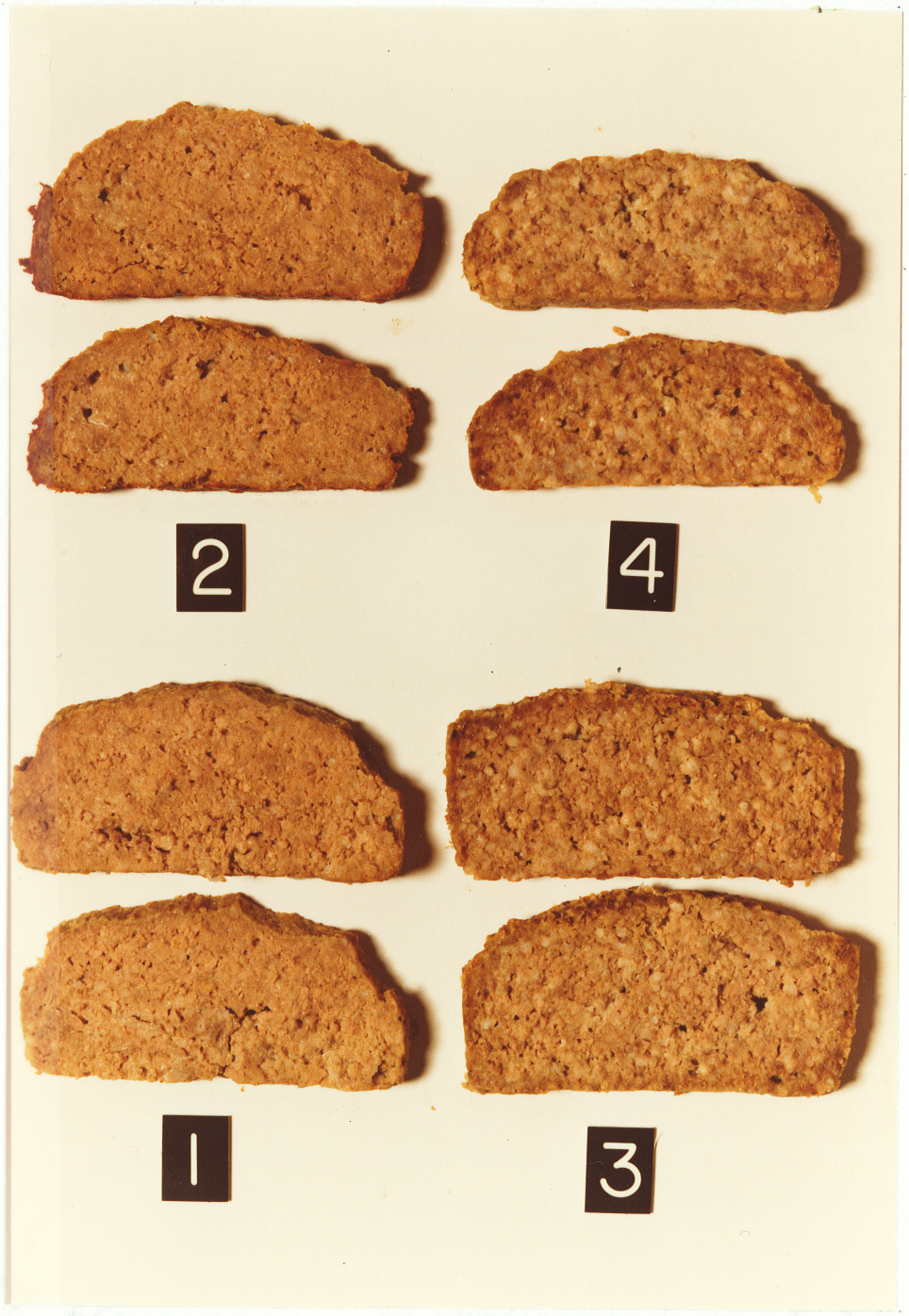

2
4
1
3

Figure 12

Meat Loaves with Pea Protein as Meat Extender

1. Pea protein at a level of 15%.

2. Pea protein at a level of 25%.

   Note the yellowish color the product developed as a result of the incorporation of the pea protein. 25% of pea protein will result in a very dense product.


2
1

Table 28

Analysis of Variance for Flavor Perception

Source of Variation	DF	SS	MS	F *
Panelists [1]	4	0.357	0.089	0.649
Products	14	1.992	0.142	1.036
Panelists/products	56	6.627	0.118	0.862
Error	148	20.324		
Total	222	29.299		

* Not significantly different ($p < 0.05$)

1 Three replications of judgement by the same five panelists.

Table 29

Analysis of Variance for Juiciness Perception

Source of Variation	DF	SS	MS	F
Panelists [1]	4	0.032	0.003	0.076 *
Products	14	2.916	0.203	1.967 *
Panelists/products	56	4.974	0.089	0.837
Error	148	15.711	0.106	
Total	222	23.633		

* Significantly different ($p < .05$)

1 Three replications of judgement by the same five panelists.

structure of the treated texturized fababean protein concentrate, is distinctly different from the untreated protein. A more dense untreated protein will tend to absorb less moisture than the porous treated protein and hence, higher loss during processing.

Firmness of the product, according to the evaluation of the five panelists in the present study, does not depend on the level of the meat extender incorporated into the meat loaves, but on the source of the plant protein. From Tables 31 and 32, it is obvious that there is no specific trend established in regard to the influence of the TPP added, but it related to the source with oats adding the least firmness to the product. Since FBPC, PPC and SPC were heat treated during texturization and physical, as well as chemical changes took place, while oats were mechanically rolled without the application of excessive heat, it is probable that method of texturization used in the present study, caused an enhancement in firmness.

A trend similar to the one established in firmness (or toughness) was found to take place in chewiness and grittiness (Tables 33, 34, 35 and 36, respectively). Treatments with treated and untreated fababean plus soy, show a

Table 30

Treatment Means for Juiciness [1] Perceived by Panel

Meat Extender	Level (%)	Treatment Mean
Soy	15	1.362 a
Soy	25	1.093 ab
Soy	5	1.001 abc
Oats	5	0.985 abc
Pea	5	0.932 abc
F.B. - T	5	0.917 abc
F.B. - T	25	0.910 abc
F.B. - T	15	0.895 abc
Pea	15	0.796 abc
Pea	25	0.781 abc
Oats	15	0.758 abc
F.B. - UT	25	0.619 bc
Oats	25	0.573 bc
F.B. - UT	5	0.546 bc
F.B. - UT	15	0.532 c

[1] Treatments with the same letter are not significantly different ($p < .05$).

Table 31

Analysis of Variance for Firmness Perception

Source of Variation	DF	SS	MS	F
Panelists [1]	4	0.088	0.022	0.195
Products	14	2.631	0.188	1.666 *
Panelists/products	56	2.183	0.089	0.845
Error	148	16.697	0.113	
Total	222	21.599		

* Significantly different ($p < .05$).

1 Three replications of judgement by the same five panelists.

Table 32

Treatment Means for Firmness [1] Perceived by Panel

Meat Extender	Level (%)	Treatment Mean
F.B. - T	15	1.322 a
Soy	5	1.295 a
F.B. - T	25	1.218 ab
Soy	15	1.193 ab
F.B. - T	5	1.129 abc
F.B. - U	5	1.117 abc
Pea	25	1.055 abc
Pea	15	1.052 abc
F.B. - U	15	1.041 abc
Pea	5	0.982 abcd
Soy	25	0.941 abcd
Oats	5	0.740 bcd
F.B. - U	25	0.672 cd
Oats	25	0.667 cd
Oats	15	0.592

1 Treatments with the same letter are not significantly different ($p < .05$).

Table 33

Analysis of Variance for Chewiness Perception

Source of Variation	DF	SS	MS	F
Panelists [1]	4	0.051	0.013	0.593
Products	14	0.727	0.052	2.434*
Panelists/products	56	0.947	0.017	0.792
Error	148	3.159	0.021	
Total	222	4.884		

* Significantly different ($p < .05$).

1 Three replications of judgement by the same five panelists.

Table 34

Treatment Means for Chewiness [1] Perceived by Panel

Meat Extender	Level (%)	Treatment Mean
Soy	5	1.271 a
Soy	15	1.175 ab
F.B. - T	15	1.173 ab
F.B. - T	5	1.163 ab
Soy	25	1.141 abc
F.B. - U	5	1.117 abc
F.B. - T	25	1.106 abc
F.B. - U	15	1.028 abcd
Pea	15	0.980 bcd
Pea	25	0.958 bcd
F.B. - U	25	0.936 bcd
Pea	5	0.928 bcd
Oats	25	0.897 cd
Oats	5	0.838 d
Oats	15	0.823 d

1 Treatment with the same letter are not significantly different ($p < .01$).

Table 35

Analysis of Variance for Mouthfeel Perception

Source of Variation	DF	SS	MS	F
Panelists [1]	4	0.069	0.017	0.723
Products	14	0.746	0.053	0.656
Panelists/products	56	9.486	0.169	2.077 *
Error	148	12.069	0.081	
Total	222	22.370		

* Significantly different ($p < .05$).

1 Three replications of judgement by the same five panelists.

Table 36

Treatment Means for Mouthfeel [1] Perceived by Panel

Meat Extenders	Level (%)	Treatment Mean
F.B. - U	15	1.217 [a]
Oats	25	1.142 [b]
F.B. - U	5	1.093 [b]
F.B. - T	15	1.088 [b]
Soy	15	1.044 [b]
Pea	15	1.043 [b]
F.B. - T	25	1.041 [b]
F.B. - U	25	1.033 [b]
Soy	25	1.024 [b]
Pea	5	0.986 [b]
Soy	5	0.962 [b]
Oats	5	0.958 [b]
Pea	25	0.819 [b]
Oats	15	0.813 [b]
F.B. - T	5	0.729 [b]

[1] Treatment with the same letter are not significantly different ($p < .05$).

Table 37

Analysis of Variance for Grittiness Perception

Source of Variation	DF	SS	MS	F
Panelists [1]	4	0.102	0.025	0.224
Products	14	4.789	0.342	3.011 *
Panelists/products	56	2.667	0.048	0.419
Error	148	16.815	0.114	
Total	222	24.794		

* Significantly different ($p < .05$).

1 Three replications of judgement by the same five panelists.

Table 38

Analysis of Variance for Total Acceptability

Source of Variation	DF	SS	MS	F
Panelists [1]	4	1.004	0.251	1.168
Products	14	5.142	0.368	1.710 *
Panelists/products	56	12.282	0.219	1.021
Error	148	31.798	0.215	
Total	222	50.226		

* Significantly different ($p < .05$).

1 Three replications of judgement by the same five panelists.

Table 39

Treatment Means for Total Acceptability [1] of Product by Panelists

Meat Extender	Level (%)	Treatment Mean
F.B. - U	15	2.505 a
F.B. - T	15	2.007 b
F.B. - T	5	1.923 b
F.B. - U	25	1.916 bc
Oats	15	1.578 bc
F.B. - U	5	1.488 bc
Soy	5	1.365 cd
Pea	15	1.230 cd
Pea	25	1.018 cd
F.B. - T	25	1.014 cd
Soy	15	1.007 cd
Oats	5	0.966 de
Oats	25	0.922 de
Pea	5	0.901 e
Soy	25	0.747 e

[1] Treatment with the same letter are not significantly different ($p < .05$).

high degree of chewiness while oats added less to the texture of the product.

The degree of mouthfeel or the sensation felt in the mouth and throat after swallowing, show some degree of correlation to the juiciness of the products. Meat loaves containing 5% of TPP left the least mouthcoating and from Table 30, it can be seen that four out of five treatments with 5% level received high values for their juiciness. This correlation suggests that the higher the moisture retention capacity of the product, the lower the unpleasant sensation of a fatty film in the mouth.

Grittiness was found to be high in sampleswwhich contained 15% and 25% meat extenders and mainly texturized fababean (Table 37). Panelists rated oats very low in grittiness and the main reason for that could be the soft texture the oats obtain when rehydrated.

The attribute that gave a total picture of the extended meat loaves, was the total acceptability of the product by the panelists. From Tables 38 and 39, it can be seen that products containing treated and untreated fababean protein concentrates in a texturized form were more readily accepted. Oats was rated low as a meat extender

on its total acceptability. Sensory evaluation is a complex method for evaluating a product and many aspects of the product might influence the decision of a panelist. In the present study, the color of the product, the consistency of the meat loaves and granulation were all affected by the meat extender (Figures 9, 10, 11 and 12) and as a result, influence to a certain degree, the judgement of the panel.

GENERAL DISCUSSION AND CONCLUSION

Four different sources of plant proteins were evaluated chemically and nutritionally as meat extenders. Protein concentrates were incorporated into the meat in various levels and studies of biological values plus sensory evaluation of eight parameters were done.

One of the major aspects of the present work was to evaluate the performance of fababean protein concentrates in a treated and untreated state. Fababeans are a relatively new source of protein for human diet in North America and have not been exposed to the market as yet. The fababean protein concentrate is a high source of protein (63 - 68%) and after ethanol extraction, loses almost all its hay-beany (bitter) flavor. The texturization of the treated fababean concentrate generated a high protein product with a bland flavor and a porous texture which enables a high water absorption capacity. Because of a continuing increase in meat prices and the advantages of using meat extenders (high juiciness level, less shrinkage of product and improved texture) a growing

interest in plant proteins as meat extenders has resulted. The major source of protein to be investigated and used in meat products, has been soybean protein concentrate in powder as well as texturized form. In Canada, there was an attempt to introduce texturized soy product as an extender in ground beef in 1973 without much success (Vaisey et al., 1975). On the other hand, many housewives are using rolled oats, cracker crumbs, etc., as extenders in many meat products being prepared at home. It is possible that the bland flavor and the semi-texturized form of these materials promoted their use over other highly texturized and/or nutritional plant proteins. A partial lack of consumer knowledge and the limited work done on meat extenders, other than soybean protein, prevented the exposure of the housewife to highly nutritional and more acceptable meat extenders, such as the texturized treated fababean protein concentrate.

In the present study, the texturized fababean was found to be more acceptable as a meat loaf extender than soybean protein concentrate, peas and rolled oats. Rolled oats formed a mushy consistency when rehydrated and mixed with the meat. The white color of the oats caused

a noticeable change in the accepted meat color (Figure 11) with the result of some deterrent effect on the panelists. The availability of a new product with better nutritional as well as organoleptic characteristics, should focus more attention of scientists and industry to the great potential in the fababean.

One of the limitations of the fababean, as is the case with many other legumes, is the low level of the sulfur containing amino acids (methionine and cystine). Body requirements for sulfur rely essentially on methionine and cystine supplies. These amino acids therefore, play an essential part in the human diet. Since methionine and cystine are the limiting amino acids in the fababean as in other legumes, there is a restriction on the utilization of other amino acids. Supplementation of proteins with complementary proteins, is one possible answer to the problem of a limiting amino acid. In the current study, 110 g of whole eggs were added to the meat loaves prepared for nutritional and sensory evaluation. Eggs, as are other animal related proteins, are high in cystine and methionine (5.9 g/16g nitrogen in eggs vs 2.9 in soy and 2.4 in fababean). The addition of the eggs to the meat loaf,

which is a common procedure followed by many housewives, increased the level of the sulfur amino acids and hence, the chemical score. Supplementation of proteins increases the availability of essential, as well as non-essential amino acids and the biological value of the diet. Supplementation could be done by mixing predetermined quantities of more than one plant protein which will complement each other and give optimal biological value. In the search after a better nutritional performance of the diet, the sensoric side of the product must not be neglected. There is a good potential for texturized plant protein in decreasing drip loss plus shrinkage with the improvement in the texture of the product. The utilization of one or more sources of plant proteins would improve the nutritional contribution of the product to the daily requirement of essential nutrients and at the same time, will promote acceptability.

The PER values obtained when rats were fed meat loaves as sources of proteins in their diets, were considerably higher than when fed plant proteins or raw mixtures of meat and plant proteins. When untreated texturized fababean was used as the meat extenders, PER

values were 2.4 for the raw materials and 3.6 for the finished product, which included eggs and binder as supplementary sources of amino acids. Values obtained are in line with PER values obtained by feeding meat and egg products (Altschul, 1964). The use of plant proteins as meat extenders gave higher PER values than obtained from 100% meat diet. From the present study, several conclusions can be drawn:

(a) Fababean protein concentrate can replace any existing meat extender (including soy) with better results both from the viewpoint of nutrition and consumer acceptance.

(b) The supplementation of a diet deficient in amino acids by one or more sources of protein, will increase the biological value of the diet in question, to a desirable level.

(c) Supplementation of meat products with plant protein concentrates will diminish the effect of the limiting amino acids and hence, will increase the chemical score of the components of the product if fed separately. It will also replenish the loss of amino acids during the various stages of processing.

(d) The use of plant proteins as meat extenders is a growing concern and interest among all levels of the food industry as well as the consumer. The search for new, inexpensive and better sources of proteins, is in full swing and this study supports the basic concept of the importance and the benefits in utilizing a rich source of supplementary amino acids.

(e) The negative relationship between the level of tyrosine and the predicted PER value demonstrates a very important aspect of future methods for biological evaluation of food products. If the equation used is proven to have a high degree of accuracy, this simple and fast method can be used by industry on a regular basis. In order to verify the accuracy of the equation and to establish negative correlation between tyrosine and PER, a feeding trial should be initiated in which rats will be fed diets containing various controlled levels of tyrosine.

More work should be done on improving the texture of the finished product to make it compatible with other pure meat products. In depth investigation of the functional properties of the plant proteins should be done in a mixture of ground meat and texturized plant

protein. Much work has been done on functional properties of meat extenders in the emulsion form, but because of the increase in the demand for other types of meat products, emphasis should be put on other processing methods of meat and meat extenders and their effects on functional properties.

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Appendix A

Weight Gain, Feed Intake and PER Values for the Four Week Period - Bioassay 1 *

Diet	(a) Weight Gain (gr)	(b) Protein Intake	PER (a/b)	Adjusted PER	Feed Intake (gr)	Feed/Gain
Casein	122.06	40.63	3.00	2.50	406.3	3.33
Meat	319.39	44.46	3.13	2.60	444.6	3.19
Meat + 20% T-F.B.	123.97	43.79	2.83	2.36	437.9	3.53
Meat + 40% T-F.B.	109.47	41.98	2.61	2.17	419.8	3.83
Meat + 20% U-F.B.	130.77	44.12	2.96	2.46	441.3	3.37
Meat + 40% U-F.B.	117.60	44.66	2.63	2.19	446.6	3.79
Meat + 20% Soy	126.06	42.12	2.99	2.49	421.2	3.34
Meat + 40% Soy	122.28	42.47	2.88	2.40	424.7	3.47
Meat + 20% Pea	124.12	43.00	2.88	2.40	430.0	3.46
Meat + 40% Pea	120.32	42.73	2.81	2.34	427.3	3.55

* Mean values for eleven rats per group.

Appendix B

Weight Gain, Feed Intake and PER Values for the Four Week Period - Bioassay 2 *

Diet	(a) Weight Gain (gr)	(b) Protein Intake	PER (a/b)	Adjusted PER	Feed Intake (gr)	Feed/Gain
Casein	96.3	35.3	2.72	2.50	352.8	3.66
Meat	130.2	39.9	3.26	2.99	398.9	3.06
Meat + 15% Soy	100.6	39.1	2.99	2.74	391.4	3.88
Meat + 25% Soy	130.3	41.5	3.14	2.88	415.6	3.19
Meat + 15% T-F.B.	118.4	37.5	3.15	2.89	375.5	3.17
Meat + 25% T-F.B.	114.9	35.3	3.25	2.98	353.3	3.07
Meat + 15% U-F.B.	137.9	34.4	4.00	3.67	343.7	2.49
Meat + 25% U-F.B.	126.9	34.6	3.66	3.37	345.8	2.72
Meat + 15% Oats	128.0	39.9	3.21	2.95	398.9	3.11
Meat + 25% Oats	118.2	38.6	3.06	2.81	386.2	3.26
Meat + 15% Pea	123.2	40.2	3.06	2.81	402.0	3.26
Meat + 25% Pea	115.2	38.1	3.02	2.77	380.8	3.30

* Mean values for eleven rats per group.

Appendix C

Levels (%) of Amino Acids in Diets * and Excreta * Samples - Bioassay No. 1

Amino Acid	Casein		Meat		FB-T 40%	
	Diet	Feces	Diet	Feces	Diet	Feces
Lysine	0.855	0.848	0.781	0.928	0.813	1.049
Histidine	0.306	0.239	0.288	0.257	0.295	0.275
NH_3	0.271	1.094	0.195	0.602	0.264	0.872
Arginine	0.377	0.652	0.562	0.760	0.930	0.870
Aspartic Acid	0.820	1.709	0.928	1.622	1.196	1.863
Threonine	0.453	0.825	0.408	0.802	0.456	1.338
Serine	0.530	1.397	0.318	0.631	0.454	0.705
Glutamic Acid	2.552	3.706	1.521	2.043	1.964	2.196
Proline	1.204	0.641	0.387	0.702	0.511	0.665
Glycine	0.212	0.771	0.461	1.089	0.552	1.093
Alanine	0.368	0.932	0.592	1.002	0.584	1.124
Cystine	0.056	0.356	0.114	0.389	0.107	0.389
Valine	0.749	1.100	0.480	0.843	0.612	1.010
Methionine	0.325	0.367	0.201	0.328	0.151	0.372
Isoleucine	0.615	1.156	0.480	0.750	0.541	0.883
Leucine	1.066	1.053	0.801	1.130	0.964	1.389
Tyrosine	0.388	0.338	0.154	0.390	0.250	0.541
Phenyalanine	0.578	0.548	0.358	0.680	0.473	0.746

Amino Acid	FB-U 40%		Soy 40%		Pea 40%	
	Diet	Feces	Diet	Feces	Diet	Feces
Lysine	0.549	1.032	0.630	1.312	0.781	1.282
Histidine	0.205	0.281	0.238	0.344	0.271	0.324
NH_3	0.179	0.679	0.198	0.758	0.203	0.856
Arginine	0.613	0.773	0.590	0.971	0.722	1.053
Aspartic Acid	0.800	1.867	0.980	2.310	1.051	2.122
Threonine	0.296	0.882	0.364	1.109	0.404	1.038
Serine	0.296	0.742	0.344	0.811	0.364	0.817
Glutamic Acid	1.256	2.128	1.518	2.745	1.661	2.631
Proline	0.310	0.653	0.424	0.791	0.404	0.831
Glycine	0.352	1.009	0.454	1.582	0.455	1.366
Alanine	0.410	1.084	0.516	1.469	0.560	1.299
Cystine	0.094	0.407	0.125	0.456	0.099	0.480
Valine	0.355	0.956	0.488	1.278	0.498	1.202
Methionine	0.142	0.380	0.192	0.584	0.159	0.527
Isoleucine	0.353	0.785	0.440	1.180	0.478	1.056
Leucine	0.618	1.294	0.743	1.777	0.813	1.597
Tyrosine	0.148	0.506	0.194	0.568	0.206	0.526
Phenyalanine	0.288	0.715	0.398	0.930	0.423	0.893

* Values are mean value of two replications.

Appendix D

Levels (%) of Amino Acids in Diets * and Excreta * Samples - Bioassay No. 2 **

Amino Acid	N-Free		FB-U Powder		FB-T Powder		Pea Powder	
	Diet	Feces	Diet	Feces	Diet	Feces	Diet	Feces
Lysine	Trace	0.779	0.612	0.911	0.509	1.111	0.755	1.754
Histidine	-	0.273	0.225	0.311	0.247	0.317	0.244	0.454
NH_3	0.100	0.832	0.182	1.825	0.284	1.903	0.220	1.850
Arginine	-	0.828	1.018	1.015	1.002	0.888	0.898	1.283
Aspartic Acid	0.021	1.478	1.193	2.026	1.151	1.834	1.132	2.456
Threonine	0.005	0.633	0.357	0.972	0.373	0.976	0.316	1.117
Serine	0.009	0.603	0.458	0.767	0.429	0.549	0.293	0.691
Glutamic Acid	0.045	1.941	1.824	2.548	1.846	2.246	1.651	3.243
Proline	-	0.613	0.478	0.730	0.406	0.675	0.420	0.786
Glycine	0.009	0.742	0.451	1.241	0.424	1.063	0.434	1.463
Alanine	0.018	0.683	0.448	1.362	0.426	1.076	0.448	1.627
Cystine	-	0.393	0.116	0.491	0.099	0.427	0.119	0.526
Valine	-	0.715	0.549	1.130	0.533	2.033	0.938	2.665
Methionine	-	0.167	0.050	0.388	0.047	0.340	0.078	0.598
Isoleucine	-	0.589	0.508	1.001	0.463	0.954	0.458	1.457
Leucine	0.020	1.018	0.886	1.469	0.851	1.387	0.782	2.273
Tyrosine	-	0.295	0.132	0.513	0.198	0.408	0.164	0.443
Phenylalanine	-	0.566	0.453	0.831	0.450	0.784	0.476	1.187

Amino Acid	Soy Powder		Meat		FB-U Extruded		FB-T Extruded	
	Diet	Feces	Diet	Feces	Diet	Feces	Diet	Feces
Lysine	0.688	1.923	0.636	0.887	0.574	1.109	0.430	1.057
Histidine	0.280	0.541	0.234	0.247	0.279	0.296	0.240	0.272
NH_3	0.249	1.565	0.142	1.233	0.260	1.422	0.196	1.469
Arginine	0.730	1.467	0.466	0.761	1.112	0.948	0.894	0.949
Aspartic Acid	1.176	3.787	0.584	1.850	1.100	2.360	0.959	2.356
Threonine	0.350	1.454	0.248	0.809	0.301	1.071	0.272	1.022
Serine	0.319	0.939	0.148	0.527	0.298	0.845	0.296	0.795
Glutamic Acid	1.886	4.879	1.002	2.191	1.710	2.547	1.507	2.468
Proline	0.531	1.273	0.252	0.703	0.440	0.671	0.384	0.649
Glycine	0.448	1.796	0.371	1.179	0.427	1.098	0.370	1.112
Alanine	0.464	1.908	0.400	0.941	0.426	1.247	0.370	1.198
Cystine	0.158	0.587	0.100	0.473	0.115	0.439	0.091	0.501
Valine	1.043	2.936	0.675	0.956	1.006	1.304	0.848	1.321
Methionine	0.140	0.696	0.238	0.264	0.062	0.389	0.057	0.346
Isoleucine	0.516	1.654	0.361	0.727	0.479	1.047	0.404	1.024
Leucine	0.860	3.115	0.641	1.126	0.859	1.479	0.740	1.489
Tyrosine	0.179	0.527	0.097	0.313	0.152	0.523	0.140	0.578
Phenylalanine	0.515	1.473	0.276	0.676	0.427	0.821	0.363	0.829

Amino Acid	Pea Extruded		Soy Extruded		Casein	
	Diet	Feces	Diet	Feces	Diet	Feces
Lysine	0.824	1.567	0.659	1.566	0.967	0.819
Histidine	0.269	0.420	0.271	0.439	0.348	0.249
NH_3	0.233	1.263	0.330	1.098	0.329	0.922
Arginine	0.965	1.309	0.740	1.270	0.448	0.683
Aspartic Acid	1.245	3.236	1.308	2.926	0.919	2.078
Threonine	0.335	1.498	0.356	1.568	0.441	0.876
Serine	0.316	1.036	0.327	1.125	0.429	1.572
Glutamic Acid	1.735	3.291	2.021	3.347	2.741	3.419
Proline	0.429	0.847	0.539	0.895	1.316	0.645
Glycine	0.466	1.463	0.466	1.427	0.233	0.833
Alanine	0.471	1.679	0.486	1.620	0.392	0.957
Cystine	0.128	0.508	0.157	0.575	0.066	0.423
Valine	1.046	1.746	0.546	1.651	0.810	1.226
Methionine	0.090	0.532	0.138	0.628	0.478	0.298
Isoleucine	0.491	1.464	0.521	1.393	0.653	1.219
Leucine	0.840	1.991	0.869	2.119	1.309	1.063
Tyrosine	0.164	0.759	0.159	0.718	0.3444	0.392
Phenylalanine	0.515	1.120	0.540	1.194	0.613	0.547

* Values are mean value of two replications.
** Values are mean value of five samples.