THE INFLUENCE OF INORGANIC NUTRITION ON THE NUTRITIONAL VALUE OF SANILAC PEA BEANS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
DON F. WAGNER
1968

This is to certify that the

thesis entitled

THE INFLUENCE OF INORGANIC NUTRITION ON THE NUTRITIONAL VALUE OF SANILAC PEA BEANS.

presented by

Don F. Wagner

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Soil Science

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Major professor

D. June 7. 1968

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ABSTRACT

THE INFLUENCE OF INORGANIC NUTRITION ON THE NUTRITIONAL VALUE OF SANILAC PEA BEANS

by Don F. Wagner

Samples of 'Sanilac' pea beans (*Phaseolus vulgaris* L.) grown on various soil levels of phosphorus, zinc and iron, in 1966 and 1967, were analyzed for amino acids and nitrogen content. Nutritive values were subsequently estimated by procedures given in the 1965 FAO/WHO joint report. The amino acid compositions were determined by ion exchange methods using acid hydrolyzed seed meal. The nitrogen content of the pea bean samples was determined by a micro-Kjeldahl procedure.

Protein scores, based on the sulfur-containing amino acids, indicated that the nutritional values of pea beans were not constant. The magnitude of the differences between protein scores were great enough to be nutritionally important.

Soil treatments and environmental conditions that favor increased zinc uptake by the plant tended to affect the amino acid profile of the pea bean by causing an increase in the methionine content. Phosphorus and iron soil treatments did not greatly affect the amino acid composition of the pea bean.

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The nitrogen content of the pea bean was not affected by soil treatments, but a 16-17% difference between crops grown in 1966 and 1967 was found. Protein scores were not affected by differences found in the nitrogen content.

Nutritional problems, arising from the fact that the nutritional value of this major food crop is somewhat dependent upon environmental conditions, are discussed.

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Don F. Wagner

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science

1968

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ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. J. F. Davis, professor of Soil Science, who, as major professor, provided the encouragement and personal counsel that made this dissertation possible.

Special thanks are due Dr. B. D. Knezek for his untiring interest and guidance throughout the course of this investigation and for his assistance in the preparation of this manuscript.

The author also wishes to express appreciation to Dr. S. L. Bandemer and to Jadwiga Nowik for their invaluable assistance with amino acid determinations.

Thanks are also extended to Dr. R. E. Lucas, Dr. R. S. Bandurski, and Dr. E. J. Benne, members of the Guidance Committee, and to the faculty, staff and fellow students of the Department of Soil Science for their aid, cooperation and friendship.

The author also expresses his appreciation to the Michigan Bean Commission of the Michigan Department of Agriculture for the financial support given to this study.

Finally, and especially, the author expresses his thanks for the patience, understanding, encouragement and aid of his wife, Ellie, which made this thesis possible.

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INTRODUCTION

World food supplies is a topic for much discussion. Editorials, magazines, books, and news programs have all, at one time or another, tried to cover the various aspects of this major subject, but the vastness of the problem is almost too great to be comprehended.

Availability of food is only one facet of the nutritional problems facing the people of the world. The nutritional value of food that is available must also be considered and understood. In many areas of the world, large populations are dependent upon plant materials as their principle source of dietary protein. For the most part, vegetable proteins are deficient in one or more of the essential amino acids (43, 47), and seeds are relatively poor sources of dietary protein (12). Altschul *et al.* (1) state that

> "seeds are the major source of proteins; the cereals furnish over 100 million tons of proteins annually, most of which is consumed by humans. One of the exciting possibilities for increasing world protein food supplies and reducing their cost to humans lies in the

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> capability of using seed proteins directly as human food. But this requires a greater understanding of the nature of proteins and the sophistication to create forms which will be nutritionally adequate, cheap and interesting."

Flodin (13) concluded that if available proteins were properly balanced with respect to their amino acid composition, a possible 50 to 100% increase in dietary protein could be effected without increasing the amount of food grown.

There are at least three ways in which plant proteins can be nutritionally improved. Protein quality can be improved by fortification with purified amino acids, by increasing the amount of animal protein consumed, or by directly improving the quality of plant protein. It may also be possible to improve the diet by introducing new food crops into a particular area, which will produce a higher quality protein. VanEtten and co-workers have looked at the amino acid composition of a large number of plant species in an attempt to find sources of nutritionally high-quality vegetable protein (47, 48, 49, 50).

At first glance, it appears that supplementation of low quality plant proteins would be the easiest and the most economical program to follow, but with further consideration many drawbacks are found. Phillips (30) found that the e

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the estimated average intake of animal protein was equal to or exceeded the desirable amount of about 30 grams per day in only 18 of 58 countries studied. Such low intakes of animal protein are often due to several factors such as low availability, high cost and food customs. Evans and Bandemer (12) point out that production of animal protein is relatively inefficient when contrasted with plant protein production and that more plant protein can be produced on a given unit of land. It therefore appears that increasing the intake of animal proteins is somewhat remote in many protein deficient areas. Fortification of low quality proteins with purified amino acids may be restricted by many of the same limitations involved in the use of animal proteins. The introduction of new sources or crops may prove to be very time consuming or impractical.

Many investigations have been conducted to study possible effects of soil fertility levels on the amino acid composition of plants, but conclusions drawn from these studies are quite varied and confusing. Most of these studies have involved nitrogen, phosphorus, and potassium fertility levels and their effect on the free amino acid composition of vegetative portions of plants (8, 15, 25, 45). It is the proposition of this study that if amino acid composition of plants can be effectively changed, that more pronounced changes may occur at various levels of available micronutrients. It is also proposed that if changes in the amino acid composition are to have any meaning in food value, the total amino acid composition of the edible portion of a food crop that could make up the basel portion of a diet should be studied.

With these thoughts in mind, a program was set up to ascertain if phosphorus, zinc, and iron nutrition might influence the nutritional value of pea bean seed.

LITERATURE REVIEW

Amino Acid Requirements for Growth

One of the first to show that the amino acid composition of various proteins is quite different was Osborne (37). Around 1900 Osborne found drastic differences between the amino acid compositions of gliadin of wheat and zein of maize. Gliadin had a very low lysine content and zein was almost devoid of lysine and tryptophan (26, 27). With discoveries that proteins from various sources could be quite different in their amino acid make up, experiments were initiated to determine the importance of individual amino acids in animal nutrition. The first unequivocal evidence that certain amino acids could not be synthesized by animals, but had to be supplied from an outside source, came from the laboratory of Osborne and Mendel (28). Using purified zein it was demonstrated that both tryptophan and lysine were essential for the growth of young rats. Since these early investigations, extensive experiments with animals, including humans, by many investigators have shown that ten amino acids are essential for rat growth and eight are ess-

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essential for man. Rose (37) lists L-tryptophan, L-phenylalanine, L-lysine, L-threonine, L-valine, L-methionine, L-leucine, and L-isolencine as the essential amino acids for man. Two additional amino acids, L-histadine and L-arginine are necessary for maximum growth of the rat, but these are not required for the maintenance of nitrogen equilibrium in normal human adults. Rose (37) summarizes much of the early work conducted to determine the amino acid composition of proteins and essentiality of individual amino acids.

It is difficult to establish minimum requirements for the intake of essential amino acids, due to the fact that so many factors are involved. One of the more important is the proportion of amino acids in any particular protein source. For example Bricker, Mitchell and Kinsman (6) found in studies with young adult women that 74.4 grams of wheat flour protein per day was required to maintain nitrogen balance for a 70 kg adult, but only 43.0 grams of milk protein was needed. Such differences in the nutritional value of different proteins may be due to a shortage of one or more of the essential amino acids or to an inbalance of amino acids.

Rose (36) was the first to completely determine the amounts of various essential amino acids required for sati

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satisfactory mammalian growth. The Rose data show that the essential amino acid requirements for growth of the weanling rat based on percent of diet are: lysine 1.0, leucine 0.8, valine 0.7, phenylalanine 0.7, methionine 0.6, isoleucine 0.5, histidine 0.4, argine 0.2 and tryptophan 0.2. Research conducted by several other investigators, as reviewed by Flodin (13) and the 1965 FAO/WHO[1] report (55), has continued to add evidence that the proportion in which amino acids are ingested is of paramount importance.

Flodin (13) also reviews the need to provide all essential amino acids simultaneously in desirable proportions if they are to be used efficiently. He brings out the fact that high quality protein should be available with each feeding. If a low quality protein is fed at one time and a high quality protein at another, the end result is a loss in effectiveness of the protein of both feedings instead of supplementing each other. This phenomenon could defeat programs that are established in such a way that one source of protein is supplied at one time and a second protein or supplement is fed at a different time.

In 1957 the Food and Agricultural Organization of the United Nations (FAO) (14) published what is known as

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[1]Food and Agricultural Organization of the United Nations and The World Health Organization.

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the FAO provisional pattern for amino acids. This provisional pattern was intended as a guide to be used in evaluating protein problems in areas of the world faced with protein deficiency problems. It was recognized, however, that more was involved in protein nutrition than simply suppling all essential amino acids in amounts equal to, or greater than, some amount believed to be a so-called minimum daily requirement. Thus, in 1965, a report of a joint FAO/WHO expert group was published which placed much more emphasis on the pattern of essential amino acids present in a protein source (55). The following statement from this report sums up the importance of the amino acid balance of proteins.

> "When protein containing foods are fed at the level needed to meet the total protein requirement, the over-all pattern of available amino acids is more important in determining quality than simply the absolute amount of each of the essentials. It is true that the quality of protein is greatly influenced by its content of essential amino acids and that supplementations with the limiting essential amino acids may produce nutritional benefit. It is probable that this improvement in protein quality results mainly from a change to a more balanced amino acid pattern."

Mineral Nutrition and Amino Acid Composition

Many people believe that the amino acid composition of the proteinaceous material produced by an organism is

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strictly controlled by genetic factors. To a large extent this is no doubt true. An important example of man's ability to manipulate the genetic template of an organism in such a way that the protein quality of the plant is improved may be found with recent breeding programs designed to develop high lysine corn.

On the other hand, the amino acid composition of the same species and varieties of plants can be changed by environmental conditions. Pleshkov and Savitskaite (33) found a twofold difference in total protein content of wheat grain depending on the variety and growing conditions, but fertilizer applications did not affect the amino acid composition of the total or alcohol-soluble proteins. Pleshkov (32) states that fertilizers affect the total composition of free amino acids in plants but do not affect the amino acid composition of plant proteins. Pálfi (20) found no qualitative differences in the free amino acid compositions of wheat shoots at different rates of nitrogen, but during late stages of development found marked quantitative differences in asparagine, alanine, glutamine and γ-aminobutyric acid content. Thompson, Morris, and Gering (45), studing turnip plants grown under conditions of normal mineral nutrition and with deficiencies of N, P, S, K, Ca, or Mg, found that there were considerable

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differences in protein levels between deficient and normal leaves but that the relative composition of the protein fraction was only slightly affected. However, non-protein amino acids were profoundly influenced by mineral nutrition, suggesting that the lack of various macronutrients affected the metabolism of amino acids in many ways. Mulder and Bakema (75) found that N, P, K, and Mg nutrition had a pronounced effect on yield and protein content of potato tubers but that the amino acid composition of the protein was not affected. Mozhaeva, Tavrorskaya and Pleshkov (24) obtained similar results working with early potato tubers and high rates of manure and fertilizer.

Renner, Bentley and McEhoy (35) found that sulfur containing fertilizers produced highly significant increases in leucine, isoleucine, valine, methionine and histidine in the protein of first year barley after alfalfa. After the third year of barley, increases of leucine and methionine were found. According to Völker (53) there is a close correlation between the proportion of single amino acids in the raw protein in the grain of wheat and barley and the nitrogen content of the dry matter. With increasing nitrogen content the proportions of arginine, lysine, alanine, threonine, glycine, aspartic acid and valine decrease in the raw protein, while glutamic acid, pehnylalanine and leucine

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increase. Therefore, the effect of late application of nitrogen on the composition of amino acids depends mainly upon an increase in the content of raw protein. This increase in raw protein is probably due to an increase in the prolanin fraction of the protein. Prolanin is very poor in lysine, thus the protein in the total grain will contain a low relative lysine content.

Savitskaite and Pleshkov (40) found that the free amino acids in the grain of wheat were greatly affected by levels of nitrogen and phosphorus. They state that low phosphorus doubled the total free amino acid content, increasing in particular, the contents of glutamic and aspartic acid, asparagine, serine, glutamine and arginine. High nitrogen also increased the free amino acid content, particularly by increasing the contents of glutamine, arginine, aspartic acid and asparagine. Larsen and Nielsen (17) found that increasing nitrogen rates increased the content of glutamic acid and proline and that the content of arginine and lysine in wheat protein decreased. Michael (20) found that different protein fractions of wheat, barley, oats and rice change with varing nitrogen applications and that increasing amounts of nitrogen favor the formation of the reserve proteins, glutelin and prolamin (corresponding to glutenin and gliadin in wheat). This observation supports tha

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the suggestion made by Völker (53) and would also suggest mineral nutrition does not change the amino acid composition within a particular protein but can affect the amount of a protein that may be present in a seed.

Tsanava (46) reported that the sum of the concentration of essential amino acids in horse bean seed was greatest with normal rates of N, P, and K or with low rates of nitrogen. In oat grain, however, the total concentration of essential amino acids was greatest with high rates of nitrogen or with low rates of phosphorus.

Sauberlich, Chang, and Salmon (39) found that the rate of nitrogen fertilization and variety influenced considerably the protein and amino acid composition of the corn kernel. They found that as the percent protein in the kernel increased that leucine, alanine, phenylalanine and proline increased when calculated on percent of total protein. Conversely, arginine, glycine, lysine, and tryptophan decreased. MacGregor, Taskovitsh and Martin (19) found similar results. According to Vlasyuk (51), NO_3 forms of nitrogenous fertilizers decreased the leucine content of maize grain when compared to other nitrogen fertilizer materials and Mn applications increased the leucine content.

According to Khai and Pleshkov (16), mineral nutrition can affect the total protein content of kidney beans. They

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found that phosphorus and especially potassium deficiency caused a substantial reduction in the content of water soluble proteins and a corresponding increase in proteins soluble in potassium chloride solutions. Khai and Pleshkov (16) concluded that insufficient phosphorus or potassium nutrition of plants could result in a decrease in the assimilability of kidney bean protein if a relationship exists between protein solubility and the degree of assimilability by animals.

In research with Saginaw and Sanilac varieties of pea beans Woods (54) found that the tryptophan content of the seed of both varieties increased when zinc was applied at successively later dates, however no meaningful relationships seemed to exist between the zinc content of the seed and tryptophan or protein content. Woods reported that the tryptophan composition ranged from 1.82 to 2.48 percent of the total protein.

Ramaiah, Rao and Chokkanna (34) found an accumulation of free amino acids in zinc deficient coffee leaves along with a lower amount of protein. DeKock and Morrison (9) found that iron affected free amino acids composition of plants in a similar manner. Such findings indicate that zinc and iron may be involved in protein synthesis.

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Feeding Value as Related to Protein Content

Miller, Aurand and Flach (21) state that increasing the protein content of corn may decrease the protein quality of the grain due to the fact that the additional protein may be zein which is very low in lysine and tryptophan. However, they found that the quality of protein was not changed with increases in the amount of protein within the range of 8.48 to 14.12%. Dobbins et al. (11) found that the zein portion of corn protein does increase as the total amount of protein increases, but they found no differences in the feeding value of corn containing 8.6, 11.2, and 13.5% protein when rats where fed a diet containing equal amounts of corn protein equated at 15% with supplement. In feeding trials with pigs, they found that when the same amount of corn was fed with varying rates of supplement to increase the protein content of the diet to 15%, the low protein corn out performed the corn containing a higher percentage of protein. Dobbins (10) evaluated high and low protein corn by the biological value method and found that as the percent protein in corn increased, the biological values decreased.

Sauberlich et al. (39) found that the protein content of low protein varieties of corn was markedly increased by nitrogen fertilization and when fed to rats in diets containing equal amounts of corn protein, the low protein corn out performed the high protein corn. On the other hand, when diets were made in such a way that equal amounts of corn were fed, the high protein corn out performed the low protein corn.

A more complete review of the effects of environmental conditions and breeding programs on the protein content of corn and the relationships between the protein content and nutritional value of the protein has been made by Mitchel, Hamilton and Beadles (22).

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MATERIALS AND METHODS

Treatments and Preparation

Bean samples used in this study were collected from selected experimental plots located in Saginaw County, Michigan. Experimental procedures, soil properties and management practices used in this plot area are given by Brinkerhoff *et al*. (7) for 1966 and Vinande *et al*. (50) for the 1967 crop.

In 1966 dry pea beans (*Phaseolus vulgaris* L. var. Sanilac) were collected at harvest time from four replications of six zinc and iron treatments grown on high phosphorus 448 kg P/ha) experimental plots and five zinc and iron treatments from plots receiving low phosphorus rates (112 kg P/ha). A complete listing of treatments is given in Table 1. Replications of each treatment were composited in the field, on a volume basis, by combining one pint of seed from each replication. Composited samples were cleaned by hand and ground in a Wiley mill to pass a 40 mesh screen. After grinding, bulk samples were thoroughly mixed and stored until analyses could be made.

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Table 1. Soil treatments used to study possible affects of phosphorus, zinc, and iron on nutritional value of Sanilac pea bean in 1966.

Treatment[a] P	Zn	FE	Carrier	Code[b]
	kg/ha			
448	-	-	-	H-O
448	-	11.2	$FeSO_4$	H-FS
448	3.36	-	$ZnSO_4$	H-ZS
448	0.67	-	ZnNTA[c]	H-ZN
448	-	0.67	FeNTA	H-FN
448	0.67	0.67	Zn and FeNTA	H-FZN
112	-	-	-	L-O
112	3.36	-	$ZnSO_4$	L-ZS
112	0.67	-	ZnNTA	L-ZN
112	-	0.67	FeNTA	L-FN
112	0.67	0.67	Zn and FeNTA	L-FZN

[a] 336 kg/ha of 8-32-16 applied 1 inch to side and 1 1/2 inch below seed. Additional P added to plots receiving 448 kg P/ha plowed down.

[b] This code used throughout remainder of text to designate treatments.

[c] Zinc nitrilotriacetic acid.

In 1967, samples for laboratory study were collected from four replications of six zinc and iron treatments, which had received high phosphorus applications (448 kg P/ha). A complete listing of these treatments is given in Table 2. Samples were hand cleaned and ground in a Wiley mill to pass a 40 mesh screen. After grinding, composite samples were prepared by taking 50 grams of meal from each replication and thoroughly mixing. Amino acid analyses were carried out on the composite samples while nitrogen determinations were made on the replicated samples.

Nitrogen Analysis

Nitrogen analysis was accomplished by the micro-Kjeldahl procedure given in Aminco Reprint No. 104 (2) with slight modifications. Approximately 50 mg of bean meal was weighed and transferred to a 30 ml digestion flask using ashless paper. One-half gram of a mixture of 1 part HgO and 12.5 parts K_2SO_4 was added to the meal and paper in the digestion flask followed by the addition of 1.5 ml of concentrated H_2SO_4. Digestion was carried out on an Labconco Model-A Electric Digestion Rack.[1] The mixture was heated for an additional 20 minutes after clearing, allowed to cool and then diluted with 10 ml of water to prevent formation of

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[1]Available from Matheson Scientific Inc., Chicago, Illinois.

Table 2. Soil treatments used to study possible affects of zinc and iron on nutritional value of Sanilac pea bean in 1967.

Treatment Zn	Treatment FE	Carrier	Code
kg/ha			
-	-	-	0 Fert[a]
-	-	-	Fert[b]
3.36	-	$ZnSO_4$	ZS
0.67	-	ZnNTA	ZN
-	0.67	FeNTA	FN
0.67	0.67	Fe and Zn NTA	FZN

[a]This treatment received no fertilizer.

[b]All remaining treatments received 336 kg/ha of 8-32-16 applied 1 inch to the side and 1 1/2 inch below seed plus 336 kg/ha of P as 0-47-0 plowed down.

K_2SO_4 crystals. An Aminco mico-Kjeldahl distillation assembly[1] was used for the ammonia distillation. The distillate was collected in 10 ml of 2% boric acid solution containing 4 drops of indicator. The indicator was prepared by dissolving 100 mg of methyl red and 200 mg of bromcresol blue in 100 ml of 95% ethanol. After distillation, the boric acid solution was titrated with 0.02 N standardized HCl. Nitrogen determinations were made in duplicate and each set of samples included a reagent blank determination. The calculation of percent nitrogen was on an air dry basis. Crude protein content was calculated by multiplying nitrogen by 6.25.

Amino Acid Analysis

For total amino acid analyses, bean meal samples were hydrolyzed according to the procedure of Evans and Bandemer (12). Amino acid content of the hydrolyzates was determined using a Technicon amino acid analyzer based on the Piez and Morris (31) modification of the Spackman, Stein, Moore (44) method. Cystine was determined by the procedure of Scharn, Moore and Bigwood (41) as modified by Bandemer and Evans (3).

For ethanol soluble amino acids, 10 g samples

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[1]Available from American Instrument Company, Silver Springs, Maryland.

of bean meal were weighed into 100 ml polyethylene centrifuge tubes, followed by an addition of 50 ml of 70% v/v ethanol. The tubes were stoppered and shaken for 30 minutes in a vertical position on a wrist action shaker. After shaking, the solid material was centrifuged at a relative centrifical force of 1100, and the supernatent decanted into a 250 ml round bottom flask. This extraction procedure was performed three times. After extraction, the ethanol was evaporated with the aid of a flash evaporator[1] at a temperature of $40^{o}C$. Forty milliliters of 0.1 N HCl were added to the residue remaining in the flask after evaporation in three equal increments. The flask was shaken for 10 minutes after each addition of HCl and the solution transferred to a 250 ml separatory funnel. Fifty milliliters of ether was then added to the flask in two 25 ml increments, shaken for 10 minutes and transferred to the separatory funnel. The HCl solution and ether layers were gently shaken together and allowed to separate. The HCl solution was drawn off and filtered through No. 2 Whatman filter paper into a 50 ml volumetric flask. The filter paper was then washed with 0.1 N HCl and volume of solution brought up to 50 ml.

Soluble amino acids were chromatographically

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[1]Available from Laboratory Glass and Instruments Corporation, New York, New York.

separated by the method of Moore, Spackman, and Stein (23), using 150 and 15 cm columns[1] and a fraction collector. A RSCO 1205 fraction collector[2] equipped with a drop counting unit was used. Ion exchange resins which met the specifications of Moore _et al_. (23) as well as all other reagents used in this procedure are commercially available.[3] The drop counting unit was adjusted to deliver 2 ml fractions. Measurement of the fraction size was accomplished by weighing the amount of solution delivered to individual test tubes. Delivery volumes were checked periodically during amino acid analyses.

It was found that a constant flow rate through the 150 cm column could not be established during the first trial runs. This problem was corrected by making all buffers with distilled water that had been passed through a cation-anion exchange resin and a charcoal column. In addition to making sure that all solutions were as free as possible from foreign

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[1]Columns which met the specifications of Moore and co-workers available from Scientific Glass Apparatus Company, Bloomfield, New Jersey.

[2]Available from Matheson Scientific Inc., Chicago, Illinois.

[3]Aminex MS, Fraction C and Aminex MS Fraction D ion exchange resins available from Bio-Rad Laboratories, New York, New York.

material it was necessary to use a millipore filter in the line between the column and the reservoir containing the buffer. With these precautions it was possible to maintain the flow rate at about 10 ml per hour at 25 mm Hg pressure. The same precautions were used when operating the 15 cm column. Flow rates with this column were about 30 ml per hour when operated at 20 mm Hg pressure.

Test tubes used were selected from standard stock 19 x 150 mm rimless tubes and matched to ± 1% transmittance by the procedure outlined in the Bausch and Lomb reference manual (4).

The method of Rosen (38) with minor modifications as given by Lawrence and Grant (18) was used to determine the minhydrin color yield of the fractions from the column. Standard leucine samples were run at regular intervals to check the performance of the method.

A calibration standard containing one micromole of each amino acid determined[1] was used to calibrate each column. A series of leucine standards from 0 to 0.45 micromoles was run each time a sample or known standard was chromatographed and fractionated. By plotting micromoles of leucine verses percent transmittance, a standard calibration curve was

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[1]Obtained from Dr. Bandermer, Department of Biochemistry, Michigan State University, East Lansing.

obtained. With this calibration curve, the percent transmittancy value for each 2 ml fraction collected from the fraction collector was converted to micromoles of leucine and plotted verus the elution volume. Several calibration standard runs were made to determine the peak areas of each amino acid before any unknown samples were analyzed. The peak area for each amino acid was calculated, or integrated, by multiplying the width of the peak at half the height times the height (area of triangle). Measurements were made in millimeters. The resulting areas were the standard areas for each column and are given in Tables 3 and 4.

Table 3. Peak areas and standard errors for chromatographed synthetic mixture containing 1 μM each of neutral and acidic amino acids.

Amino Acid	Column No. 1[a]		Column No. 2[a]	
	Mean	SE	Mean	SE
Aspartic acid	6266	± 97	5910	± 179
Threonine	5775	± 160	5723	± 56
Serine	6165	± 212	6008	± 140
Glutamic acid	6218	± 120	6078	± 94
Proline	1493	± 102	1287	± 135
Glycine	5762	± 134	5605	± 244
Alanine	5911	± 115	5653	± 250
Cystine	5962	± 157	5510	± 386
Valine	6014	± 281	5298	± 289
Methionine	5874	± 196	6026	± 336
Isoleucine	6558	± 240	5960	± 69
Leucine	6518	± 228	5911	± 123
Tyrosine	6333	± 135	5914	± 98
Phenylanlanine	6511	± 301	5956	± 81

[a]Means for five determinations.

Table 4. Peak areas and standard errors for chromatographed synthetic mixture containing 1 μM each of basic amino acids and ammonia.

Amino Acid	15 cm Column[a]	
	Mean	SE
Lysine	6830	± 217
Histidine	6033	± 146
Arginine	5336	± 180
Ammonia	5469	± 165

[a]Means for seven determinations.

RESULTS AND DISCUSSION

Nitrogen and Crude Protein Content

The percentages of nitrogen and crude protein of Sanilac pea beans grown on soils receiving different rates of phosphorus, zinc, and iron are shown in Table 5.

The nitrogen and crude protein content was not greatly affected by any of the treatments, however, some trends do occur. In 1966, beans grown on plots receiving a combination of zinc and iron contained higher percentages of nitrogen and crude protein than beans from other treatments. Phosphorus did not affect the nitrogen and crude protein content. This is substantiated by comparing data from similar zinc and iron treatments on the two levels of phosphorus. Beans grown in 1966 had a higher nitrogen and crude protein content than those from the 1967 season. The average crude protein content from all treatments in 1966 was 24.5% while in 1967 the average was 20.1%.

Such fluctuations in the nitrogen and crude protein content may be an important factor in nutrition, particularly in areas where a limited quantity of food is available to

Table 5. Nitrogen and crude protein content of Sanilac pea beans as affected by phosphorus, zinc and iron fertilizer treatments in 1966 and 1967.

Treatments[a]	% Nitrogen	% Crude Protein[b]
1966		
H-O	3.7	23.1
H-FS	4.0	25.0
H-ZS	3.9	24.4
H-ZN	3.9	24.4
H-FN	3.8	23.7
H-ZFN	4.1	25.6
Mean	3.9	25.0
L-O	3.8	23.7
L-ZS	3.8	23.7
L-ZN	3.9	24.4
L-FN	3.8	23.7
L-ZFN	4.0	25.0
Mean	3.7	24.1
1967		
O Fert	3.2	20.0
Fert	3.4	21.2
ZS	3.2	20.0
ZN	3.2	20.0
FN	3.2	20.0
FZN	3.1	19.4
Mean	3.2	20.1

[a]See Tables 1 and 2 for description of treatment code.

[b]Percent N X 6.25 = percent crude protein.

feed the population. For example, the 1965 FAO/WHO report (55) states that the protein requirement for an adult in terms of grams per kilogram body weight per day is approximately 0.71 g. Therefore, an adult weighing 68 kg would need to consume 200 g of food containing 24% protein compared with 240 g of food containing 20% protein to satisfy the FAO/WHO standard. This comparison assumes that both sources have equally digestable proteins. The FAO/WHO report makes these requirements in terms of a reference, or high quality protein. The protein quality of the pea bean has a rating of about one-half that of the reference material, therefore, about twice as much bean protein as compared with the reference source is needed to meet minimal requirements. Protein requirements for infants, children and adolescents are higher than for adults, on a gram protein per kilogram body weight per day basis. Thus, improper protein nutrition could easily reach a critical stage with a combination of short food supplies and low protein sources.

Amino Acid Composition of Pea Beans

The amino acid contents of pea beans grown on various levels of phosphorus, zinc and iron are presented in Tables 6, 7, and 8. Amino acid data given in these tables are expressed in grams of amino acid per 16 g nitrogen (g/16 g N).

Table 6. Total amino acid composition of Sanilac pea beans grown on high soil phosphorus levels in 1966.

Amino Acids	1957 FAO Provisional Pattern (14)	Treatments[a] H-O[b]	H-FS	H-ZS	H-ZN	H-FN	H-ZFN
		(grams per 16 grams nitrogen)					
Essentials							
Isoleucine	4.2	4.6	4.3	4.0	4.8	4.7	4.0
Leucine	4.8	7.4	7.1	7.1	7.6	7.9	6.8
Lysine	4.2	6.6	6.6	5.6	7.1	6.7	5.6
Phenylalanine	2.8	5.5	5.2	5.0	5.7	5.7	4.9
Tyrosine	2.8	2.2	2.2	2.0	2.3	2.2	2.0
Cystine	2.0	0.8	0.9	0.7	0.7	0.7	0.7
Methionine	2.2	0.9	0.9	1.4	0.8	1.0	0.9
Threonine	2.8	4.1	3.9	3.6	4.2	3.9	3.7
Valine	4.2	5.3	4.9	4.6	5.4	5.1	4.4
Typtophan	1.4	Not Determined					
Nonessentials							
Alanine		4.0	3.7	3.6	4.0	4.1	3.3
Arginine		7.0	7.5	6.4	7.3	6.8	6.0
Aspartic Acid		10.5	10.0	10.5	10.7	11.0	9.3
Glutamic Acid		16.0	16.4	15.5	17.8	17.4	14.7
Glycine		3.9	3.6	3.5	4.2	4.0	3.4
Histidine		2.8	2.7	2.3	2.8	2.9	2.5
Proline		3.6	3.4	4.0	3.8	3.8	3.2
Serine		5.5	5.3	5.0	5.6	5.7	5.1
S-Methyl cysteine		1.2	1.0	1.3	1.1	1.1	1.1
Ammonia		1.4	1.6	1.2	1.5	1.5	1.3

[a] Average of two determinations.
[b] See Table 1 for complete description of treatment code.

Table 7. Total amino acid composition of Sanilac pea beans grown on low soil phosphorus levels in 1966.

Amino Acids	1957 FAO Provisional Pattern (14)	Treatments[a]				
		L-O[b]	L-ZS	L-ZN	L-FN	L-ZFN
		(grams per 16 grams nitrogen)				
Essentials						
Isoleucine	4.2	4.2	4 5	4.3	4.8	4.4
Leucine	4.8	7.4	8.0	7.3	8.1	7.4
Lysine	4.2	5.8	6.4	6.2	6.7	6.3
Phenylalanine	2.8	5.1	5.5	5.2	5.6	5.3
Tyrosine	2.8	2.0	2.3	2.2	2.2	2.2
Cystine	2.0	0.7	0.6	0.7	0.7	0.7
Methionine	2.2	1.1	1.1	1.1	0.9	0.9
Threonine	2.8	4.0	4.2	4.0	4.2	3.9
Valine	4.2	4.6	5.3	5.0	5.6	5.2
Typtophan	1.4	Not Determined				
Nonessentials						
Alanine		3.8	4.0	3.4	4.1	4.0
Arginine		6.4	6.8	6.4	6.7	6.6
Aspartic acid		10.7	10.7	10.6	10.5	10.3
Glutamic acid		15.4	18.0	16.3	17.9	16.9
Glycine		3.7	4.0	3.4	3.9	3.8
Histidine		2.7	2.8	2.7	2.9	2.8
Proline		3.2	3.3	3.2	3.6	3.7
Serine		5.5	5.8	5.5	6.1	5.4
S-Methyl cysteine		1.0	1.0	1.2	1.2	1.2
Ammonia		1.3	1.4	1.4	1.5	1.4

[a]Average of two determinations
[b]See Table 1 for complete description of treatment code.

Table 8. Total amino acid composition of Sanilac pea beans grown in 1967.

Amino Acids	1957 FAO Provisional Pattern (14)	Treatments[a]					
		O Fert[b]	Fert	ZS	ZN	FN	FZN
		(grams per 16 grams nitrogen)					
Essentials							
Isoleucine	4.2	5.0	4.7	5.0	4.7	4.8	5.0
Leucine	4.8	7.9	7.5	7.4	7.4	7.3	8.0
Lysine	4.2	7.2	6.7	6.8	6.8	6.9	6.6
Phenylalanine	2.8	5.7	5.4	5.6	5.3	5.5	5.6
Tyrosine	2.8	2.7	2.6	2.6	2.5	2.6	2.6
Cystine	2.0	1.0	0.8	0.8	1.0	1.0	1.0
Methionine	2.2	1.0	0.8	1.0	1.0	1.4	1.3
Threonine	2.8	5.0	4.8	4.6	4.8	4.6	5.0
Valine	4.2	6.1	5.6	5.8	5.4	5.7	6.0
Typtophan	1.4	Not Determined					
Nonessentials							
Alanine		4.7	4.2	4.6	4.3	4.4	4.6
Arginine		6.4	5.7	5.8	5.4	5.6	5.9
Aspartic acid		11.3	9.9	11.4	11.3	11.2	11.9
Glutamic acid		17.8	19.2	18.0	18.2	15.9	18.0
Glycine		4.1	4.3	4.2	3.8	3.9	4.2
Histidine		2.7	2.7	2.7	2.6	2.7	2.6
Proline		4.1	4.1	4.8	3.5	4.5	4.0
Serine		6.7	6.8	8.9	6.4	6.1	6.4
S-Methyl cysteine		1.3	1.4	1.6	1.4	1.3	1.4
Ammonia		1.7	1.6	1.6	1.5	2.0	1.6

[a] Average of two determinations
[b] See Table 2 for complete description of treatment code.

Such an expression is approximately equal to grams of amino acid per 100 g protein since proteins contain an average of 16% nitrogen. Expression of protein amino acid composition as g/16 g N is, therefore, also the percentage of a particular amino acid present in a protein. The FAO provisional reference pattern of essential amino acids (14) is included in these tables for purposes of comparison.

Tyrosine is included in the FAO provisional pattern even though it has not been shown to be essential for mammalian nutrition. Apparently, animals are able to synthesize sufficient amounts of tyrosine to meet their needs. Perhaps a portion of the tyrosine requirement is met by phenylalanine or by a conversion of phenylalanine to tryosine. For these reasons, the total amount of aromatic amino acids, which are primarily phenylalanine and tyrosine, is considered when evaluating the nutritional value of a protein source.

Evans and Bandemer (12) and Woods (54) reported that the tryptophan content of Sanilac pea beans was greater than the 1.4 g/16 g N recommended by the FAO provisional pattern (14). On the basis of these reports (12, 54) and because of the difficulty in determination, typtophan analyses were not made.

Data given in Tables 6, 7, and 8 show methionine and cystine to be the most limiting amino acids in pea beans. These results agree with previous reports by other investigators (12). Levels of methionine ranged from 0.8 to 1.4 g/16 g N, while the variability in cystine content was much narrower. Cystine content ranged from 0.8 to 1.0 g/16 g N. Recommended levels given by the FAO provisional pattern for methionine and cystine are 2.2 and 2.0 g/16 g N respectively, which is approximately twice as high as that found in pea beans analyzed.

The cystine content was so consistant for all samples that it did not appear to be influenced by any of the treatments, however, more cystine was found in beans grown in 1967 as compared to those grown in 1966. The average amount of cystine in the 1966 crop was about 0.7 g/16 g N while the 1967 crop had an average content of about 0.9 g/16 g N.

The variability in the methionine content suggests that factors other than the genetic make up of the pea bean can influence the amount of this sulfur-containing amino acid found in plant proteins. Some trends showing a relationship between the methionine content and soil treatments do appear.

Zinc uptake data of five week old plants (7) indicate that high phosphorus applications will depress the amount of zinc taken up by the pea bean plant. The same data show that zinc sulfate applications may overcome the depressing effects of high phosphorus fertilization. Methionine content of pea bean protein tends to follow the pattern of zinc uptake indicating that zinc may be involved in regulating the amount of methionine found in the pea bean seed. In 1966, beans grown on areas receiving only 336 kg/ha of a 8-32-16 fertilizer tended to have a higher methionine content than beans grown on areas receiving 336 kg/ha of 8-32-16 plus 336 kg/ha of elemental phosphorus. The highest methionine content was found in beans grown on high phosphorus treatments receiving zinc sulfate. In 1967, the methionine content was about the same as that for beans grown on the low phosphorus plots in 1966. These data may be the result of the higher levels of residual zinc present in the 1967 plot area as compared with the 1966 plot area, therefore, the high phosphorus application in 1967 did not greatly reduce zinc uptake. The application of FeNTA[1] and Zn and FeNTA tended to increase zinc uptake in 1967 (51). This increase in zinc uptake is reflected in an increased methionine content. The use of different zinc and iron carriers did not appear to affect the methionine content of pea beans.

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[1]Iron nitrilotriacetic acid.

All other essential amino acids were found in amounts greater than those given by the FAO provisional pattern (14), therefore, changes in the concentrations of these amino acids are of little concern as far as the nutritional value of the pea bean is concerned. On the other hand, if soil treatments were reflected in the concentration of any of the amino acids, a more complete understanding of the function of plant nutrients would be gained.

Isoleucine, lysine, phenylalanine, threonine and valine did not appear to be affected by Zn or Fe treatments, but the contents of these amino acids were affected by the different growing seasons. All of these amino acids were found in somewhat greater amounts in the 1967 crop compared with 1966.

The nonessential amino acids show about the same seasonal response as the essentials. Treatments did not greatly affect the amino acid content of the bean seed. Exceptions to this were glutamic acid and proline. In the case of these amino acids, zinc treatments tended to cause an increase in their concentration.

Essential amino acids expressed in milligrams of amino acid per gram of nitrogen (mg/g N) are given in Tables 9, 10, and 11. Since these figures were obtained by multiplying values from Tables 5, 6, and 7 by 100 and dividing

Table 9. Essential amino acid composition of Sanilac pea beans grown on high soil phosphorus levels in 1966. (milligrams of amino acid per gram of total nitrogen)

Amino Acid	Hen's Egg (55)	Treatments[a]					
		H-O[b]	H-FS	H-ZS	H-ZN	H-FN	H-ZFN
Isoleucine	415	288	269	250	300	284	250
Leucine	553	463	444	444	475	494	425
Lysine	403	413	388	350	444	418	350
Total "Aromatic" Amino Acids	627	482	463	437	500	494	431
Phenylalanine	365	344	325	312	356	356	306
Tyrosine	262	138	138	125	144	138	125
Total S-Containing Amino Acids	346	106	112	132	94	107	100
Cystine	149	50	56	44	44	44	44
Methionine	197	56	56	88	50	63	56
Threonine	317	256	244	225	263	244	231
Tryptophan	100	113[c]	113	113	113	113	113
Valine	454	331	306	288	338	319	275
Total Essential Amino Acids	3215	2452	2339	2239	2527	2473	2175

[a]Average of two determinations.

[b]See Table 1 for complete description of treatment code.

[c]Calculated from data from Evans and Bandemer (12).

Table 10. Essential amino acid composition of Sanilac pea beans grown on low soil phosphorus levels in 1966. (milligrams of amino acid per gram of total nitrogen)

Amino Acid	Hen's Egg (55)	Treatments[a]				
		L-O[b]	L-ZS	L-ZN	L-FN	L-ZFN
Isoleucine	415	263	281	269	300	275
Leucine	553	463	500	456	506	463
Lysine	403	363	400	388	418	394
Total "Aromatic" Amino Acids	627	444	488	463	488	469
Phenylalanine	365	319	344	325	350	331
Tyrosine	262	125	144	138	138	138
Total S-Containing Amino Acid	346	113	107	113	100	100
Cystine	149	44	38	44	44	44
Methionine	197	69	69	69	56	56
Threonine	317	250	263	250	263	244
Tryptophan	100	113[c]	113	113	113	133
Valine	454	288	331	313	350	325
Total Essential Amino Acids	3215	2297	2438	2365	2538	2383

[a]Average of two determinations

[b]See Table 1 for complete description of treatment code.

[c]Calculated from data from Evans and Bandemer (12).

Table 11. Essential amino acid composition of Sanilac pea beans grown in 1967. (milligrams of amino acid per gram of total nitrogen)

Amino Acid	Hen's Egg (55)	Treatments[a]					
		O-Fert	Fert[b]	ZS	ZN	FN	FZN
Isoleucine	415	313	294	313	294	300	313
Leucine	553	494	468	463	463	456	500
Lysine	403	450	418	425	425	432	413
Total "Aromatic" Amino Acids	627	525	501	513	487	507	513
Phenylalanine	365	356	338	350	331	344	350
Tyrosine	262	169	163	163	156	163	163
Total S-Containing Amino Acids	346	126	100	113	126	151	144
Cystine	149	63	50	50	63	63	63
Methionine	197	63	50	63	63	88	81
Threonine	317	313	300	288	300	288	313
Tryptophan	100	113[c]	113	113	113	113	113
Valine	454	381	350	363	338	358	375
Total Essential Amino Acids	3215	2715	2544	2591	2546	2605	2684

[a]Average of two determinations.

[b]See Table 2 for complete description of treatment code.

[c]Calculated from data from Evans and Bandemer (12).

by 16, all values have the same relationships as discussed above. By expressing the amino acid content in this manner, however, it is possible to evaluate the protein quality of pea beans by following the procedures of the 1965 FAO/WHO report (55).

The 1965 FAO/WHO report (55) concluded that a revision of the 1957 FAO pattern was needed. Experimentation as reviewed by the report, showed that the proportion of typtophan and sulfur-containing amino acids in the FAO provisional pattern was too high. The net result of revisions made, resulted in a pattern that closely resembled the essential amino acid pattern of whole hen's egg. Thus, the essential amino acid pattern of this protein source has been adopted for reference purposes by FAO/WHO.

When comparing the essential amino acid pattern for eggs with values obtained in this study, the sulfur-containing amino acids of pea beans are the most limiting, but isoleucine, leucine and valine were also present in deficient levels in all samples studied. The lysine content of beans tended to fall below the reference pattern with zinc treatments in 1966. The one exception is with the zinc chelate treatment. This treatment gave the highest level of lysine for all treatments studied. In 1967, all values for lysine were above the reference level.

It appears that an inverse relationship exists between lysine and methionine, at least in the 1966 crop. The lowest level of methionine, 50 mg/g N, corresponds to the highest level of lysine, 444 mg/g N. The highest level of methionine, 88 mg/g N, corresponds to the lowest level of lysine, 350 mg/g N. The same relationship is seen in the 1967 data but in this case it is not as clear or as consistent.

The amount of total essential amino acids also shows a tendency to be decreased by zinc treatments. As in the case of individual amino acids, this tendency is not completely consistent but it seems that soil treatments may be a factor influencing the total essential amino acid content.

Ethanol Soluble Amino Acids

Amounts of 70% v/v ethanol extractable amino acids in bean meal were determined on a limited number of treatments from the 1966 crop. The results are shown in Table 12.

As generally expected, the amounts of ethanol soluble amino acids present in bean meal were so low that this fraction would not play an important role in determining the nutritional value of the bean. However, the literature indicates that the fertility of the soil may be reflected

Table 12. Amino acids extracted from pea bean meal with 70% v/v ethanol.[a]

	Treatment[b]										
	H-O	H-FS	H-ZS	H-FN	H-FZN	Mean	L-O	L-ZS	L-ZN	L-FZN	Mean
						mg/g					
Aspartic acid	0.51	0.56	0.45	0.40	0.56	0.50	0.48	0.35	0.53	0.47	0.46
Threonine	0.19	0.07	0.27	0.18	0.31	0.20	0.23	0.15	0.19	0.20	0.19
Serine	0.74	0.75	0.65	0.66	0.99	0.76	0.81	0.75	0.92	0.95	0.86
Glutamic acid	1.06	1.03	1.26	0.97	1.50	1.16	1.13	0.96	1.15	1.09	1.08
Proline	0.13	0.15	0.05	0.05	0.08	0.09	0.22	0.05	0.14	0.05	0.11
Glycine	0.03	0.04	0.03	0.03	0.04	0.03	0.07	0.02	0.04	0.04	0.04
Alanine	0.10	0.09	0.11	0.14	0.12	0.11	0.14	0.11	0.12	0.12	0.12
Valine	0.11	0.09	0.11	0.19	0.06	0.11	0.13	0.13	0.15	0.12	0.13
Methionine	0.03	0.01	0.02	0.04	0.03	0.03	0.03	0.02	0.10	0.04	0.05
Isoleucine	0.04	0.07	0.07	0.05	0.04	0.05	0.05	0.07	0.09	0.05	0.06
Leucine	0.08	0.08	0.08	0.08	0.07	0.08	0.07	0.09	0.05	0.07	0.07
Tyrosine	0.04	0.02	0.04	0.07	0.04	0.04	0.04	0.04	0.07	0.04	0.05
Phenylalanine	0.08	0.08	0.11	0.13	0.10	0.10	0.11	0.16	0.18	0.11	0.14
Lysine	0.04	0.10	0.04	0.12	0.04	0.07	0.07	0.03	0.04	0.03	0.04
Histidine	0.31	0.42	0.23	0.31	0.33	0.38	0.29	0.20	0.30	0.33	0.28
Arginine	2.35	3.14	2.11	1.48	2.37	2.29	1.85	2.40	2.35	2.62	2.30
Ammonia	0.02	0.04	0.01	0.04	0.01	0.02	0.02	0.03	0.02	0.01	0.02
Cystine	0.01	0.01	0.02	0.02	0.09	0.03	0.02	0.02	0.01	0.04	0.02
Total	5.87	6.75	5.66	4.96	6.78	6.05	5.76	5.58	6.45	6.38	6.02

[a]See Table 1 for description of treatment code.

[b]Average of two determinations.

in the concentrations of soluble amino acids found in plant material.

The data obtained in this study, do not show any large differences that could be related to any of the soil treatments because the variability inherent in sampling and analyses was greater than differences found in the soluble amino acid composition, but some general trends do appear.

A comparison of the total amount of soluble amino acids extracted from pea beans grown on the two rates of phosphorus indicate that phosphorus fertilization did not affect the soluble amino acid fraction. For example, the amount of amino acids extracted from beans grown on the H-O and L-O plots was 5.87 and 5.76 mg/g of meal respectively.

When comparing all zinc and iron treatments, slightly greater amounts of amino acids were extracted from beans grown on H-FS, H-FZN, L-ZN and L-FZN treatments.[1] These data indicate that zinc and iron may be directly or indirectly involved in amino acid or protein metabolism.

The increase in soluble amino acids is probably not the result of an increase of any particular amino acid, but the result of an increase in the concentration of all amino

[1]See Table 1 for details of treatment code.

acids.

It was hoped that soluble amino acids might show larger differences in their concentrations as a result of soil treatments and possibly substantiate some trends shown by total amino acid analyses but this did not prove to be the case.

Climatic Effects on Nitrogen Content and Amino Acid Composition

Differences in the nitrogen and protein content of the bean seed as noted for the two different seasons (Tables 6, 7, and 8) may have been the result of two extremes in weather during the growing seasons. In 1966, a fairly dry growing season was experienced, while the 1967 season was dominated by excessive amounts of rain during the early part of the season and cool temperatures throughout the year. It is quite possible, therefore, that a greater amount of nitrogen was "mineralized" and available for plant growth during the 1966 season and that this increase in available nitrogen resulted in a high nitrogen and crude protein content of the bean in 1966.

Data from both 1966 and 1967 seasons indicate that fluctuations in the concentrations of amino acids may be due to climatic as well as soil environmental conditions.

With the decrease in the percent of crude protein present in the bean from the 1967 season, it was observed that many amino acids increase in percentage present in the protein. In the case of the essential amino acids, isoleucine, lysine, tyrosine, cystine, threonine and valine tend to show an increase. Leucine, phenylalanine and methionine appear not to be affected by the different climatic conditions.

Generally, percentages of nonessential amino acids in the protein increased with a decrease in the percentage of crude protein in the bean. The one notable exception was arginine which was consistantly lower in beans grown in 1967. Glycine and histidine values were not noticably different between the two crops.

These data indicate that the lower crude protein content in beans grown in 1967 might be the result of a particular protein, or group of proteins, rich in arginine, glycine, histidine, methionine, phenylalanine, and leucine not accumulating in the seed. Such proteins may be storage proteins which accumulate as the result of excessive nitrogen uptake.

Protein Evaluation

Several systems have been proposed to evaluate the nutritional value of proteins and foods. The most reliable method for measurement and expression of protein quality is a biological evaluation system. The 1965 FAO/WHO report (55) summarizes some of the most useful biological procedures. Biological evaluation of protein quality is often slow and costly when compared with chemical procedures, and is most useful after chemical procedures have been used to screen proposed diets or protein sources. Early methods of chemical evaluation of protein were usually conducted by comparing the amino acid composition of a protein of unknown quality with a reference amino acid pattern. The proportion or balance of essential amino acids of a protein source is now generally recognized to be important in determining the nutritional value of a food material or particular diet. FAO/WHO places much more emphasis on the proportion of essential amino acids and suggest that more precise information, regarding the nutritional value of a protein source, may be obtained from chemical analyses, if an amino acid to total essential amino acid ratio is used. This ratio is known as the A/E ratio. The A/E ratio is determined by dividing the amount of a particular amino acid present in a protein by the total amount of essential amino acids present in the same protein. For example, in the case of whole hen's egg (Table 9) the methionine content is 197 mg/g N and the amount of total essential amino acids is 3.215 g/g N. The A/E ratio of 61 is obtained by dividing 197 mg methionine/g N by 3.215 g total essential amino acids.

A/E ratios for whole hen's egg and Sanilac pea beans grown on different phosphorus, zinc and iron soil fertility treatments are given in Tables 13, 14, and 15.

The data given in Tables 13, 14, and 15 show that the A/E ratios for most of the essential amino acids are not greatly affected by soil applications of phosphorus, zinc or iron and that the A/E ratios for beans grown in 1966 and 1967 are very similar. There is much more variability in the A/E ratios for methionine across the zinc and iron treatments than for the other essential amino acids, especially for beans grown on plots receiving 448 kg/ha of phosphorus, as compared with beans grown on plots receiving 112 kg/ha of phosphorus. In 1966, the A/E ratio for methionine in pea beans grown on the high phosphorus plots ranged from 20 to 39. In 1967 the A/E ratio for methionine ranged from 21 to 34. When these values are compared with 61, which is the A/E ratio for the methionine content of whole hen's egg, it is seen that pea bean seed is quite low in this essential amino acid, and that the nutritive value of pea bean is about one-half that of the reference protein source.

Zinc fertilization of pea bean tended to give a higher A/E value than other treatments studied. In 1966,

Table 13. A/E ratios for essential amino acids of Sanilac pea beans grown on high soil phosphorus levels in 1966.[a] (milligrams per gram of total essential amino acids)

Amino Acid	Hen's Egg[c]	Treatments					
		H-O[b]	H-FS	H-ZS	H-ZN	H-FN	H-ZFN
Isoleucine	129	117	115	112	119	115	115
Leucine	172	189	190	198	190	200	195
Lysine	125	168	166	156	176	169	161
Total "Aromatic" Amino Acids	195	197	198	195	198	200	198
Phenylalanine	114	140	140	139	141	144	141
Tyrosine	81	56	59	56	57	56	57
Total S-Containing Amino Acids	107	43	48	59	37	43	46
Cystine	46	20	24	20	17	18	20
Methionine	61	23	24	39	20	25	26
Threonine	99	104	104	100	104	99	106
Tryptophan	31	46	48	50	45	46	52
Valine	141	135	131	129	134	129	126
Protein Score[a] Based on Methionine and Cystine	100%	40%	44%	55%	34%	40%	43%

[a]Calculated as described by FAO/WHO (55).

[b]See Table 1 for complete description of treatment code.

[c]From 1965 FAO/WHO report (55).

Table 14. A/E ratios for essential amino acids of Sanilac pea beans grown on low soil phosphorus levels in 1966.[a] (milligrams per gram of total essential amino acids)

Amino Acid	Hen's Egg[c]	Treatments				
		L-O[b]	L-ZS	L-ZN	L-FN	L-ZFN
Isoleucine	129	114	113	114	118	115
Leucine	172	202	201	193	199	194
Lysine	125	158	161	164	165	165
Total "Aromatic" Amino Acids	195	194	197	196	192	197
Phenlalanine	114	139	139	137	138	139
Tyrosine	81	54	58	58	54	58
Total S-Containing Amino Acids	107	49	43	48	39	42
Cystine	46	19	15	19	17	18
Methionine	61	30	28	29	22	24
Threonine	99	109	106	106	104	102
Tryptophan	31	49	45	48	45	47
Valine	141	125	133	132	138	136
Protein Score[a] Based on Methionine and Cystine	100%	45%	40%	44%	36%	39%

[a]Calculated as described by FAO/WHO (55).

[b]See Table 1 for complete description of treatment code.

[c]From 1965 FAO/WHO report (55).

Table 15. A/E ratio for essential amino acids of Sanilac pea beans grown in 1967.[a] (milligrams per gram of total essential amino acids)

Amino Acid	Hen's Egg[c]	Treatments					
		O Fert	Fert[b]	ZS	ZN	FN	FZN
Isoleucine	129	115	116	121	115	115	117
Leucine	172	182	184	179	182	175	186
Lysine	125	166	164	164	167	166	154
Total "Aromatic" Amino Acids	195	193	197	198	191	195	191
Phenylalanine	114	131	132	135	130	132	130
Tyrosine	81	62	65	63	61	63	61
Total S-Containing Amino Acids	107	46	39	44	49	58	54
Cystine	46	23	19	19	24	24	23
Methionine	61	23	21	25	25	34	31
Threonine	99	115	118	111	118	111	117
Tryptophan	31	42	44	44	44	43	42
Valine	141	140	138	140	133	137	140
Protein Score[a] Based on Methionine and Cystine	100%	43%	36%	41%	45%	54%	50%

[a] Calculated as described by FAO/WHO (55).

[b] See Table 2 for complete description of treatment code.

[c] From 1965 FAO/WHO report (55).

the H-ZS, L-O, L-ZS and L-ZN[1] treatments gave the highest A/E ratios for methionine. In 1967, the highest A/E ratios, based on methionine, were found for beans grown on the FN and FZN soil treatments. It is not known why the H-ZN treatment gave the lowest A/E ratio for methionine; however, non-zinc treatments that did show relatively high A/E ratios were treatments where an improvement in zinc uptake usually occurred (7, 51).

The 1965 FAO/WHO report (55) outlines a procedure for scoring a protein based on the essential amino acid, or group of amino acids, which limits the nutritional value of the protein source being studied. This scoring procedure predicts the relative efficiency of a protein in comparison with a reference protein. The FAO/WHO report (55) also suggests that when the essential sulfur-containing amino acids limit the nutritional value of a protein, such a protein should be scored on the bases of the total amount of all of these amino acids present. This is done because the sulfur-containing amino acids tend to complement each other and it is difficult to effectively separate them biologically when each one is present in a food source.

Protein scores, based on methionine and cystine,

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[1]See Tables 1 and 2 for description of treatment code.

for Sanilac pea beans grown on varying phosphorus, zinc and iron soil treatments are presented in Tables 13, 14, and 15. Based on whole hen's egg, which is given a protein score of 100%, the protein scores of pea beans studied in this investigation varied from 34-55%. These data give a clear indication that the nutritive value of pea beans does not remain constant and that environmental conditions play a role in determining the protein quality of this crop.

It appears that environmental conditions which affect the nitrogen and crude protein content of pea beans do not affect their nutritional value. As show in Table 5, the nitrogen and crude protein contents of beans were higher in the 1966 crop when compared with the 1967 crop; however, the protein scores for the beans grown during these two years are quite similar. Therefore, conditions that will increase the crude protein content of the bean will not necessarily cause a decrease in the protein quality.

The factors affecting the protein scores determined in this study are not known, but soil environment conditions that favor increased zinc uptake by the plant may be involved.

SUMMARY AND CONCLUSION

The influence of inorganic nutrition on protein quality of dry pea bean seed grown under field contitions as evaluated by parameters of total and ethanol soluble amino acids, and crude protein content was investigated in this study. Conclusions drawn were:

1. Protein scores show that the nutritional value of pea beans was not consistent when this crop was grown under different environmental conditions.

2. Crude protein content of pea beans was not affected by P, Zn or Fe.

3. Environmental factors which affect nitrogen availability may affect crude protein content of pea beans.

4. An increase or decrease in the crude protein content of pea beans did not affect the nutritional value of protein produced.

5. The nutritional value of pea bean protein was limited by the methionine and cystine content.

6. Zinc fertilization or other soil factors which favor Zn uptake tended to increase the percentage of methionine in

pea bean protein.

7. The total amount of ethanol soluble amino acids present in pea bean seed was affected slightly by Zn and Fe fertilizer treatments.

Findings of this study show that soil and atmospheric environmental conditions do have an influence on the protein content and nutritive value of pea beans, even though, conditions which favor improved bean quality were not determined to any degree of precision. Wide fluctuations in the nutritive value of basic food crop could mean the difference between adequate or inadequate nutrition of people living in areas where high quality protein is in short supply. Research on the problem of improving the protein quality of major food crops must continue, but more complete information regarding the quality of protein produced by the same crop under different environmental conditions should also be carefully studied.

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