Bioavailability of three homopoly amino acids in growing rats and chicks
by Nancy Creviston Dew

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Home Economics
Montana State University

Abstract:
A basic application of genetic engineering to improve protein quality of a substance is the incorporation of homopoly amino acids (HPAAs) into the original protein. Growth trials were conducted with growing rats and chicks to determine if three HPAAs, poly-L-lysine (PLL), poly-L-methionine (PLM), and poly-L-trypotophan (PLT), were nutritionally available sources of the respective amino acids. The control diet provided all amino acids in the free L-form. Other diets deviated from the control by providing lysine as PLL, methionine as PLM, tryptophan as PLT, or by being void of lysine, methionine, or tryptophan. Chick trials lasted two weeks. Rat trials lasted nine days and included a nitrogen balance study. The rats and chicks fed the control and PLL diets grew, while those fed PLM, PLT, and diets void of an amino acid demonstrated depressed or no growth. These trials present evidence that PLL is an available source of lysine and PLM is not an available source of methionine. Due to difficulties in quantitative analysis, no conclusions can be drawn regarding PLT.

It is suggested from this data that protein quality could be improved by the incorporation of PLL into the protein. The incorporation of PLM would be of no value.

BIOAVAILABILITY OF THREE HOMOPOLY AMINO ACIDS IN GROWING RATS AND CHICKS

by

Nancy Creviston Dew

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Home Economics

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1982

APPROVAL

of a thesis submitted by

Nancy Creviston Dew

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

November 17, 1982
Date

Rosemary K Laurman
Chairperson, Graduate Committee

Approved for the Major Department

November 24, 1982
Date

Margaret Briggs
Head, Major Department

Approved for the College of Graduate Studies

12-6-82
Date

Michael P. Malone
Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his/her absence, by the Director of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Nancy Carrington Dunn

Date 12-2-82

ACKNOWLEDGEMENTS

"God never makes us conscious of our weakness except to give us of His strength."[1] My deepest thanks and praise go to Christ for providing the strength and talents necessary for completion of this goal.

I am grateful to Ross Laboratories for providing partial support of funds for this thesis project.

Knowing Dr. Rosemary Newman has been a rewarding component of my education and my sincere gratitude is due her for providing challenges and direction in conquering them. My thanks also go to Dr. C. W. Newman and Dr. J. M. Jaynes for sharing the idea of this project and for their guidance in experimental design and preparation of this thesis. Dr. Jacquelynn O'Palka also deserves thanks for her ideas concerning this thesis and accomplishment of career goals. Dr. N. J. Roth, Gayle Watts and Donna Soderberg are warmly thanked for their kindness and help during experimentation and laboratory analysis. I am thankful to Evelyn Richard for the proficient typing of this manuscript. My warmest appreciation is extended to Mrs. Elizabeth Bellingham and the food service staff of Bozeman Deaconess Hospital. Without their help and support, the completion of this degree would have been impossible.

I would like to thank my parents and family for their loving support and prayers. Most of all, I thank my husband, Randy, for his support, technical help, tireless understanding and invaluable encouragement.

---

[1] Apples of Gold, compiled by Jo Petty. C. R. Gibson, Co., Norwalk, CN.

TABLE OF CONTENTS

TABLE OF CONTENTS, Continued

LIST OF TABLES

LIST OF TABLES, Continued

LIST OF FIGURES

ABSTRACT

A basic application of genetic engineering to improve protein quality of a substance is the incorporation of homopoly amino acids (HPAAs) into the original protein. Growth trials were conducted with growing rats and chicks to determine if three HPAAs, poly-L-lysine (PLL), poly-L-methionine (PLM), and poly-L-trypotophan (PLT), were nutritionally available sources of the respective amino acids. The control diet provided all amino acids in the free L-form. Other diets deviated from the control by providing lysine as PLL, methionine as PLM, tryptophan as PLT, or by being void of lysine, methionine, or tryptophan. Chick trials lasted two weeks. Rat trials lasted nine days and included a nitrogen balance study. The rats and chicks fed the control and PLL diets grew, while those fed PLM, PLT, and diets void of an amino acid demonstrated depressed or no growth. These trials present evidence that PLL is an available source of lysine and PLM is not an available source of methionine. Due to difficulties in quantitative analysis, no conclusions can be drawn regarding PLT. It is suggested from this data that protein quality could be improved by the incorporation of PLL into the protein. The incorporation of PLM would be of no value.

Chapter 1

INTRODUCTION

The shortage of food proteins that contain balanced quantities of all the essential amino acids increases with expanding world population. This fact forces a growing number of people to become dependent on plant protein sources, all of which are deficient in one or more essential amino acids. Three of the most common limiting amino acids in plant proteins are lysine, methionine, and tryptophan. Populations whose diets depends on plants as the major protein source would benefit from supplementing the diet with amino acids that are limiting in plants. An obvious method of supplementation for the purpose of improving protein quality is simply adding the chemically synthesized L-amino acid to a food product. However, this is costly and the nutritional value of the amino acid may be lost in processing (Carpenter, 1973). Other methods for accomplishing this improvement in protein quality include: 1) fermented food products produced with an organism excreting increased quantities of the limiting amino acids; 2) addition of another substance, whose protein contains complimentary amounts of the limiting amino acids, into the diet; and 3) genetic modification of the protein itself. Recombinant DNA procedures and genetic engineering techniques are methods that are capable of producing a multitude of novel protein products.

Before such protein products can be introduced to a food supply market, their biological availability and possible toxicity must be determined. As some novel proteins do not occur naturally, their

biological value may be questionable and the possibility exists that normal gastrointestinal processes may not be effective in digesting and absorbing them. One elementary example of a genetically engineered protein now available for assay is the homopoly amino acid.

This thesis was designed to assess the biological availability of three homopoly amino acids, poly-L-lysine, poly-L-methionine, and poly-L-tryptophan. This was accomplished by analysis of growth data of weanling rats and chicks fed these peptide polymers as the sole source of the respective amino acid.

Chapter 2

LITERATURE REVIEW

Introduction

The search for economical sources of high quality protein is vigorous and includes exploration of conventional and unconventional protein sources and methods of modification for the purpose of improving their quality. One such method is the genetic alteration of a protein source which would result in a protein specifically engineered for high biological value for a specific animal species. The most basic alteration for improvement of an existing protein source would be the incorporation of polymers of the limiting amino acid, or a homopoly amino acid. The nutritional value of homopoly amino acids will be determined by their physiological availability. Physiological availability is partially determined by protein quality and by the fate of the protein in digestion and absorption.

Protein Quality

Several methods for determining protein quality have been developed. Biological value for an organism is based on the proportion of absorbed nitrogen retained, or the protein's ability to support growth and maintenance (Mitchell, 1923). Net protein utilization is a measurement combining biological value with the digestibility of the food protein (Bender and Miller, 1953). Protein efficiency ratio is simply determined by dividing an animal's weight gain by its protein intake (Osborne, et. al. 1919). Although these methods have

been extensively used, they do meet with criticism and different methods for measuring protein quality have been developed and evaluated (Pellett, 1978; Hegsted and Chang, 1965).

The method of the amino acid score involves comparison of the amino acid composition of food proteins to an amino acid reference pattern (FAO/WHO, 1973). A protein deficient in one or more limiting amino acids will produce a lower amino acid score than a protein with an adequate amino acid pattern. Amino acid imbalances, or amino acids in disproportionate amounts, can also have quality lowering effects on a food's protein value.

The most basic form of measuring the biological availability of a protein is the measurement of growth of an animal fed the protein in question versus a protein known to be of good value. The purpose of the measurement is not to determine the protein's quality per se, but to determine if the protein source can be digested and utilized by the animal.

Protein Digestion

Protein digestion is a product of a variety of factors, including pH, hormones, and enzymes. The pH of the environment is important for activation of a zymogen or for providing the optimal environment an enzyme needs to be functional. Hormone secretions serve as a stimulus for organ function and the release of enzyme rich secretions. The enzymes act as catalysts in the actual degradation of the consumed proteins to the end products of free amino acids and peptides. A more detailed discussion of these functions follows.

pH

The major function pH plays in the digestion of proteins is in its effect on the enzymes necessary to cleave the peptide bonds. In the stomach, a pH 5 or below is necessary for initial activation of pepsinogen to pepsin (Tang, 1976), which itself is then capable of catalyzing the reaction of pepsinogen to pepsin. When the stomach contents are emptied into the duodenum, the chyme must be neutralized or its acidity will render pancreatic enzymes inactive. Controlled by secretin and pancreozymin released from the duodenal wall, a mechanism exists which stimulates alkaline secretions from the pancreas that effectively neutralize the acid chyme from approximately pH 2 to 6.5 (Florey, et al. 1941; Harper, 1959) within the first fifteen inches of the duodenum (Iber, 1980).

Hormones

The presence of protein in the antral portion of the stomach stimulates the production of gastrin in the gastric mucosa (Woodward, 1960). Gastrin is absorbed into mucosal capillaries and causes the fundic portion of the stomach to secrete the highly acidic gastric juice (Jacob and Francone, 1974). As chyme enters the duodenum, the presence of polypeptides and acid stimulate the release of secretin and cholecystokinin-pancreozymin (CCK-PZ) by duodenal mucosa. Secretin stimulates the release of an enzyme deficient alkaline pancreatic juice which serves to dilute the acidity of the chyme. CCK-PZ stimulates the production and secretion of the enzyme rich pancreatic juices (Jacob and Francone, 1974). CCK-PZ may also be responsible for

stimulating the release of enterokinase by the intestinal mucosal epithelial cells (Kim and Freeman, 1977). The pancreatic juices reach the duodenum via the major pancreatic duct (Jacob and Francone, 1974).

Enzymes

The first enzyme involved in protein digestion is pepsin, whose activation of the low pH environment is the result of the liberation of a 44 residue peptide from the amino-terminal end of its zymogen (Tang, 1976). Pepsin, an acid (or carboxyl) protease, has broad specificity but does exhibit some partiality toward peptide bonds adjacent to the aromatic amino acids (phenylalanine, tyrosine, and tryptophan), methionine, and leucine (Sanger and Tuppy, 1951). The proteolytic activity of pepsin initiates protein digestion, producing shorter peptides, but few free amino acids.

After the stomach, the remainder of protein degradation occurs in the small intestine. The enzymes released from the pancreas are in the zymogen forms of trypsinogen, chymotrypsinogen, proelastase, and procarboxypeptidases (Gitler, 1964). Intestinal glands lining the crypts of Lieberkuhn produce enterokinase and aminopeptidase; the former is secreted from the gland and the latter is liberated from sloughed mucosal cells (Jacob and Francone, 1974).

Activation of Pancreatic Enzymes

The first and therefore most critical activation is that of trypsinogen by enterokinase. As the pancreatic juices enter the duodenum, enterokinase is secreted and hydrolyzes a lysine-

isoleucine peptide bond in trypsinogen, thus activiting it to trypsin (Yamashina, 1956). Trypsin is then capable of activating chymotrypsinogen and proelastase to the active enzymes of chymotrypsin and elastase. Trypsin also activates carboxypeptidase A and carboxypeptidase B from their zymogens.

Substrate Specificity

Trypsin generally hydrolyzes the peptide bonds adjacent to lysine or arginine. Chymotrypsin is preferential to those bonds involving the aromatic amino acids, phenylalanine, tyrosine, and tryptophan (Ryle and Porter, 1959). Elastase exhibits partiality to the neutral aliphatic amino acids (glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, and methionine) (Naughton and Sanger, 1961).

Made obvious by the name, carboxypeptidases cleave one amino acid at a time from the peptide starting from the terminal carboxyl group. Carboxypeptidase A will hydrolyze any carboxyl-terminal peptide bond except those involving lysine or arginine. Carboxypeptidase B specificaly cleaves only lysine and arginine residues from this site (Neurath, 1960). The aminopeptidases attack the peptide from the amino terminal end and hydrolyze peptide bonds removing one amino acid at a time (Smith, 1960).

Completion of Protein Digestion

The digestion of proteins continues as the luminal contents pass through the small intestine. Some peptidase activity has been

documented in the rat ileal luminal contents, probably due to the enzymes liberated from sloughed mucosa, and not to secreted enzymes from the pancreas or intestine itself (Silk et al., 1976). As the digestive enzymes break the appropriate peptide bonds and the protein sources become oligo-, tetra-, tri-, and di- peptides and more free amino acids, these products make their way to the mucosa lining the small intestine. The majority of protein digestion and absorption, which occurs prior to and in the upper jejunum, is rapid (Kim and Freeman, 1977). Nixon and Mawer (1970a), using human subjects, showed that some absorption from a milk protein meal occurred in the duodenum and that 80 percent of the dietary protein was absorbed in the first fifty to one hundred centimeters of the jejunum. However, some products of a protein meal were found in the human jejunal and ileal fluids four or more hours after ingestion (Adibi and Mercer, 1973).

Transport

Nixon and Mawer (1970b) found that after a milk protein meal, some amino acids (lysine, valine, arginine, tyrosine, phenylalanine, methionine, and leucine) were absorbed rapidly in the free form. However, proline, glycine and the dicarboxylic acids which remained peptide linked much longer, were shown in a different study (Nixon and Mawer, 1970a) to be absorbed. This finding led these authors to believe that transport, and subsequent absorption of these amino acids occurred in a peptide form. The following discussion will outline current knowledge and theories of transport mechanisms

concerning free amino acids and peptides.

Transport of Free Amino Acids-Specific Carrier Systems

Transport of free amino acids across the intestinal membrane is thought to occur with the use of specific carriers. There is some agreement that four specific transport systems (Table 1) exist, (Gray and Cooper, 1971; Saunders and Isselbacher, 1966). These systems are outlined in Table 1.

Adibi and Gray (1967) demonstrated that affinity for the neutral amino acid system is greatest for methionine, followed in decreasing order by isoleucine, leucine, valine, phenylalanine, tryptophan, and threonine.

Some structural aspects are important for all the transport systems to work, as illustrated in Figure 1 (Saunders and Isselbacher, 1966). The correct stereoisomerism is required; that is the L-form for all amino acids except methionine, in which case the D-form is also acceptable. The carboxyl group attached to the alpha carbon atom must remain free and can not be substituted. The alpha amino group must also remain free, however substitution of the hydrogen of the amino group is acceptable resulting in reduced transport. Amino acids with the amino group in the beta rather than alpha position will be accepted for proper transport (Randall and Evered, 1964).

Table 1. Intestinal Amino Acid Transport Mechanisms

Type	Amino acid transported	Type of transport	Relative rate
Neutral (Monoamino-monocarboxylic)	Aromatic (tyrosine, tryptophan, phenylalanine Aliphatic (glycine,[a] alanine, serine, threonine, valine, leucine, isoleucine) Methionine, histidine, glutamine, asparagine, cysteine	Active Na+-dependent	Very rapid
Dibasic (diamino)	Lysine, arginine, ornithine, cystine	Active, partially Na+-dependent	Rapid (10% of neutral)
Dicarboxylic	Glutamic acid, aspartic acid	Carrier-mediated, ?active, partially Na+-dependent	Rapid
Imino acids and glycine	Proline, hydroxyproline glycine[a]	Active, ?Na+-dependent	Slow

[a]Shares both the neutral and imino mechanism with low affinity for the neutral (Gray and Cooper, 1971).

Competition exists among the amino acids sharing each transport system (Christensen, 1963). The amino acid with the highest affinity for the carrier would have the highest absorption rate and would inhibit the transport of the amino acids with lower affinity. Other

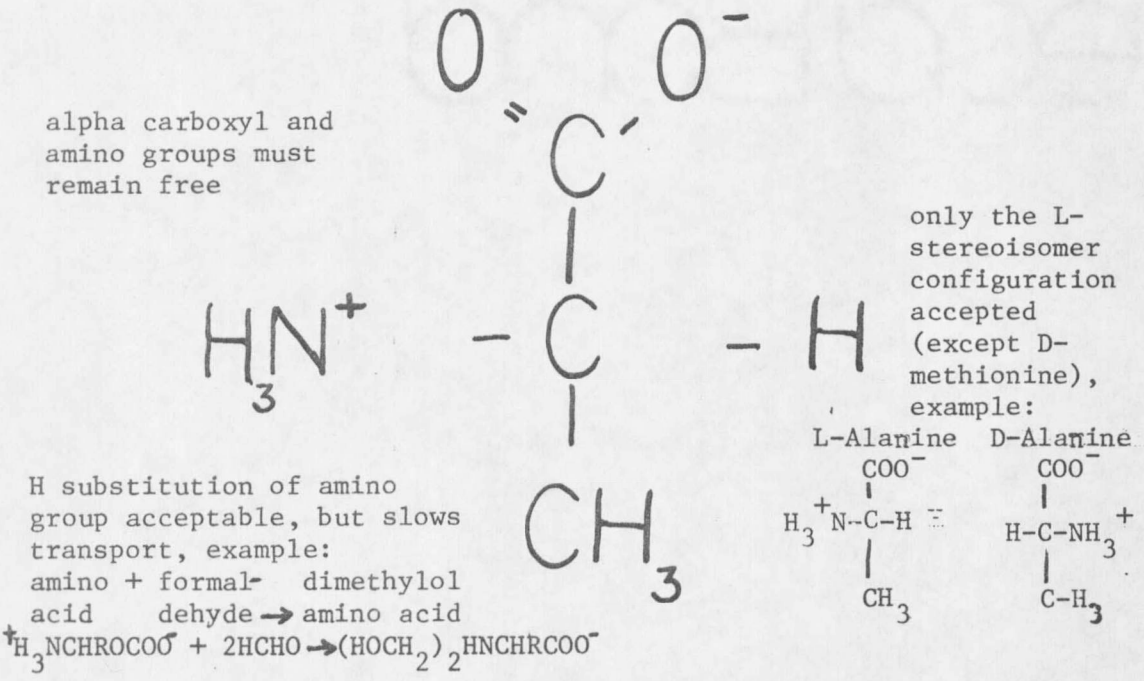

Figure 1. Structural Aspects Important to Function of Free Amino Acid* Transport System.

than affinity for the system, selection of an amino acid for transport is on a "first come, first serve" basis (Adibi and Gray, 1967), so amino acids present in limited amounts would have decreased opportunity for transport compared to those present in larger quantity.

Transport of Peptides

More is known regarding the transport of amino acids than is known about peptide transport partly because of the large number of amino acid combinations forming peptides.

Conflicting data exists concerning the number and differing qualities of carriers for peptides. Due to inconsistencies in experimental design and model peptides used, it is difficult to decide if peptide transport is a product of passive diffusion and a dependency on carriers with the possibility of competition existing among carriers, or if the latter alone is the case. Silk (1981) summarizes characteristics of di- and tri- peptide transport. First, the system of transport of unhydrolyzed peptides is different and separate from those systems for free amino acid absorption. Silk's reasoning for this comes from two pieces of evidence. The first stems from data gathered on individuals with genetic disorders of amino acid transport. Subjects with Hartnup's disease have a transport defect involving some neutral amino acids (histidine, leucine, tyrosine, tryptophan, phenylalanine, serine, methionine, glycine, and glutamine),

and those with cystinuria cannot transport cystine and dibasic amino acids (Matthews, 1975). Oral tolerance tests (oral ingestion followed by estimations of plasma amino acids) showed, in the case of Hartnup's disease, that affected amino acids are poorly absorbed as free amino acids, but absorption from peptides containing these amino acids was within the normal range (Asatoor et al., 1970; Navab and Asatoor, 1970). Similar results were obtained with studies of the defect of cystinuria (Hellier et al., 1972; Asatoor et al., 1972). These findings suggest that the defect is in the entry, rather than exit, mechanism of the mucosal cell, as an exit defect would impair the absorption of amino acids from peptides as well as from the free form (Matthews, 1975). The second evidence comes from studies which show that uptake of di- or tri- peptides is not significantly inhibited by the addition of large concentrations of free amino acids to peptide solutions (Adibi and Soleimanpour, 1974; Addison et al., 1975).

Competition for Transport

The studies on kinetics of peptide transport show conflicting results. Addision et al., (1974) used everted hamster jejunum to show that competition does exist between peptides for transport. They found that carnosine (β-alanyl-L-histidine) transport was inhibited by equimolar concentrations of glycylglycine, glyclyglyclyglycine, glyclysarcosine, glyclyproline, methionylmethionine, and prolyhydroxyproline. However, carnosine uptake was not inhibited by lysyllysine or α-glutamylglutamic acid, nor did the latter two peptides affect each other's uptake.

Similar work performed by Taylor et al., (1980), also with everted hamster jejunum, showed different results. In this study the pH was adjusted to pH 5 to reduce brush border and/or intramedium hydrolysis of lysyllysine. They found that at high concentrations, glyclysarcosine and lysyllysine would competively inhibit each other's uptake. A study by Matthews et al. (1979) with the same conditions showed competitive inhibition between glyclysarcosine and glutamylglutamic acid. The conclusion was reached that the neutral dipeptide glyclysarcosine, the acidic dipeptide glutamylglutamic acid, and the basic peptide lysyllysine all share one transport system. This demonstrates a great difference between peptide and amino acid transport which is affected by the net charge of the amino acid side chain (Schultz and Curran, 1970). In addition, Taylor et al. (1980) and Matthews et al. (1979) found that the influx of these peptides would not conform to simple Michaelis-Menton kinetics, as do free amino acids. This finding is suggestive of the theory that two different components are at work in the transport of these peptides.

Cytosol and Brush Border Peptidases

The barrier between the lumen and portal circulation is the intestinal membrane. As few peptides are found in the portal circulation, one may hypothesize that the peptides that are transported into the mucosal cells must be hydrolyzed there to their constituent amino acids. Two areas of the mucosal cell, the cytosol and the brush border fractions, have been shown to contain the highest

peptidase activity level (Kim and Freeman, 1977). The enzymes from these fractions are aminopeptidases (Peters, 1973) and their functions have been verified by several studies. Addison et al., (1975) used hamster jejunum; Peters (1973) experimented with guinea-pig intestinal mucosa; Nicholson and Peters (1978) and Nicholson and Peters (1979) performed trials with human jejunum. All of these studies demonstrated that di- and tri- peptides are mainly hydrolyzed by the cytosol fraction, whereas larger peptides are broken down by the brush border enzymes. Kim et al. (1972) described the physio-chemical properties and electrophoretic mobilities of these two amino peptidases. A summary of properties and substrate specifications is offered by Kim and Freeman (1977). Approximately 10 percent of dipeptide substrate activity occurs in the brush border but up to 60 percent of tripeptidase and nearly all of tetrapeptidase and hexapeptidase activity occurs in this fraction (Peters, 1973). The cytosol hydrolase specificity is nearly the opposite, with very little tetrapeptidase activity but up to 90 and 95 percent of tripeptidase and dipeptidase activity, respectively, as shown in Table 2.

Peptide Absorption

The function of the peptidases is to break down the peptides to constituent amino acids which can be absorbed into portal circulation. Described in this way, the functioning of the transport system begins in the lumen and ends once the peptides reach their appropriate peptidases in the cytosol or brush broder. Following hydrolysis,

Table 2. Comparison of the Properties of Intestinal Cytosol and Brush Border Peptide Hydrolases.

	CYTOSOL	BRUSH BORDER
Substrates	Percentage of Total Cellular Activity	
Dipeptides	80-95%	5-12%
Tripeptides	30-90%	10-60%
Tetra- and higher peptides	NIL to low	90%
Proline-containing dipeptides	Hydrolyzed	Not readily hydrolyzed
Chain length of substrate	2, 3, (4)	2, 3, 4, 5, 6, 7, 8 -
End terminal specificity	Amino peptidase	Amino peptidase

Adapted from Kim, Nicholson, and Curtis, 1974.

absorption occurs. This representation may not only be simplified, but may also find little or no agreement in the literature. In fact, a precise, commonly used definition of processes involved in absorption could not be found. As stated by Matthews (1975)

> Much confusion may arise from the use of the term 'absorption' by different authors in different senses. Absorption may be used to mean the whole process of absorption-removal from the intestinal lumen, transport across the intestinal wall, and entry into the blood or lymph, including any hydrolysis occurring during the process and its meaning may even include the process of intralumen digestion as in 'protein absorption.' It is also often used to mean only one part of the whole process....

Thus a distinct differentiation between transport and absorption is not available, as the former is frequently included in the latter.

The same proposed modes of peptide absorption are presented by

both Kim et al. (1974) and Silk et al. (1973). One method is by the direct trasnport system. The other is characterized by mucosal surface hydrolysis of peptides followed by absorption of the liberated amino acids. Silk (1981) and Matthews (1975) agree that a dual hypothesis utilizing both modes is more likely. Thus, peptides with a low affinity for brush border peptidases would be transported into the cell and those with a high affinity for these peptidases would be hydrolyzed and absorbed via one of the free amino acid transport systems.

All of the aforementioned experiments were performed using specific di-, tri-, tetra-, and hexa- peptides. The peptides studies may be chosen due to availability and expense, or their net charge; but generally these peptides were found in conventional protein sources.

Protein Sources

Common protein sources for human consumption are animal and plant proteins. Domestic animals are generally provided with cereals, legumes, and byproducts of the meat industry, such as blood meal, to meet their protein needs. Some unconventional proteins being developed for animal, and ultimately human, consumption are fish protein concentrate, leaf protein concentrate, solid wastes, and single cell protein.

Animal Protein Sources

Animal protein sources have been historically considered to be of good quality because they contain all the amino acids considered

essential to man. In 1935, the mixed commission of the League of Nations reported that for humans some quantity of animal protein was essential (Aylward and Jul, 1975). Although it is now recognized that adequately planned meat free diets can provide a complete protein source (Register and Sonnenberg, 1973), the superior protein quality of meat cannot be disputed.

The production of animal protein finds merit in that foods that are either unfit or undesirable to humans can be eaten by the animal and converted into an acceptable form of high quality protein (Jones, 1974a). The commercial production of animal protein finds controversy from those who believe that grains should be used directly for human consumption and not as feed for livestock production. McGill and Pye (1980) report that the production of one pound of beef, pork, turkey, chicken, and eggs requires 16, 6, 4, 3, and 3 pounds of grain and soy, respectively. The cost of feed constituents can also make the cost of feeding the animal prohibitive. Storage of animal protein presents another problem due to its sensitivity to biodeterioration (Jones, 1974a).

Plant Protein Sources

Unlike proteins from animal sources, those from plants do not contain all the essential amino acids, so their quality is dependent on the quantity and availability of one or more limiting amino acids. A brief discussion follows on cereals and legumes, including pulses, as sources of proteins.

Lysine is the first limiting amino acid of many cereals, including

wheat, barley, rice, millets, and sorghum. The location of the protein in regard to seed or kernel structures influences the quality of some cereals. For example, in barley and sorghum the protein prolamine is low in lysine causing a low lysine proportion in the total grain. In the maize kernel, the embyro is twice as high in protein as the endosperm; unfortunately the embyro is only 10 percent of the entire kernel. The content of crude fiber, branched polysaccharides (such as B-glucans), and tannins (polyphenolic compounds) reduce digestibility, lowering protein quality (Inglett, 1977).

Legumes and pulses differ from cereals in that they are first limiting in methionine, and second limiting in phenylalanine. With the exception of peanuts, legumes contain an adequate amount of lysine (Wolf, 1977). Along with proteins and other nutrients, legumes may also be a source of toxins or other detrimental substances unless properly treated. Protease inhibitors, such as trypsin inhibitor, have been found in many legumes, including raw soybeans, navy and lima beans and peanuts. Phytohemagglutinins, which agglutinate red blood cells, are present in raw soybeans, black, kidney, and mung beans. Goiterogens, cyanogens and substances with anti-vitamin and metal-binding properties found in various legumes are discussed by Liener (1975).

The combining of legumes with cereals will provide a complete protein source as the deficiencies of one are found in adequate amounts in the other. These complimentary proteins should be consumed together to assure the concurrent presence of all the

essential amino acids (Burr, 1975). The effect of combining legumes with cereal sources was demonstrated by Bressani (1973). Protein efficiency ratios (PER) were obtained using rats fed diets containing a cereal as the sole source of protein and then substituting the black bean for 10 percent of the cereal, without changing total protein of the diet. Protein efficiency ratios of each grain diet was 2.15, 0.87, 0.88, 1.05, and 1.60 for rice, maize, sorghum, wheat, and oats, respectively. The inclusion of the bean increased each PER to 2.32, 1.40, 1.39, 1.73, and 2.37, respectively.

Fishmeal and Fish Protein Concentrate

Fish meal is the solid fraction remaining from whole or fresh-fish discard after the oil has been pressed out (Jones, 1974b). The quality grade of fish meal is below the standard for human consumption, but can be used as a supplementary source of protein in animal diets (Aylward and Jul, 1975).

Fish protein concentrate (FPC) is the result of an attempt to produce an economical concentrated source of protein for humans that is resistant to biodeterioration. The constituents which must be removed for safe storage are water, to reduce microbial spoilage, and lipid, to reduce oxidative changes (Tannenbaum, 1971). A discussion of problems in utilizing FPC for human consumption is presented by Richardson (1975). Fish protein concentrate does hold advantages as it provides an inexpensive, stable protein with an excellent balance of essential amino acids. It is high in

lysine with tryptophan, threonine, and sulphur amino acids in adequate amounts.

Leaf Protein Concentrate

The process of deriving protein concentrate from forage plants supplies a new source of protein for monogastrics by separating the fibrous, lignin containing portion of the plant from the proteinous portion. Alfalfa has been subjected to this process and studied extensively as a source of leaf protein concentrate (LPC) due to its high protein yield, its non-requirement of annual seeding and cultivation (Jorgensen, 1975), and as it is the best forage crop for large areas of the world (Kohler and Lyon, 1977).

Soluble white LPC, one fraction of alfalfa concentrate, has nitrogen digestibility and protein efficiency ratios comparable to casein; however, improved values for these parameters were obtained when the soluble white LPC was supplemented with methionine and lysine, the first and second limiting amino acids. The addition of bisulfite after grinding and before juice expression produced soluble white LPC values greater than casein (Bickoff et al., 1975). The bisulfite inhibits the oxidation of polyphenols to quinones, which react with the protein (Kohler and Lyon, 1977) and leads to a breakdown of some essential amino acids.

A disadvantage of LPC is that different varieties of leaves contain undesirable materials (Kohler and Lyon, 1977). Leaf protein concentrate has, however, been found to be nutritionally available to rats, poultry, swine, sheep, and dairy calves (Jorgensen, 1975)

and will undoubtedly find a permanent place in the protein market.

Wastes As Protein Sources

The recycling of wastes from agricultural, domestic and industrial sources can provide protein for animal consumption (Wedin and Hodgson, 1980). Sunde (1975) fed dried poultry waste (DPW) at concentrations from 10 to 30 percent of the total diet at the expense of soybean meal, to pullets and laying hens. The presence of DPW in the diets moderately decreased the efficiency of feed conversion and slightly lowered body weight. No effect was detectable on egg size, production, or mortality. The protein in DPW was also found to be acceptable to sheep. Jones (1974c) reported that 10 percent of the waste from chicken farms in the United Kingdom was being fed to ruminants providing 2 percent nitrogen, or 12 to 14 percent protein equivalent in rumens.

McGovern (1975) reported on the use of waste and residues of the forest industry as substrates for conversion to protein. This would occur by using microorganisms capable of using the partially delignified cellulose of the wood as a carbon source. The microorganisms would then be used as single cell protein.

Single Cell Protein

The microorganisms being considered as sources of single cell protein (SCP) include yeast, bacteria, algae, and fungi. Providing protein from one of these sources has many advantages over the conventional supplies of protein, plants and animals. Microorganisms

can increase their mass exponentially due to a very short generation time. They are excellent sources of a variety of vitamins and minerals depending on the organism. They can be easily subjected to genetic modification and are relatively high in protein, containing between seven and twelve percent nitrogen on a dry weight basis. Their production can be based on a wide variety of raw materials, with no dependency on agricultural input, may be performed in a relatively small area, and will produce a limited amount of waste requiring disposal (Kihlberg, 1972).

Kihlberg reported on many studies that have been performed testing the nutritional value of the microorganisms mentioned above in rats, chicks, swine, and man. The bulk of this research has involved yeast feeding trials. More recent work (Schulz and Oslage, 1976) has focused on animal growth trials using yeast and bacteria grown on unconventional nutrient substrates such as methanol, ethanol, alkanes, aldehydes, organic acids, or the mixture of hydrogen and carbon dioxide. Since the nutrient composition of the substrate affects that of the organism, those organisms grown on the new substrate sources must be tested to determine nutritional value and the possibility of toxicity (deGroot, 1976).

A problem of digestibility in feeding trials using any SCP is created by the organism's cell wall. Tannenbaum and Miller (1967) improved the apparent digestibility of B. Megaterium from 56 to 67 by breaking the cell wall. The cell wall can be broken by heating (ie., cooking or autoclaving), drying, acid hydrolysis, or use of

enzymes; however, each of these methods can also be responsible for deteriorating protein quality. Another problem concerning nutritional value of SCP is the high content of nucleic acids, which is approximately proportional to growth rate of the cell mass (Schulz and Oslage, 1976). Consumed nucleic acids are depolymerized and converted to nucleosides in the intestine (Kihlberg, 1972). After absorption, the nitrogen of the purine and pyrimidine bases may be used again for nucleic acid synthesis, or it may be metabolized to uric acid (in man and poultry) or allantoin (in some mammals and reptiles). In species possessing the enzyme uricase which oxidizes uric acid to allantoin, a high nucleic acid consumption is not a problem as allantoin is soluble and an easily excretable metabolite. In species not possessing this enzyme, high consumption can lead to gout, evidenced by increased levels of urine uric acid and plasma uric acid, resulting in urate precipitating in tissues and joints (Tannenbaum, 1971).

Fungi

Fungi as a protein source is discussed by Wu and Stahmann (1975). Higher fungi, mushrooms, are readily accepted by consumers world wide. The protein content of mushrooms has been reported to be half that of yeast, but the proportion of protein in the fungi can be manipulated by the carbon to nitrogen ratios of the substrate. One of the greatest advantages of fungi is that over 1,000 species

are capable of degrading lignin. The use of fungi in combination with organisms of higher protein yield on lignocellulosic substances could be a viable alternative to chemical or enzymatic delignification techniques to make these carbon sources available. Another alternative is that once the fungi decomposes the lignin, the fungi could be used as a human protein source, feeding the remaining cellulose to animals. VanderWal (1976) reported on SCP of fungal origin being fed to chickens and pigs. The nitrogen digestibility coefficient for the fungi in chickens was 59.3 and in pigs, 71.0.

Algae

The use of algae for human consumption appears to be common in some nations, including Japan and Mexico. In Japan, chlorella is processed into tablets or extracts in the amounts of 500 tons per year as reported by Jones in 1974(d). The algae spirulina has been harvested and added to food products. Cultured algae could be used with no further processing as a slurry in fish farms. An added advantage to the use of algae as a nutritional source for animals and humans is that by using sewage or industrial waste as substrate, human water supplies can be spared or cleaned (Jones, 1974d). Disadvantages of these substrates are the possible incorporation of toxic substances and the indigestibility of cell walls. Both of these problems would be alleviated if the algae protein were isolated and extracted (Mitsuda et al., 1969).

Yeasts

Of the SCP sources mentioned, yeast has been given the most attention and the best public acceptance, as it has been functional in foods and beverages for a very long time. Yeast is high in lysine and contains adequate amounts of other essential amino acids except methionine and cysteine (VanderWal, 1976). A recent study (Ashraf, 1981) demonstrated the value and safety of yeast as a protein source. Yeast SCP, supplemented with methionine, was used to replace 50 or 100 percent of soybean meal in a multigeneration rat study. Results indicated that yeast as a source of essential amino acids was available and could support normal fetus growth, milk production, post-weaning growth and body weight maintenance of dams. Schulz and Oslage (1976) demonstrated that both strain of yeast (or bacteria) used and type of substrate affect nutritive value.

Bacteria

Pseudomonas grown on methanol produced good apparent digestibility (87.2%) and utilizable protein (62.2%) values for rats (Schulz and Oslage, 1976).

Agren et al. (1974) used rats to test the value of yeast and bacterial SCP grown on a chemically pure hydrocarbon fraction, using casein and commercial stock diets as controls. Yeast had no effect on mortality, general condition or behavior of the rats; however,

bacterial SCP caused weight loss, bleeding at the nose and eyes, increased mortality, and organ abnormalities. The striking difference between this and the study by Schulz and Oslage (1976) is probably due to the differences of substrate and strain.

McCoy (1975) discussed the use of aerobic thermophilic bacteria as economical and nutritive sources of SCP. Bacteria are at a disadvantage compared to other SCP sources due to susceptibility to phage, high nucleic acid content, and small size causing high cell recovery cost. Their advantages include faster growth rate and higher total protein and sulphur amino acid contents compared to other SCP sources. Since DNA sequencing information was established with the use of the bacterium, Escherichia coli, this bacterium is frequently used for study concerning recombinant DNA and construction of desired polypeptides (Wetzel, 1980).

Genetic Engineering of Proteins

Supplementing a protein quality by changing it genetically to include more of the limiting amino acid is now a realistic alternative to conventional protein supplementation techniques. One way to achieve this improved protein product would be to insert repeating codons for the limiting amino acid into the DNA from which the plant's protein is produced. When expressed, these repeating codons would result in polymers of this amino acid, a homopoly amino acid, being produced along with the original protein to jointly become the improved protein.

Kangas et al. (1982) were successful in incorporating repeated codons for proline in E. coli. When expressed, this alteration resulted in a protein containing polymers of proline. The assumption was made by these authors that this would improve protein quality; however, the nutritional value was not tested. A suggestion was presented that this method, insertion of repeating codons for an amino acid, could be used to increase the amount of any limiting amino acid to improve protein quality. An illustration cited was the use of poly-L-methionine enriched single cell protein (such as E. coli) to supplement soybean feed for poultry. The simplest available subject for the genetic engineer to work with would be a single cell organism such as E. coli. As single cell proteins are currently used in the animal feed industry to supplement grain proteins, the suggestion made by Kangas et al. is a viable one. These authors have demonstrated a procedure of inserting repeated codons that is reliable; however, adequate bioavailability of the produced protein has not been shown.

Homopoly Amino Acids

The product of repeating codons, a homopoly amino acid, is a polymer consisting solely of that particular amino acid. This type of structure does not occur naturally and a minute amount of research has been done using a homopoly amino acid as the subject. Newman, et al. (1980) fed poly-L-lysine to growing rats to test its nutritive

value and found that the growth of these rats was not significantly different from the growth of those fed the free amino acid, L-lysine. This research was the first published evidence that homopoly amino acids are biologically available.

Boebel and Baker (1982) fed poly-L-methionine to chicks with strikingly different results. They found this homopoly amino acid to have no, or extremely limited, biological availability. The difference between these two studies may be due to species specificity. Another possible explanation could be that these synthetic protein sources are unique substances which may or may not be biologically available to any species due to the presence or absence of enzymes and/or transport systems.

Chapter 3

MATERIALS AND METHODS

Homopoly Amino Acids

Poly-L-lysine (PLL), poly-L-methionine (PLM), and poly-L-tryptophan (PLT), were synthesized by Sigma Chemical Company.[1] The molecular weights (as stated by Sigma) were 4,000 to 15,000, 30,000 to 50,000, and 15,000 to 50,000 for PLL, PLM, and PLT, respectively. The average number of amino acid units in one molecule were 41, 285, and 167 units of L-lysine (Lys), L-methionine (Met) and L-tryptophan (Trp), respectively. Poly-L-lysine was purchased as a precipitated hydrogen bromide salt. The bromide was removed by dissolving the peptide in 100 ml of water and filtering the solution through a Diaflow ultra-filtration apparatus with a 10,000 daltons membrane filter. When the volume had decreased to 10 ml, it was again diluted to 100 ml and filtered a second time. The ultra-filtrate was than lyophilyzed.

When calculating diets, the amount of each homopoly amino acid (HPAA) added was adjusted to the difference of adding the polymer versus the free amino acid due to the weight of the water molecule released for each peptide bond formed. Each HPAA was analyzed for purity by Sigma Chemical Company. The PLL contained 65 percent Lys, PLM contained 96 percent Met, and PLT contained 25 percent Trp.

---

[1] Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri 63178.

Rat Trials - General

Weanling female Holtzman Sprague-Dawley rats were individually housed and for two days prior to trial initiation were provided water and fed ad libitum a diet consisting of autoclaved cornmeal, casein, corn oil, vitamin and mineral supplements, calcium carbonate, and antibiotic (Table 3).

Table 3. Percentage Composition of Initial Diet Fed to Rats Prior to Trials 1 and 2.

Item	Percent
Cornmeal, autoclaved	84.07
Casein	6.00
Corn oil	5.00
Vitamin mixture[1]	2.00
Mineral mixture[2]	2.00
Calcium carbonate[3]	0.80
Antibiotic[4]	0.03
Total	100.00

[1]Vitamin diet fortification mixture, ICN nutritional biochemicals, Cleveland, Ohio.

[2]Salt mixture, bernhart tomarelli - modified. ICN nutritional biochemicals, Cleveland, Ohio.

[3]Reagent grade.

[4]Oxytetracycline, 200 grams per kg.

Rats were then allotted into seven treatment groups based on initial weight. The average weight of each treatment group was 65.6 grams and varied no more than ± 0.5 grams. The rats were assigned cage positions in such a manner as to assure that no variation due to cage location occurred. In both trials 1 and 2, four rats were fed one of seven diets, thus combined there were eight subjects per diet. Trial 2 was a replication of trial 1, the only difference being the diets for both trials were prepared prior to trial 1 so that the ration mixtures were stored under refrigeration for five weeks before being fed in trial 2.

Water was provided ad libitum and feed was restricted to ten grams per day during the test period. The rats were housed in the cages described for the growth trial (see Environmental Conditions) for the first five days of the trial. On the sixth day, they were moved to the cages described for the nitrogen balance trial, where they remained until the end of the nine day trial. The growth data were based on the entire nine days, with the first five days being an adaptation period and the final four days the nitrogen balance study. On the final day of each trial, rats were sacrificed by inducing hypoxia using carbon dioxide as the oxygen replacement.

Environmental Conditions

Rats were housed in individual woven wire cages in an environmentally controlled room at 22°C with light automatically regulated to provide twelve hours of continuous light and twelve hours of continuous darkness. Cages for the growth trial were contained on

a rack with six vertical and five horizontal rows on each side. Feed was placed in a metal cup with a hole in the cover, contained in an open ceramic crock to reduce wastage and allow for daily weigh back of unconsumed feed. Fresh water was provided daily in glass bottles with sipping tubes.

Standard metabolism cages were used for the nitrogen balance study. These were located on racks with four vertical and two horizontal rows on each side. Feed was provided in a glass cup on the outside of the cage to which entry was made available via a metal tunnel, and water was provided as described above.

Diets

All diets (Tables 4 and 5) were purified and identical except for the source or lack of one specific amino acid. Diet 1 provided all amino acids in the free L-form. Diet 2 provided Lys solely as PLL. Diet 3 provided Met solely as PLM. Diet 4 provided Trp solely as PLT. Diets 5, 6, and 7 were Lys free (LF), Met free (MF), and Trp free (TF), respectively. Glutamic acid was used as the nitrogen replacement in the last three diets to compensate for the absent amino acid. Cysteine was used in all diets to provide 25 percent of the sulfur amino acid requirements. All diets were formulated to provide 10 percent protein and at least 100 percent of NRC guidelines for all known essential nutrients (NRC, 1979). Nitrogen (N) determination of each diet was made by Kjeldahl analysis and was multiplied by 6.25 for protein content of the diet.

Table 4. Percentage Composition of Diets Fed to Rats in Trials 1 and 2 Testing the Nutritional Value of Three Homopoly Amino Acids vs L-amino Acids.

Diets	Percent									
	Premix[1]	Poly-L-Lysine[2]	Poly-L-Methionine[2]	Poly-L-Trypophophan[2]	L-Lysine HCl[3]	L-Methionine	L-Cysteine[4]	L-Tryphtophan	L-Glutamic Acid	Cornstarch
Control	25.53	-	-	-	0.87	0.45	0.15	0.15	-	72.85
Poly-L-Lysine	25.53	0.615	-	-	-	0.45	0.15	0.15	-	73.105
Poly-L-Methionine	25.53	-	0.396	-	0.87	-	0.15	0.15	-	72.904
Poly-L-Tryptophan	25.53	-	-	0.137	0.87	0.45	0.15	-	-	72.863
Lysine Free	25.53	-	-	-	-	0.45	0.15	0.15	0.70	73.02
Methionine Free	25.53	-	-	-	0.87	-	0.15	0.15	0.45	72.85
Tryptophan Free	25.53	-	-	-	0.87	0.45	0.15	-	0.15	72.85

[1] For composition of premix, see Table 5.

[2] Percentage of diet based on respective amino acid requirement adjusted to new molecular weight of polymer versus the free amino acid. Molecular weight and average number of units per polymer are 4,000 to 15,000 and 41 for poly-L-lysine, 30,000 to 50,000 and 285 for poly-L-methionine, and 15,000 to 50,000 and 167 for poly-L-tryptophan.

[3] 0.87% L-lysine-HCl provides 0.70% L-lysine.

[4] 0.15% L-cysteine equals 25% of sulphur amino acid requirement.

Table 5. Composition of Premix Incorporated in Rat Diets.

Item	g
Corn oil	5.00
Alphacel[1]	5.00
Mineral mixture[2]	2.00
Vitamin mixture[3]	2.00
Calcium carbonate[4]	0.80
Antibiotic[5]	0.13
L-Arginine	0.60
L-Asparagine - H_2O[6]	0.45
L-Glutamic acid	4.00
L-Histidine	0.30
L-Isoleucine	0.50
L-Leucine	0.75
L-Phenylalinine	0.40
L-Tyrosine	0.40
L-Proline	0.40
L-Threonine	0.50
L-Valine	0.60
L-Glycine	0.57
L-Alanine	0.57
L-Serine	0.56
Total	25.53

[1]ICN nutritional biochemicals non-nutritive cellulose.

[2]Salt mixture bernhart tomarelli - modified. ICN nutritional biochemicals, Cleveland, Ohio.

[3]Vitamin diet fortification mixture, ICN nutritional biochemicals, Cleveland, Ohio.

[4]Reagent grade.

[5]Oxytetracycline, 200 grams per kg.

[6]Provides 0.40 percent asparagine.

Growth and Nitrogen Balance Trials

Rats were weighed every morning at which time the feed not consumed was measured prior to giving the daily portion. Data collected concerning weight gain and feed consumption were used to calculate protein efficiency ratio (PER), feed/gain (F/G), and nitrogen intake (NI). Calculations were made individually and then averaged per treatment group.

After the five day adjustment period the rats were moved to metabolism cages with collection funnels. The tip of the collection funnel was positioned so the urine would be collected in a 125 ml flask containing 25 ml of 5 percent sulfuric acid. A small amount of glass wool was positioned in the top of the flask so that feces could not drop into the urine flask. This flask was placed in a 600 ml beaker containing 50 ml of 5 percent sulfuric acid which was the collection container for the feces. In both cases, the 5 percent sulfuric acid was used to prevent ammonia N losses. The collection funnels were rinsed with water twice daily.

At the end of each trial, the bottom of the cages and the collection funnels were rinsed with water, which was collected in the urine flasks. Urine samples were covered and refrigerated. The feces were digested and homogenized by adding 100 ml of concentrated sulfuric acid to each beaker containing collected feces, which was then loosely covered and held at room temperature until analysis could be performed. Nitrogen determination was made on all samples by Kjeldahl analysis. Urine was diluted with water to 200 ml in

volumetric flasks and a 15 ml aliquot taken as a sample. Digested feces were diluted with water to 500 ml in volumetric flasks, using 100 ml aliquots as samples. Data gathered from these analyses were used to calculate total urinary nitrogen (UN) loss and total feces nitrogen (FN) excretion.

Values obtained were used to compute true digestibility (TD), biological value (BV), and net protein utilization (NPU). The calculations were made individually and averaged for treatment groups. Metabolic fecal nitrogen (MFN) was calculated as 1.35 mg N/gm dry matter. Endogenous urinary nitrogen (EUN) was considered to be 16.14 mg/rat/day. These values were made available by Stobart (1977).

Liver Data

Livers were removed from all rats immediately following sacrifice. Individual livers from both trials were immediately weighed, frozen and later subjected to N determination by Kjeldahl analysis. Liver weights were used to calculate liver as a percent of body weight. Nitrogen determinations were used to express the percent N of liver.

Chick Trials - General

Newly hatched broilers were obtained from the H & N Company, Redmond, Washington. Upon arrival, chicks were randomly assigned to treatment and control groups, banded and given their assigned diet. Water was provided ad libitum overnight, with the growth trial beginning the following day.

Trial 3 involved feeding the control, PLL, LF, PLT, and TF diets each to eight chicks. In trial 4, seven chicks were fed the control diet and eight were fed the PLM and MF diets. Due to a phenomenon observed in trial 3, a fifth trial was initiated to test the effect of the absence or source of tryptophan on the duration of heartbeat following death. Treatment groups were housed in community cages of a battery brooder. Feed and water were provided ad libitum. On the final day of all trials, the chicks were sacrificed by cervical dislocation.

Environmental Conditions

The trials were conducted in a room thermostatically controlled at 27°C, with continuous lighting. Electrical heating units in each cage provided warmth up to 35°C. The community cages were constructed of woven wire sides and bottoms. Newspaper was placed in the bottom of the cages the first five days of the trials to provide more consistent flooring.

Diets

The purified diets (Tables 6 and 7) were formulated to meet at least 100 percent of the NRC requirements (NRC, 1977) for all known essential nutrients. Major diet constituents were cornstarch, corn oil, free amino acids, vitamin and mineral supplements. All diets were identical except for the source or absence of test amino acids. As suggested by Sasse and Baker (1973), L-proline was added at 0.4 percent of the diet at the expense of glutamic acid. Cysteine

Table 6. Percentage Composition of Diets Fed to Chicks in Trials 3 and 4 Testing the Nutritional Value of Three Homopoly Amino Acids vs L-amino Acids.

Diets	Percent									
	Premix[1]	Poly-L-Lysine[2]	Poly-L-Methionine[2]	Poly-L Tryptophan[2]	L-Lysine HCl[3]	L-Methionine	L-Cysteine[4]	L-Tryptophan	L-Glutamic Acid	Cornstarch
Control	40.674	-	-	-	1.50	0.70	0.23	0.23	-	56.666
Poly-L-Lysine	40.674	1.054	-	-	-	0.70	0.23	0.23	-	57.112
Poly-L-Methionine	40.674	-	0.616	-	1.50	-	0.23	0.23	-	56.75
Poly-L-Tryptophan	40.674	-	-	0.21	1.50	0.70	0.23	-	-	56.686
Lysine Free	40.674	-	-	-	-	0.70	0.23	0.23	1.20	56.966
Methionine Free	40.674	-	-	-	1.50	-	0.23	0.23	0.70	56.666
Tryptophan Free	40.674	-	-	-	1.50	0.70	0.23	-	0.23	56.66

[1] For composition of premix, see Table 7.

[2] Percentage of diet based on respective amino acid requirement adjusted to new molecular weight of polymer versus the free amino acid. Molecular weight and average number of units per polymer are 4,000 to 15,000 and 41 for poly-L-lysine, 30,000 to 50,000 and 285 for poly-L-methionine, and 15,000 to 50,000 and 167 for poly-L-tryptophan.

[3] 1.50 % L-lysine-HCl provides 1.20% L-lysine.

[4] 0.23% L-cysteine equals 25% of sulphur amino acid requirement.

Table 7. Composition of Premix Incorporated in Diets Fed to Chicks.

Item	g
Corn oil	10.00
Alphacel[1]	3.00
Mineral mixture[2]	4.00
Vitamin mixture[3]	1.10
Sodium bicarbonate[4]	1.50
70% Choline chloride solution[5]	0.1785
Selinium mixture[6]	0.05
Zinc carbonate - sucrose mixture[7]	0.05
Ferric citrate	0.03
Manganese sulfate	0.003
L-Arginine	1.44
L-Glycine	1.5
L-Histidine	0.45
L-Isoleucine	0.80
L-Leucine	1.35
L-Tyrosine	0.62
L-Phenylalanine	0.72
L-Threonine	0.75
L-Valine	0.82
L-Proline	0.40
L-Glutamic acid	11.89
Total	40.674

[1]ICN nutritional biochemicals non-nutritive cellulose.

[2]Salt mixture bernhart tomarelli - modified. ICN nutritional biochemicals, Cleveland, Ohio.

[3]Vitamin diet fortification mixture, ICN nutritional biochemicals, Cleveland, Ohio.

[4]Reagent grade.

[5]Provides 1.04 grams choline per kilogram feed.

[6]Selinium limestone mixture, 0.05% selinium, 36.0% calcium, provides 0.1 mg selinium per kilogram feed.

[7]58 grams zinc carbonate (62.15% Zn) to 300 grams sucrose.

provided 25 percent of the sulfur amino acid requirement. In trials 3 and 4, diet 1 was the control and provided all amino acids in the free L-form. Diet 2 provided Lys solely from PLL. Diet 3 provided Met solely from PLM. Diet 4 provided Trp solely from PLT. Diets 5, 6, and 7 were LF, MF, and TF, respectively. All diets were formulated to contain 23 percent protein. Glutamic acid was added in diets 5, 6, and 7 to replace N from the absent amino acids. Nitrogen determination was performed for all diets exactly as described for the rat diets.

Growth Trials

For two weeks, chicks were individually weighed and feed consumption measured each day prior to adding additional feed. As an entire group was housed in one cage, feed consumption data reflects the group consumption and was divided by the number of chicks per group for individual values. PER and F/G were calculated from weight gains and averaged feed consumption in all groups.

Liver Data

Immediately following sacrifice, livers were removed and individually frozen. Frozen livers were weighed and analyzed for N content by Kjeldahl analysis. Individual liver weights were used to calculate liver as a percent of body weight. Nitrogen determinations were used to express the percent N of liver.

Heartbeat Experiment

Trial 5 included the feeding of a diet void of Trp and other

diets providing the amino acid in the free L-form, as PLT, and a combination of these two, or as the dipeptide, tryptophyl-L-tryptophan (Table 8). The diets were identical to those fed for the growth trials, deviating only in the source or absence of Trp.

Trial 5 was designed to measure the occurrence of continued heartbeat following death, and to investigate if this occurrence could be correlated with the presence of PLT in the diet. Five chicks were assigned to each of the diets above. Individual weights and group feed consumption were measured each day of the nine day trial.

Hearts were observed immediately following death and until palpitations ceased. Every five minute period of beating was assigned a value of one, thus a heart continuing to beat for twenty minutes was given a value of 4. Individual values were then averaged per treatment group. Weight gain and averaged feed consumption data were used to calculate F/G and PER for all groups.

Statistical Analysis

The data were analyzed by analysis of variance (Nie et al., 1975) and means separated by Newman-Keuls multiple range test (Snedecor and Cochran, 1967) when significant differences were detected.

Table 8. Percentage Composition of Diets Fed to Chicks Comparing Various Sources or Absence of Tryptophan in Relation to Duration of Heartbeat Following Death.

Diets	Percent								
	Premix[1]	Poly-L-Trypho-phan[2]	L-Lysine HCl[3]	L-Meth-ionine	L-Cys-teine	L-Try-ptophan	Trypto-phyl-L-Tryptophan	L-Glu-tamic Acid	Corn-starch
Control	40.674	-	1.50	0.70	0.23	0.23	-	-	56.666
Poly-L-Tryptophan	40.674	0.21	1.50	0.70	0.23	-	-	-	56.686
Tryptophan Free	40.674	-	1.50	0.70	0.23	-	-	0.23	56.666
Poly-L-Tryptophan	40.674	0.21	1.50	0.70	0.23	0.23	-	-	56.456
Tryptophan-L-Trypto-phan	40.674	-	1.50	0.70	0.23	-	0.23	-	56.666

[1]For composition of premix, see Table 7.

[2]Percentage of diet based on tryptophan requirement adjusted to new molecular weight of polymer versus the free amino acid. Molecular weight and average number of units per polymer are 15,000 to 50,000 and 167 for poly-L-tryptophan.

[3]0.87% L-lysine-HCl provided 0.70% L-lysine.

Chapter 4

RESULTS

Rat Growth Trials 1 and 2

The combined data of rat growth trials 1 and 2 are shown in Table 9. No difference ($P > 0.05$) was detected in the weight gain of rats fed PLM, PLT, LF, MF, or TF diets. Weight gain of rats fed the control and PLL diets was higher ($P < 0.05$) than the gain of rats fed the other diets with the control diet gaining faster ($P < 0.05$) than those fed the PLL diet.

Total feed consumption per rat was not different ($P > 0.05$) for the control and PLL groups, however consumption in these groups was higher ($P < 0.05$) than that of all other diet treatments. Total feed consumption was not different ($P > 0.05$) between PLM and MF fed rats, yet values from these two groups were lower ($P < 0.05$) than those from the LF and TF groups, the latter two being similar and not different ($P > 0.05$). Total feed consumption of rats fed the PLT diet was not different ($P > 0.05$) from those fed PLM, MF, LF, or TF diets.

The percent protein of the diets fed to rats in trials 1 and 2 is given in Table 10. Analysis of data collected concerning F/G, PER, and NI produced identical results for all three measurements. No difference ($P > 0.05$) was noted between the control and PLL diet fed rats, however these two groups produced higher values than all other groups. The values for all three measurements were not different ($P > 0.05$) among the PLM, PLT, LF, MF, and TF groups.

Table 9. Comparative Performance[1] of Rats Fed Three Homopoly Amino Acids vs L-amino Acids in Synthetic Diets, Trials 1 and 2.

Treatments	Number of Rats	Body Weight Gain	Feed Consumed	Nitrogen Intake	Feed/ Gain	PER[2]
		g	g	g	kg/g	
Control	8	$23.0^{a}\pm2.56$*	$85.37^{a}\pm5.18$	$0.6427^{a}\pm0.04$	$3.74^{a}\pm0.36$	$2.59^{a}\pm0.24$
Poly-L-Lysine	8	$17.25^{b}\pm2.43$	$88.12^{a}\pm2.03$	$0.6464^{a}\pm0.00$	$5.19^{a}\pm0.70$	$1.94^{a}\pm0.28$
Poly-L-Methionine	8	$-5.12^{c}\pm2.17$	$38.5^{c}\pm7.07$	$0.3130^{b}\pm0.03$	$9.74^{b}\pm6.74$	$-1.23^{b}\pm0.54$
Poly-L-Tryptophan	8	$-5.62^{c}\pm1.51$	$44.75^{bc}\pm4.13$	$0.3172^{b}\pm0.04$	$8.51^{b}\pm2.44$	$-1.24^{b}\pm0.40$
Lysine Free	8	$-6.00^{c}\pm2.00$	$54.62^{b}\pm5.97$	$0.3783^{b}\pm0.06$	$10.17^{b}\pm4.05$	$-1.13^{b}\pm0.37$
Methionine Free	8	$-7.25^{c}\pm1.67$	$37.87^{c}\pm3.72$	$0.3019^{b}\pm0.04$	$5.54^{b}\pm1.67$	$-1.91^{b}\pm0.54$
Tryptophan Free	8	$-5.75^{c}\pm2.38$	$52.62^{b}\pm6.8$	$0.3660^{b}\pm0.06$	$11.74^{b}\pm8.97$	$-1.14^{b}\pm0.52$

^{a,b,c}Means in the same column with unlike superscripts are significantly different ($P < 0.50$).

*Standard deviation.

[1]Means represent combined valued from Trials 1 and 2.

[2]Protein efficiency ratio.

Table 10. Percent Protein[1] of Diets Fed to Rats in Trials 1 and 2 Testing the Nutritional Value of Three Homopoly Amino Acids vs L-amino Acids.

	Con-trol	Poly-L Lysine	Poly-L Methio-nine	Poly-L-Trypto-phan	Lysine Free	Methio-nine Free	Trypto-phan Free
Percent Protein[2]	10.99	10.68	10.68	10.88	10.32	10.75	10.53

[1]All values given on a dry matter basis.

[2]Protein determined by Kjeldahl analysis.

Rat Nitrogen Balance Trials 1 and 2

The combined data of rat nitrogen balance trials 1 and 2 are shown in Table 11. The highest UN values, which were also similar ($P > 0.05$), were produced by rats fed the PLL and LF diets. These values were different ($P < 0.05$) than the lowest values representing the control, PLM, and MF diet fed rats, which were also similar ($P > 0.05$). Rats fed PLT and TF diets produced a medium set of values that were not different ($P > 0.05$) from any other group.

Fecal nitrogen (FN) values from rats fed the PLL diet were higher ($P < 0.05$) than those of rats fed the PLT, MF, and TF diets. Fecal nitrogen of rats fed control, PLM, and LF diets was not different ($P > 0.05$) than that of those fed any other diet.

No differences ($P > 0.05$) was shown between any treatment groups for values concerning true digestibility.

The highest BV figure was produced by the group fed the control diet although it was not different ($P > 0.05$) from the rats fed PLL, PLM, MF, and TF diets. The BV of the LF diet was lower ($P < 0.05$) than the BV of the control, PLL, PLM, and MF diets but was not different

Table 11. Comparative Nitrogen Utilization[1] by Rats Fed Three Homopoly Amino Acids vs L-amino Acids, Trials 1 and 2.

Treatments	Urinary Nitrogen	Fecal Nitrogen	True Digestibility	Biological Value	Net Protein Utilization
Control	$0.1246^{b} \pm 0.01$*	$0.0758^{ab} \pm 0.02$	$95.99^{a} \pm 3.36$	$90.38^{a} \pm 1.87$	$86.73^{a} \pm 2.65$
Poly-L-Lysine	$0.2258^{a} \pm 0.06$	$0.1177^{a} \pm 0.06$	$89.92^{a} \pm 8.99$	$72.84^{ab} \pm 8.03$	$64.98^{bc} \pm 4.26$
Poly-L-Methionine	$0.1101^{b} \pm 0.03$	$0.0559^{ab} \pm 0.04$	$90.39^{a} \pm 12.38$	$84.07^{ab} \pm 8.77$	$75.31^{ab} \pm 7.89$
Poly-L-Tryptophan	$0.1714^{ab} \pm 0.04$	$0.0391^{b} \pm 0.03$	$95.57^{a} \pm 9.96$	$65.33^{bc} \pm 11.51$	$62.76^{bc} \pm 14.91$
Lysine Free	$0.2389^{a} \pm 0.04$	$0.0609^{ab} \pm 0.04$	$93.35^{a} \pm 8.38$	$49.98^{c} \pm 11.57$	$46.11^{c} \pm 8.48$
Methionine Free	$0.1238^{a} \pm 0.04$	$0.0465^{b} \pm 0.03$	$92.47^{a} \pm 11.32$	$78.79^{ab} \pm 13.88$	$71.95^{ab} \pm 10.15$
Tryptophan Free	$0.1692^{ab} \pm 0.05$	$0.0433^{b} \pm 0.02$	$96.33^{a} \pm 6.28$	$69.92^{abc} \pm 14.57$	$67.62^{ab} \pm 16.28$

^{a,b,c}Means in the same column with unlike superscripts are significantly different ($P < 0.05$).

[1]Means represent combined values from Trials 1 and 2.

*Standard deviation.

($P > 0.05$) from the PLT and TF diets. Biological values of the PLL, PLM, PLT, MF and TF diets were not different ($P > 0.05$).

Net protein utilization values follow a trend similar to that of BV. The best NPU was evidenced in the control group but was not different ($P > 0.05$) from the PLM, MF and TF groups. The LF group had the lowest NPU which was different from the control, PLM, MF, and TF diets but was not different ($P > 0.05$) from the PLL and PLT diets. The PLL, PLM, PLT, MF, and TF diet fed rats showed no difference ($P > 0.05$) in NPU values.

Rat Liver Data

Pooled data from trials 1 and 2 concerning liver measurements are presented in Table 12. The highest mean liver weight was found in the group receiving the control diet. However, this weight was different ($P < 0.05$) from the MF and TF groups only, which represented the lowest ($P < 0.05$) liver weights and which were not different ($P > 0.05$) from each other. The latter two treatment groups were not different ($P > 0.05$) from the other groups, with the exception of the control group.

No differences were detected from the values representing liver weight as a percent of body weight. The percent N of liver data reveals higher ($P < 0.05$) means from the PLM and MF diet fed rats compared to all other groups. The lowest mean percent N of liver occurred in the group fed the LF diet, which was not different ($P > 0.05$) from means of groups fed the PLT and PLL diets. The PLL fed group also showed a lower ($P < 0.05$) percent N of liver than the TF group, which was not different ($P > 0.05$) from the control. The control

Table 12. Comparative Effect of Three Homopoly Amino Acids vs L-amino Acids on Rat Liver Weight and Percent Nitrogen of Liver[1], Trials 1 and 2.

Treatments	Number of Livers	Liver Weight (LW) g	LW as % Body Weight	% Nitrogen of Liver
Control	8	$4.51^{a}\pm0.93$*	$5.01^{a}\pm0.90$	$16.91^{b}\pm1.89$
Poly-L-Lysine	8	$3.95^{ab}\pm1.08$	$4.79^{a}\pm1.30$	$15.78^{cd}\pm0.68$
Poly-L-Methionine	8	$3.17^{ab}\pm0.75$	$5.40^{a}\pm1.10$	$17.93^{a}\pm0.91$
Poly-L-Tryptophan	8	$3.33^{ab}\pm0.75$	$5.74^{a}\pm1.13$	$15.54^{d}\pm0.53$
Lysine Free	8	$2.94^{ab}\pm0.51$	$5.09^{a}\pm0.76$	$15.45^{d}\pm0.88$
Methionine Free	8	$2.88^{b}\pm0.74$	$5.10^{a}\pm1.29$	$18.42^{a}\pm0.96$
Tryptophan Free	8	$2.89^{b}\pm0.54$	$5.01^{a}\pm1.03$	$16.56^{bc}\pm0.67$

^{a,b,c,d}Means in the same column with unlike superscripts are significantly different ($P < 0.05$).

[1]Means represent combined values from trials 1 and 2.

*Standard deviation.

rats' percent N of liver was different ($P < 0.05$) from all other groups with an intermediate value.

Chick Growth Trials 3 and 4

Pooled data from chick growth trials 3 and 4 are shown in Table 13. The group fed the control diet gained faster ($P < 0.05$) than any other group. All other test diets resulted in less weight gain ($P < 0.05$) than the diet containing PLL.

Table 13. Comparative Performance[1] of Chicks Fed Three Homopoly Amino Acids vs L-amino Acids in Synthetic Diets, Trials 3 and 4.

Treatment	Number of Chicks Initial	Final	Body Weight Gain	Feed Consumed[2]	Adjusted Feed/Gain[3]	PER[4]
			g	g	kg/g	
Control	12	9	159.10[a] ± 31.76*	19.64 ± 6.65	1.42 ± 0.24	3.70 ± 0.65
Poly-L-Lysine	8	8	42.50[b] ± 10.88	9.66 ± 2.58	2.56 ± 0.64	1.95 ± 0.50
Poly-L-Methionine	8	7	3.00[c] ± 4.36	2.94 ± 0.89	9.73 ± 14.01	0.24 ± 0.86
Poly-L-Tryptophan	8	5	3.20[c] ± 2.17	4.41 ± 0.62	5.29 ± 1.17	0.37 ± 0.13
Lysine Free	8	8	1.25[c] ± 1.49	3.89 ± 0.76	6.31 ± 1.99	0.08 ± 0.28
Methionine Free	8	6	1.83[c] ± 3.12	3.08 ± 0.31	7.79 ± 6.52	0.16 ± 0.60
Tryptophan Free	8	7	1.29[c] ± 1.89	3.99 ± 0.70	6.03 ± 1.30	0.15 ± 0.26

[a,b,c] Means in the same column with unlike superscripts are significantly different ($P < 0.05$).

[1] Means represent combined values from trials 3 and 4.

[2] Represents mean feed consumption per chick per day.

[3] All values were adjusted by addition of a constant (8) to the gain measurement to negate effect of negative numbers.

[4] Protein efficiency ratio.

* Standard deviation.

Average daily feed consumption of chicks fed the control diet was double that of chicks fed the PLL diet, which was at least twice that of chicks fed any of the other diets. The percent protein of diets fed to chicks in trials 3 and 4 is shown in Table 14.

Table 14. Percent Protein[1] of Diets Fed to Chicks in Trials 3 and 4 Testing the Nutritional Value of Three Homopoly Amino Acids vs L-amino Acids.

	Control	Poly-L Lysine	Poly-L Methio-nine	Poly-L Trypto-phan	Lysine Free	Methio-nine Free	Trypto-phan Free
Percent Protein[2]	19.33	18.11	19.29	19.32	18.38	19.17	18.88

[1]All values given on a dry matter basis.

[2]Protein determined by Kjeldahl analysis.

Feed/gain ratios were adjusted to negate the effect of negative numbers. A constant was added to each weight gain value so that none were below a ratio of 1:1. These data showed the lowest F/G was produced by chicks fed the control diet, followed by those fed PLL. High F/G ratios were found from chicks fed PLT, TF, LF, MF, and PLM diets.

Similar results are found with PER. The highest PER was from the control group, followed by the group fed the PLL diet. All remaining diets produced very low PERs.

Growth data were also collected in trial 5 concerning the source or absence of Trp in the diet and are presented in Table 15. The largest gain was found with the chicks fed the PLT plus L-Trp diet although this figure was not different ($P > 0.05$) from the gain of

Table 15. Comparative Performance of Chicks Fed Various Sources of Tryptophan or No Tryptophan in Synthetic Diets, Trial 5.

Treatment	Number of Chicks Initial	Final	Body Weight Gain	Feed Consumed[1]	Adjusted Feed/Gain[2]	PER[3]
			g	g	kg/g	
Control	5	5	82.00[a]±14.30*	13.8	1.48±0.26	2.86±0.50
Poly-L-Tryptophan	5	5	1.80[b]±1.30	4.1	6.77±1.78	0.21±0.15
Tryptophan Free	5	5	1.60[b]±3.85	3.7	11.65±12.55	0.21±0.50
Poly-L-Tryptophan + L-Tryptophan	5	5	87.00[a]±20.70	13.3	1.39±0.37	3.15±0.74
Tryptophyl-L-Tryptophan	5	5	77.40[a]±16.73	12.2	1.40±0.29	3.05±0.66

[a,b]Means in the same column with unlike superscripts are significantly different ($P < 0.05$).

[1]Represents mean feed consumption per chick per day.

[2]All values were adjusted by addition of a constant (4) to the gain measurement to negate effect of negative numbers.

[3]Protein efficiency ratio.

*Standard deviation.

chicks fed the control diet or the diet containing the dipeptide, tryptophyl-L-tryptophan. The gains from chicks fed these three diets were different ($P < 0.05$) from the gain of those fed diets containing PLT or no Trp, which were similar ($P > 0.05$). Average daily feed consumption, adjusted F/G and PER values for chicks fed these diets followed a similar trend.

Chick Liver Data

Liver data collected following chick growth trials 3 and 4 are summarized in Table 16. Mean liver weight from chicks fed the control diet was greater ($P < 0.05$) than mean weights from chicks fed all other diets. Chicks fed PLL, PLM, and MF diets produced mean liver weights of similar size, however the former two were larger ($P < 0.05$) than mean weights from chicks fed PLT, LF and TF diets. Mean liver weight from MF fed chicks was not larger than that from chicks fed PLT, which was not different ($P > 0.05$) from mean liver weights of chicks fed LF and TF diets.

When liver weight was expressed as a percent of body weight, the values from groups fed the control, PLM and MF diets were not different ($P > 0.05$) from each other, but were higher ($P < 0.05$) than all other groups. The PLL fed chicks produced a value higher ($P < 0.05$) than the PLT, LF and TF groups and was similar to none. Chicks fed PLT, LF and TF diets produced similar ($P > 0.05$) values.

Kjeldahl anlaysis showed the livers from chicks fed PLT, LF and TF diets to contain more ($P < 0.05$) N as a percent of liver weight than

Table 16. Comparative Effect of Three Homopoly Amino Acids vs L-amino Acids on Chick Liver Weights and Percent Nitrogen of Liver[1], Trials 3 and 4.

Treatments	Number of Livers	Liver Weight (LW)	LW as % Body Weight	% Nitrogen of Liver
		g		
Control	11	$6.03^{a}\pm1.20$*	$3.04^{a}\pm0.22$	$16.62^{cb}\pm1.13$
Poly-L-Lysine	8	$1.89^{b}\pm0.32$	$2.44^{b}\pm0.15$	$17.85^{b}\pm1.04$
Poly-L-Methionine	7	$1.55^{b}\pm0.27$	$3.30^{a}\pm0.32$	$15.50^{c}\pm1.55$
Poly-L-Tryptophan	4	$0.68^{cd}\pm0.08$	$1.78^{c}\pm0.34$	$22.61^{a}\pm1.97$
Lysine Free	8	$0.60^{d}\pm0.11$	$1.75^{c}\pm0.28$	$20.42^{a}\pm2.36$
Methionine Free	6	$1.34^{bc}\pm0.42$	$3.02^{a}\pm0.49$	$11.60^{d}\pm4.82$
Tryptophan Free	7	$0.62^{d}\pm0.12$	$1.74^{a}\pm0.22$	$21.44^{a}\pm1.58$

^{a,b,c,d}Means in the same column with unlike superscripts are significantly different ($P < 0.05$).

[1]Means represent combined values from trials 3 and 4.

*Standard deviation.

PLL fed chicks, which produced livers with more N ($P < 0.05$) than those fed PLM and MF diets. The control and PLM fed chicks produced values greater ($P < 0.05$) than the MF group.

Chick Heartbeat Phenomenon

Data gathered from observing the duration of heartbeats following death are presented in Table 17. Heartbeats of chicks fed the TF diet endured longer ($P < 0.05$) than those fed the control, dipeptide, and PLT plus L-Trp diets. Duration of heartbeat of chicks fed PLT was not different ($P > 0.05$) from those fed TF or PLT plus

Table 17. Comparative Effect of Source or Absence of Tryptophan in Synthetic Diets on Chick Duration of Heartbeat Following Death, Trial 5.

	Control (L-Tryptophan)	Poly-L-Tryptophan	Tryptophan Free	Poly-L-Tryptophan + L-Tryptophan	Tryptopyl L-Tryptophan
Number of 5 minute intervals[1]	$1.00^{c}\pm0.00$*	$32.80^{ab}\pm19.38$	$38.40^{a}\pm16.21$	$18.00^{bc}\pm20.49$	$5.00^{a}\pm2.00$

^{a,b,c}Means with unlike superscripts are significantly different ($P < 0.05$).

[1]Each 5 minute interval of continued beating was given a value of 1; thus a heart beating 30 minutes following death would be given a value of 6.

*Standard deviation.

L-trp diets. The shortest duration of heartbeat was provided by chicks fed the control, dipeptide, and PLT plus L-trp diets.

Chapter 5

DISCUSSION

Growth data from rat and chick trials followed similar trends as data gathered by Newman et al. (1980) and Boebel and Baker (1982). Newman and coworkers found no growth difference between PLL and L-lys fed rats, however the results presented in this paper did show significant differences between the two groups. A logical explanation for this is found in the amount of PLL provided in the diets. Newman incorporated PLL at 118 percent of the lysine requirement of the rat based on an analysis revealing the polymer to contain 84 percent lysine after debromization. The same analysis was performed in the present study, but not until after trial initiation, thus the results are not used in diet formulation.

All diets containing HPAAs were calculated with the assumption that the polymers were 100 percent pure. In addition, the amount of each HPAA added to the diet was actually less than the respective amino acid requirement because the HPAAs were considered a more concentrated source of the amino acid than their free L-form. Thus, with the NRC requirement of Lys for rats at 0.7 percent of diet, PLL was provided at 0.615 percent of diet. Analysis of PLL by AAA Laboratories[2] and Sigma Chemical Company showed the polymer to contain 49.5 percent and 65 percent Lys, respectively. Providing Lys at 0.615 percent instead of 0.7 percent of diet from a source that was approximately only two-thirds Lys resulted in a deficiency state of this amino acid in the diet. This is undoubtedly the reason for

---

[2]AAA Laboratories, 6206 89th Ave. S.E., Mercer Island, WA 98040.

the depressed growth seen in this trial. The fact that growth did occur despite these circumstances does substantiate the conclusion drawn by Newman et al. (1980) that PLL is an available source of that amino acid for the rat.

The amount of PLM added to the diet of chicks was calculated in the same manner as that described for PLL. AAA Laboratories and Sigma Chemical Company analyzed the polymer and found it to contain 50 percent and 96 percent Met, respectively. It is unlikely, but if a deficiency state were produced by this level of Met it would be less than that for Lys found in PLL diets. Also, 25 percent of the sulfur amino acid requirement was met by cysteine. Taking these two points into consideration, had the PLM been an available source of the amino acid, some growth should have occurred. Since it did not, these results are considered to be supportive of the conclusion by Boebel and Baker (1982) that PLM is not an available source of that amino acid for the chick.

The results for PLL and PLM are in agreement with results found by two other investigators (Newman et al., 1980; Boebel and Baker, 1982). Since growth did occur with PLL fed rats and chicks, and did not occur with PLM fed chicks or rats, it is suggested that the availability of these two HPAAs is not species specific.

There are no known studies with which to compare the growth data for rats or chicks fed PLT. The amount of this HPAA was calculated and added to the diets of both species in the same manner

as that described for PLL. AAA Laboratories and Sigma Chemical Company analyses of this polymer were very different, 7 percent and 25 percent, respectively. If Trp were provided at 25 percent of the requirement, it would supply 0.06 percent of diet for chicks and 0.04 percent of diet for rats. Klain et al. (1960) fed chicks graduated levels of Trp in a crystalline amino acid diet which provided 30 percent protein and niacin at nearly four times the required level. The lowest amount of Trp provided was at 0.075 percent of diet which caused an average total weight loss of 1.0± 1.1 grams for the seven day trial. This indicates that providing Trp at only 25 percent of the requirement would not be enough to sustain the growth of chicks. Similar results were found by Young and Munro (1973) who fed rats graduated levels of Trp for nine days. The diets in their study also provided amino acids in the crystalline form, were approximately 16 percent protein and provided niacin at the recommended level. They found that providing Trp at 0.033 percent of diet caused an average weight loss of 3.5± 2.5 grams, while feeding Trp at 0.066 percent of diet produced an average weight gain of 12.5± 8.7 grams. This study indicates that supplying Trp at 25 percent of the requirement, or 0.04 percent of diet, would result in little, if any, weight gain in rats. It seems appropriate to conclude that if the PLT had contained 25 percent Trp, the results of the growth trials for rats and chicks would have been the same whether or not the polymer were an available source of the amino acid.

Since HPAAs constitute a relatively new area of study, procedures

for their amino acid assay are not entirely standardized. The results of analysis from Sigma Chemical Company are, for the purpose of this project, regarded to be more accurate than those from AAA Laboratories as analysts working for Sigma have performed many repetitions of their procedures with the intent of perfecting them. This level of expertise was not expected of AAA Laboratories. The analysis method that indicated that the PLT polymer contained 25 percent Trp was not considered to produce accurate results by Sigma Chemical Company. In several personal communications, the difficulty of hydrolyzing the PLT molecule was mentioned. When performing the anlaysis, it was noted that some residues had escaped hydrolysis. Sigma Chemical Company believes these residues are Trp.

When PLT was subjected to high pressure liquid chromotography by Sigma Chemical Company, only one peak was produced from the polymer, indicating its purity. Until the problem with hydrolysis and subsequent analysis can be overcome, the actual quantity of Trp contained in the polymer is unknown. Because of this circumstance, firm conclusions can not be drawn from the growth data of rats or chicks fed PLT. According to the studies by Klain et al. (1960) and Young and Munro (1973), 45 percent of the Trp requirement or 0.10 percent of diet for chicks and 0.07 percent of diet for rats, will allow growth in rats and chicks. Analysis of the PLT must show the polymer to contain at least 45 percent Trp, the lowest amount demonstrated to sustain growth, before the growth data gathered for this thesis project concerning Trp can be of value.

Two obvious questions arise from the points so far covered in this discussion. First, since neither the Lys nor the Met polymers contained 100 percent of the respective amino acid, then what are the remaining constituents of the polymers? Very likely, these constituents are water or a product resulting from damage to an amino acid residue that occurred during the synthesis of the polymer. A small amount of the difference between 100 percent and the amount of Lys and Met analyzed could be due to errors in the analysis, such as incomplete hydrolysis.

The second, and more significant, question is why did the polymer of Lys support growth and not the polymer of Met, and possibly not Trp? To explore the answer to this question, the subjects of solubility, enzymes, and transport systems will be considered.

The most likely answer to this question is insolubility of PLM and PLT. The difficulty of hydrolyzing PLT, which was also a problem with PLM, attests to its insolubility. If these two HPAAs were not soluble in the environments encountered in the digestive tract, enzymes would not have the opportunity to attack the molecules and digestion would not occur.

Another possible answer for the second question involves the enzymes and the number of amino acid residues per HPAA polymer. Peptide bonds adjacent to Lys can be broken by trypsin, carboxypeptidases A and B, and aminopeptideases, the latter three functioning at the respective carboxy- or amino- terminal ends (Ryle and Porter,

1959; Neurath, 1960; Smith, 1960). Carboxypeptidase A and the amino peptidases have no specificity for a particular amino acid, only its position on the polymer. Thus Lys shares the function of these enzymes with all other carboxy- and amino- terminal amino acids. Trypsin and carboxypeptidase B exhibit specificty for peptide bonds adjacent to only Lys and arginine. In addition to carboxypeptidase A and the aminopeptidases, methionine residues can be hydrolyzed from the peptide chain by elastase, which shows partiality to the nine neutral aliphatic amino acids. Tryptophan can be hydrolyzed by chymotrypsin, carboxypeptidase A and aminopeptidases, the former being specific for the three aromatic amino acids. Pepsin is capable of hydrolyzing peptides at any amino acid residue, but does exhibit preferential attack to peptide bonds adjacent to aromatic amino acids. So, not including carboxypeptidase A and aminopeptideases, there are two enzymes specific for peptide bond hydrolysis adjacent to Lys, and one enzyme performing this function adjacent to Met and Trp.

The average number of amino acid residues in one molecule were 41 of Lys, 285 of Met, and 167 of Trp for each respective polymer. Combining this information with that previously supplied regarding enzymes and number of substrates, an interesting thought comes to mind. Perhaps, since there were 41 Lys residues per polymer and the presence of two enzymes with specifity for Lys peptide bonds, the opportunity for complete hydrolysis was greater for PLL than the other two HPAAs, which contained many more residues and had available one less enzyme for hydrolysis. It would be interesting to perform a

study similar to the one discussed here, except providing Met and Trp as homopolymers with approximately twenty amino acid residues per molecule. If growth did not occur, the above proposed hypothesis that the number of residues per HPAA molecule influences digestibility, may have value.

If the polymers were broken down to dipeptides, their opportunities for transport and absorption would probably be equal. Boebel and Baker (1982) used methionylmethionine in chick trials and found it an acceptable source of that amino acid. Tryptophyltryptophan was demonstrated to be available to both rats and chicks in trials performed for this thesis, and also from work studying Hartnup's disease (Asatoor et al., 1970). In vitro work by Taylor et al. (1980) and Addison et al. (1974) testify for the ability of transport of lysyllysine by hamster jejunum. The transport of peptides appears to be dependent not on the amino acid constituents of the peptide, but the number of residues contained therein. This would then indicate that if the size of the peptide were equal, then dipeptides of Lys, Met, Trp, would all have the same chance for transport.

The results of the rat N balance trials 1 and 2 were not expected. During the trial, it was anticipated that since growth was occurring only in the control and PLL groups, then N balance data would show distinct differences between the groups growing and those losing weight. This was not the case. The results are confusing and it is difficult to draw any meaningful conclusions from them. An example of this is that the rats fed the PLM produced higher values

for TD, BV, and NPU than those fed PLL, and those fed the diet void of Trp had a TD figure greater than both these and the control groups.

A possible explanation may lie in the values obtained for UN and FN from all groups. The greatest ($P < 0.05$) UN came from rats fed the PLL, LF, and MF diets while the lowest ($P < 0.05$) UN came from those fed the control and PLM diets. Rats fed the PLT and TF diets excreted a quantity of N in the urine that was not different ($P > 0.05$) from any other group. Rats fed PLL excreted the most ($P < 0.05$) FN and those fed PLT, MF, and TF diets excreted the least ($P < 0.05$) FN. The FN values from control, PLM, and LF fed rats were not different ($P > 0.05$) from the PLL or PLT, MF, or TF fed rats. These results clearly indicate that no difference can be found between the rats that grew and those that did not grow in regard to UN and FN in trials 1 and 2. Since UN and FN are crucial values in the calculations of TD, BV, and NPU, then the lack of difference discussed above for UN and FN would produce the same confusing results for TD, BV, and NPU.

Njaa (1963) demonstrated that heavier rats excreted more nitrogen in feces than lighter rats. Causeret et al. (1965) found a positive correlation between UN and body weight. Eggum (1973) discusses the fact that heavier animals will produce lower TD and BV due to greater UN and FN than lighter animals. Eggum was not able to show significant differences in this regard with rat weight variations of at least 5 grams. However, greater variation in weight could produce significantly different TD and BVs. In trials 1 and 2,

variations in weight between the control and PLL fed rats and all other groups were at least 20 grams. This amount of difference may be enough to effect TD, BV, and NPU.

An explanation for the unusual UN and FN values may be found in problems incurred in the collection of urine and feces. The rats fed PLM, PLT, Trp, and diets void of an amino acid weighed 53-63 grams when beginning the N balance trials. They were small enough to fit their entire body in the feed cup at the end of the metal tunnel of the collection cage. Since they ate less than half of their feed each day, their coats and tails would carry some of the remaining feed from the cup to the open area of the cage, where it could fall into the collection vessels. Thus, some of the N detected in urine and feces samples could have come from the feed which fell into the samples. This occurrence would also have some effect on F/G and PER which are dependent on feed consumption data. The reason clear differences were shown for these parameters is probably due to the great differences in weight gains.

One problem with the above explanation for unexpected N balance data is that in this facility using rats of comparable size, this problem had not previously caused this noticeable an effect. Newman et al. (1980) used the same facility and cages for their work. They were able to show logical differences in TD, BV, and NPU between L-lys, PLL and LF fed rats. The size of the rats in both studies were approximately the same, so both would have had equal opportunity to carry feed back to the open area of the cage where it could fall

into collection vessels. Also, the occurence was of equal magnitude in both trials 1 and 2, so it could not be due to the behavior of one particular group of rats.

The chick trial investigating the incidence of prolonged heartbeat following death provided results contrary to expectations. The hearts which continued to beat the longest time period following death belonged to chicks fed diets with no Trp or fed PLT. However, the length of time was not significantly different between the latter group and the group fed the polymer plus L-Trp, which was not different ($P > 0.05$) from the control and dipeptide groups. This suggests that the occurrence is more likely associated with the lack of an available source of Trp in the diet than the presence of PLT.

In attempting to understand this phenomenon, the roles of Trp must be explored. The amino acid can be converted into niacinamide, however, this vitamin was provided at 1.8 times the requirement of the chick in the diet, so it is unlikely that the occurrence was due to a niacin deficiency. Tryptophan is also the precursor or serotonin which has been found to play two roles in blood coagulation. It is a stimuli for the release of platelet factor 3, which is a main component of blood coagulation chemistry (Orten and Neuhaus, 1982). It is also a powerful vasoconstrictor that locally narrows the blood vessel wall to restrict the bleeding area and is also a weak platelet aggregating agent. In chicks, serotonin can cause a depression in blood pressure, resulting in increased heart rate in live chicks (Wood, 1971). This may be due to its vasoconstrictive ability. With

no available Trp source, serotonin would be produced only in limited amounts, if at all. Considering its role in blood coagulation, the possibility exists that the prolonged contractions could be due to the lack of serotonin, so decreased coagulation or lengthened amount of time for coagulation to occur. Serotonin is also known to be a neurotransmitter. If it plays a role in rate or regularity of heartbeat, its absence may have an effect on the occurrence discussed here.

The reason this occurence was found only with the chicks may be due to methods of sacrifice. Cervical dislocations, though an effective means of termination, does not change the amount of oxygen in the blood. Thus, oxygen was not limiting for oxidative processes allowing the contraction of the heart muscle. The method of sacrifice for the rats was effective due to oxygen replacement by carbon dioxide, so that when death occurred, there was no oxygen left in the blood to allow a prolonged heartbeat.

An interesting observation was made concerning chicks fed PLL approximately one week after trial initiation. At this time in the trial, it was obvious that the chicks fed the PLL and control diets were growing while those on the other diets were not. This was suggestive, and later confirmed by the growth data, that the PLL was an available source of Lys. However, it was also noticed that the chicks fed this polymer had either ceased or greatly decreased the habit of grooming. This was handled merely as an observation, so no experimental design was pursued to demonstrate a difference in

grooming behavior between groups. Nonetheless, a lack of cleanliness was noticeable in all chicks in the PLL groups, and was not observed in any other group. It was not noticeable in rats fed PLL or any other diet.

Compulsive grooming behavior is known to be mediated by a variety of peptides (Katz, 1980). Now designated as neuropeptides, short peptides that are active in the nervous system and can function as hormones, these substances constitute a relatively new area of study (Bloom, 1981). Possibly the presence of PLL has an effect on one of these neuropeptides, eliciting a change in the normal grooming pattern. The occurence may also have been due to Lys being present in a limited amount, although this has not been documented previously to this author's knowledge. In any case, this phenomenon should be investigated more extensively to ascertain if: 1) there is a relationship between PLL and changes in grooming behavior in chicks; and 2) other behavioral or toxic effects arise from the use of PLL in this species.

Chapter 6

CONCLUSIONS

The research performed for this thesis project supports the findings of Newman et al. (1980) and Boebel and Babker (1982) that PLL is an available source of Lys for rats and that PLM is not an available source of Met for chicks. It can be further concluded from this work that the availability, or lack of availability, of these polymers is not species specific. Firm conclusions can not be drawn on the study for PLT until it is proven to contain at least 45 percent Trp.

These findings demonstrate that the insertion of repeating codons into the DNA of a protein source with the intent of producing homopolymers of the source's limiting amino acid(s) would be of value for Lys, but not Met and possibly not Trp. Genetic modification for supplementing Met in to a protein source would need to stress the incorporation of dipeptides or single units of the amino acids into the original protein as performed by Jaynes et al. (submitted for publication). The same may be true for Trp.

The unusual observations regarding prolonged heartbeat following death of Trp deficient chicks and altered grooming behavior in PLL fed chicks warrant more attention. The latter observation may be of some importance since this HPAA was found to be an available source of this nutrient.

LITERATURE CITED

LITERATURE CITED

Addison, J. M., Burston, D., Payne, J. N., Wilkinson, S. and Matthews, D. M. (1975) Evidence for active transport of tripeptides by hamster jejunum in vitro. Clin. Sci. Mole. Med. 49, 305-312.

Addison, J. M., Matthews, D. M. and Burston, D. (1974) Competition between carnosine and other peptides for transport by human jejunum in vitro. Clin. Sci. Mole. Med. 46, 707-714.

Adibi, S. A. and Gray, S. J. (1967) Intestinal absorption of essential amino acids in man. Gastroenterology 52, 837-845.

Adibi, S. A. and Mercer, D. W. (1973) Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals. J. Clin. Invest. 52, 1586-1594.

Adibi, S. A. and Soleimanpour, M. R. (1974) Functional characterization of dipeptide transport system in human jejunum. J. Clin. Invest. 53, 1368-1374.

Agren, G., Stenram, U., Nordgren, H., Eklund, A. and Glas, J. E. (1974) Some biological effects observed in rats on single cell proteins of yeast and bacterial origin. Nutr. Metab. 17, 20-36.

Asatoor, A. M., Cheng, B., Edwards, K. D. G., Lant, A. F., Matthews, D. M., Milne, M. D., Navab, F. and Richards, A. J. (1970) Intestinal absorption of two dipeptides in Hartnup disease. Gut 11, 380-389.

Asatoor, A. M., Harrison, B. D. W., Milne, M. D. and Prosser, D. I. (1972) Intestinal absorption of an arginine-containing peptide in cystinuria. Gut 13, 95-98.

Ashref, M., Vetter, R. L., Nissen, S. and Graham, D. L. (1981) Nutritional evaluation of methanol-based yeast single cell protein in a multigeneration rat study. Nutr. Rep. Int. 23, 813-824.

Aylward, F. and Jul, M. (1975) Protein Foods. In: Protein and nutrition policy in low-income countries, pp. 50-62, John Wiley and Sons, Inc., New York.

Bender, A. E. and Miller, D. S. (1953) A new brief method of estimating net protein value. Biochem J. 53, vii.

Bickoff, E. M., Booth, A. N., deFremery, D., Edwards, R. H., Knuckles, B. E., Miller, R. E., Saunders, R. M. and Kohler, G. O. (1975) Nutritional evaluation of alfalfa leaf protein concentrate. In: Protein nutritional quality of foods and feeds. Part 2. (Friedman, M., ed.), pp. 319-340, Marcel Dekker, Inc., New York.

Bloom, F. E. (1981) Neuropeptides. Sci. Am. 245, 148-168.

Boebel, K. P. and Baker, D. H. (1982) Efficacy of methionine peptides as determined by chick bioassay. J. Nutr. 112, 1130-1132.

Bressani, R. (1973) Legumes in human diets and how they might be improved. PAG Bulletin 3, 15-42.

Burr, H. K. (1975) Pulse proteins. In: Protein nutritional quality of foods and feeds. Part 2. (Friedman, M., ed.), pp. 119-134, Marcel Dekker, Inc., New York.

Carpenter, K. J. and Booth, V. H. (1973) Damage to lysine in food processing: its measurement and its significance. Nutr. Abstr. Rev. 43, 424-451.

Causeret, J., Hugat, D. and Arnouy, J. (1965) Statistical study of variations of excretion of metabolic nitrogen in faeces and endogenous nitrogen in urine in relation to body weight, intake of feed, and weight of faeces in young rats. Ann. Biol. Animale Biochim. Biophys. 5, 61. according to Eggum, Bjørn O. (1973) Experimental technique for rats. In: A study of certain factors influencing protein utilization in rats and pigs.,pp. 17-29, I Kommission hos Landhusholdningsselskabets Forlag, Rolighedsvej 26, 1958 Kobenhavn V.

Christensen, H. N. (1963) Amino acid transport and nutrition. Fed. Proc. 22, 1110-1114.

deGroot, A. P. (1976) Safety evaluation studies with single cell protein. PAG Bulletin 6, 41-44.

Eggum, Bjørn O. (1973) Experimental technique for rats. In: A study of certain factors influencing protein utilization in rats and pigs., pp. 17-29, I Kommission hos Landhusholdningsselskabets Forlag, Rolighedsvej 26, 1958 Kobenhavn V.

FAO/WHO (1973). Energy and protein requirement report of a joint FAO/WHO ad hoc expert committee. FAO Nutrition Meetings report Series No. 52, WHO Technical Report Series No. 522. pp. 55-67, FAO/WHO, Geneva.

Florey, H. W., Wright, R. D. and Jennings, M.A. (1941) The secretions of the intestine. Physiol Rev. 21, 36-69.

Gitler, C. (1964) Protein digestion and absorption in nonruminants. In: Mammalian protein metabolism Vol. 1. (Munro, H. N. and Allison, J. B., eds.), pp. 35-69, Academic Press, New York.

Gray, G. M. and Cooper, H. L. (1971) Protein digestion and absorption. Gastroenterology 61, 535-544.

Harper, A. A. (1959) Physiologic factors regulating pancreatic secretion. Gastroenterology 36, 386-391.

Hegsted, D. M. and Chang, Yet-oy. (1965) Protein utilization in growing rats. 1. Relative growth index as a bioassay procedure. J. Nutr. 85, 159-168.

Hellier, M. D., Holdsworth, C. W., Perrett, D. and Thirumalai, C. (1972) Intestinal dipeptide transport in normal and cystinuric subjects. Clin. Sci. 43, 659-668.

Iber, F. L. (1980) The gastrointestinal tract: an overview of function. In: Modern nutrition in health and disease, sixth edition (Goodhart, R. S. and Shils, M. E., eds.), pp. 35-50, Lea and Febiger, Philadelphia.

Inglett, G. E. (1977) Cereal proteins. In: Food proteins (Whitaker, J. R. and Tannenbaum, S. R., eds.), pp. 273-290, AVI Publishing Co., Westport, Conn.

Jacob, S. W. and Francone, C. A. (1974) The digestive system. In: Structure and function in man, pp. 423-462, W. B. Saunders Co., Philadelphia.

Jones, A. (1974)a. Meats. In: World protein resources, pp. 63-96, Halsted Press, New York.

Jones, A.(1974)b. Fish. Ibid., pp. 119-152.

Jones, A. (1974)c. Future Proteins. Ibid., pp. 362-374.

Jones, A. (1974)d. Algae. Ibid., pp. 219-222.

Jorgensen, N. A. (1975) Evaluation of green plant protein concentrates as animal feeds. In: Papers from a workshop on unconventional sources of protein, College of Agricultural and Life Science, pp. 141-150, University of Wisconsin, Madison.

Kangas, T. T., Cooney, C. L. and Gomez, R. F. (1982) Expression of a proline-enriched protein in Escherichia coli. Appl. Env. Micro. 43, 629-635.

Katz, R. (1980) Grooming elicited by intracerebroventricular bombesin and eledoisin in the mouse. Neuropharmacology 19, 143-146.

Kihlberg, R. (1972) The microbe as a source of food. Ann.Rev. Micro. 26, 427-466.

Kim, Y. S., Britwhistle, W. and Kim, Y. W. (1972) Peptide hydrolases in the brush border and soluble fractions of small intestine mucosa of rat and man. J. Clin. Invest. 51, 1419-1430.

Kim, Y. S. and Freeman, H. J. (1977) The digestion and absorption of protein. In: Symposium on clinical nutrition update-amino acids. (Greene, H. L., Holliday, M. A. and Munro, H. N., eds.), pp. 135-141, Am. Med. Assoc., Chicago.

Kim, Y. S., Nicholson, J. A. and Curtis, K. J. (1974) Intestinal peptide hydrolases: Peptide and amino acid absorption. Med. Clin. N. Am. 58, 1397-1412.

Klain, G. J., Scott, H. M. and Johnson, B. C. (1960) The amino acid requirement of the growing chick fed a crystalline amino acid diet. Poul. Sci. 39, 39-44.

Kohler, G. O. and Lyon, C. K. (1977) Plant protein sources. In: Food proteins (Whitaker, J. R. and Tannenbaum, S. R., eds.), pp. 516-541, Avi Publishing Co., Westport, Conn.

Liener, I. E. (1975) Effects of anti-nutritional and toxic factors on the quality and utilization of legume proteins. In: Protein nutritional quality of foods and feeds. Part 2 (Friedman, M., ed.), pp. 523-550, Marcel Dekker, Inc., New York.

McCoy, E. (1975) Thermophiles as producers of microbial protein. In: Papers from a workshop on unconventional sources of protein, College of Agricultural and Life Science, pp. 121-128, University of Wisconsin, Madison.

McGill, M. and Pye, O. F. (1980) World food patterns and protein economy. In: Issues in nutrition for the 1980s. (Tobias, A. L. and Thompson, P. J., eds.) pp. 354-376, Woodsworth, Inc., Belmont, Cal.

McGovern, I. N. (1975) Carbohydrates from forrest and forrest industry residues as substrates for protein production. In: Papers from a workshop on unconventional sources of protein, College of Agricultural and Life Science, pp. 13-35, University of Wisconsin, Madison.

Matthews, D. M. (1975) Intestinal absorption of peptides. Physiol. Rev. 55, 537-608.

Matthews, D. M., Gandy, R. H., Taylor, E. and Burston, D. (1979) Influx of two dipeptides, glycylsarcosine and L-glutamyl-L-glutamic acid, into hamster jejunum in vitro. Clin. Sci. 56, 15-23.

Mitchell, H. H. (1923) A method of determining the biological value of protein. J. Biol. Chem. 58, 873-922.

Mitsuda, H., Yasumoto, K., and Nakamura, H. (1969) A new method for obtaining protein isolates from chlorella algae, torula yeasts, and other microbial cells. In: Englineering of unconventional protein production (Bieber, H., ed), pp. 93-103, American Institute of Chemical Engineers, New York.

Naughton, M. A. and Sanger, F. (1960) Purification and specificity of pancreatic elastase. Biochem. J. 78, 156-163.

Navab, F. and Asatoor, A. M. (1970) Studies on intestinal absorption of amino acids and a dipeptide in a case of Hartnup disease. Gut 11, 373-379.

Neurath, H. (1960) Carboxypeptidases A and B. In: The enzymes, 2nd ed., Vol. 4. (Boyer, P. D., Lardy, H. and Myrback, K., eds.), pp. 11-36, Academic Press, New York.

Newman, C. W., Jaynes, J. M. and Sands, D. C. (1980) Poly-L-lysine, a nutritional source of lysine. Nutr. Rep. Int. 22, 707-715.

Nicholson, J. A. and Peters, T. J. (1978) Subcellular distribution of hydrolase activities for glycine and leucine homopeptides in human jejunum. Clin. Sci. Mole. Med. 54, 205-207.

Nicholson, J. A. and Peters, T. J. (1979) The subcellular localization of peptidase activity in the human jejunum. Eur. J. Clin. Invest. 9, 349-354.

Nie, N. H., Hull, C. H., Jenkinds, J. G., Steinbrenner, K. and Bent, D. H. (1975) SPSS: Statistical Package for the Social Sciences. 2nd ed., McGraw-Hill, Inc., New York.

Nixon, S. E. and Mawer, G. E. (1970)a The digestion and absorption of protein in man. 1. The site of absorption. Brit. J. Nutr. 24, 227-240.

Nixon, S. E. and Mawer, G. E. (1970)b The digestion and absorption of protein in man. 2. The form in which digested protein is absorbed. Brit. J. Nutr. 24, 241-258.

Njaa, L. R. (1963) A study on the Mitchell method for determination of the biological value of protein. In: Reports on technological research conerning Norwegian fish industry, Vol. IV., pp. 118, Griegs Boktrykkeri, Bergen. According to Eggum, Bjørn O. (1973) Experimental technique for rats. In: A study of certain factors influencing protein utilization in rats and pigs., pp. 17-39, I Kommission hos Landhusholdningsselskabets Forlag, Rolighedsvej 26, 1958 Kobenhavn V.

National Research Council (1978) Nutrient requirements of domestic animals. Nutrient requirement of laboratory animals. 3rd rev. ed., pp. 7-37, National Academy of Sciences, Washington, D.C.

National Research Council (1977) Nutrient requirements of domestic animals. Nutrient requirement of poultry. 7th rev. ed. National Academy of Sciences, Washington, D.C.

Orten, J. M. and Neuhaus, O. W. (1982) Blood. In: Human Biochemistry, 10th ed., pp. 434-523, C. V. Mosby Co., St. Louis.

Osborne, T. B., Mendel, L. B. and Ferry, E. L. (1919) A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. 37, 223-229.

Pellett, P. L. (1978) Protein quality evaluation revisited. Food Tech., 32, 60-79.

Peters, T. J. (1973) The hydrolysis of glycine oligopeptides by guinea-pig intestinal mucosa and by isolated brush borders. Clin. Sci. Mole. Med. 45, 803-816.

Randall, H. G. and Evered, D. F. (1964) Amino acid uptake by living cells. I. Amino group requirement by small intestine of the rat in vitro. Biochim. Biophys. Acta. 93, 98-105.

Register, U. D. and Sonnenberg, L. M. (1973) The vegetarian diet. J. Am. Diet. Assoc. 72, 253-261.

Richardson, T. (1975) Utilization of novel proteins in human foods. In: Papers from a workshop on unconventional sources of protein, College of Agricultural and Life Science, pp. 151-180, University of Wisconsin, Madison.

Ryle, A. P. and Porter, R. R. (1959) Parapepsins: Two proteolytic enzymes associated with porcine pepsin. Biochem. J. 73, 75-86.

Sanger, F. and Tuppy, H. (1951) The amino-acid sequence in the phenylalanyl chain of insulin. Biochem. J. 49, 481-490.

Sasse, C. E. and Baker, D. H. (1973) Modification of the Illinois reference standard amino acid mixture. Poul. Sci. 52, 1970-1972.

Saunders, S. J. and Isselbacher, K. J. (1966) Intestinal absorption of amino acids. Gastroenterology 50, 586-595.

Schultz, S. G. and Curran, P. F. (1970) Coupled transport of sodium and organic solutes. Physiol. Rev. 50, 637-718.

Schulz, E. and Oslage, H. J. (1976) Composition and nutritive value of single cell protein. An. Fd. Sci. Tech. 1, 9-24.

Silk, D. B. A. (1981) Peptide transport. Clin. Sci. 60, 607-615.

Silk, D. B. A., Nicholson, J. A. and Kim, Y. S. (1976) Hydrolysis of peptides within lumen of small intestine. Am. J. Physiol. 231, 1322-1329.

Silk, D. B. A., Perrett, D. and Clark, M. L. (1973) Intestinal transport of two dipeptides containing the same two neutral amino acids in man. Clin. Sci. Mole. Med. 45, 291-299.

Smith, E. L. (1960) Peptide bond cleavage (Survey). In: The enzymes, 2nd edition, Vol. 4 (Boyer, P. D., Lardy, H. and Myrback, K, eds.), pp. 1-10, Academic Press, New York.

Snedecor, G. W. and Cochran, W. G. (1967) Statistical Methods. 6th ed., Iowa State College Press, Ames, Iowa.

Stobart, J. E. (1977) Nutritional evaluation of selected high-lysine grains. Masters thesis, Montana State University, Bozeman, Montana.

Sunde, M. L. (1975) Farm wastes as feedstuffs for animals. In: Papers from a workshop on unconventional sources of protein, College of Agricultural and Life Sciences, pp. 36-52, University of Wisconsin, Madison.

Tang, I. (1976) Pepsin and pepsinogen; models for carboxyl (acid) proteases and their zymogens. Trends Biochem.Sci. 1, 205-208.

Tannenbaum, S. R. (1971) Potential for new protein sources. In: Amino acid fortification of protein foods (Scrimshaw, N. S. and Altschul, A. M., eds.),pp. 139-150, MIT Press, Cambridge.

Tannenbaum, S. R. and Miller, S. A. (1967) Effect of cell fragmentation on nutritive value of Bacillus Megaterium protein. Nature 214, 1261-1262.

Taylor, E., Burston, D. and Matthews, D. M. (1980) Influx of glycyl-sarcosine and lysyl-L-lysine in hamster jejunum in vitro. Clin. Sci. 58, 221-225.

VanderWal, I. P. (1976) Experience with SCP in animal feeding in Europe. PAG Bulletin 6, 7-18.

Wedin, W. F. and Hodgson, H. J. (1980) Feed production. In: Animal agricultural research to meet human needs in the 21st century (Pond, W. G., Merkel, R. A., McGilliard, L. D. and Rhodes, V. J., eds.), pp. 98-114, Westview Press, Boulder.

Wetzel, R. (1980) Applications of Recombinant DNA technology. Am. Sci. 68, 664-675.

Woods, J. J. (1971) Studies on the distribution and action of serotonin in the avian cardiovascular system. Ph.D. thesis, Rutgers University, New Brunswick, New Jersey.

Woodward, E. R. (1960) The role of the gastric antrum in the regulation of gastric secretion. Gastroenterology 38, 7-14.

Wu, Lung-Chi and Stahmann, M. A. (1975) Fungal protein. In: Papers from a workshop on unconventional sources of protein, College of Agricultural and Life Sciences, pp. 67-104, University of Wisconsin, Madison.

Yamashina, I. (1956) The action of enterokinase on trypsinogen. Biochimica Et Biophysica Acta. 20, 433-434.

Young, V. R. and Munro, H. N. (1973) Plasma and tissue tryptophan levels in relation to tryptophan requirements of weanling and adult rats. J. Nutr. 103, 1756-1763.

Dew, N. C.
Bioavailability of three homopoly amino acids in growing rats and chicks

DATE	ISSUED TO
[illegible]	[illegible]
GAYLORD 40	