STUDIES ON THE AMINO ACID ADEQUACY OF MILO PROTEIN FOR GROWTH OF RATS AND SWINE

by

WILSON GIDEON POND

Bachelor of Science
University of Minnesota
Minneapolis, Minnesota
1952

Master of Science
Oklahoma Agricultural and Mechanical College
Stillwater, Oklahoma
1954

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Thesis Approved:

J.C. Hillier
Thesis Advisor

A. B. Nelson

Glen Bratcher

Duane A. Benton

W. S. Newcomer

Robert MacVicar
Dean of the Graduate School

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INTRODUCTION

Of the many considerations involved in formulating a ration balanced in all known nutrients for swine, none is more complex than that of supplying the proper levels of essential amino acids. Published values for the average essential amino acid composition of various feedstuffs can be correlated with estimated requirements for the animal under specified conditions to arrive at a satisfactory amino acid balance. However, the problem is not so easily solved as this, since practical considerations pertaining to the cost and availability of the feeds otherwise suitable for use are also involved.

The large annual production of milo (grain sorghum) in the Southwest at present, and the continuing agronomic improvements point toward greater future production. Therefore it seemed desirable to obtain more information about the characteristics of milo with respect to its advantages and limitations from a nutritive standpoint for use in rations for swine. Although it is well established that the average crude protein content of milo grain is higher than that of corn, there is a paucity of published data concerning the essential amino acid makeup of milo.

It was the purpose of these investigations (1) to determine the essential amino acids most limiting in a variety of milo (Kafir 44-14) for the growth of rats and swine, and (2) to study the effect of the addition of a non-protein nitrogen source (urea) to a milo ration on the growth of rats and swine.

REVIEW OF LITERATURE

The conventional method used in studies of amino acid requirements and of amino acid adequacy of feedstuffs has been that of measuring rate of increase in body weight of young animals. To appreciate the importance of the optimum level of each essential amino acid in the diet in this regard, a brief review of published work dealing with the effects of insufficient or excess amounts of essential amino acids seems worthwhile.

The term "amino acid imbalance" is a descriptive term applied to the concept that has developed during the past decade concerning the relative amounts of amino acids available to an animal for growth or maintenance. The term has been applied to instances wherein a deficiency or an excess of one or more amino acids exists relative to the others present.

Amino Acid Deficiencies:

Forbes and Vaughan (1954) obtained weight loss, negative nitrogen balance, lethargy and the appearance of porphyrin-like pigments around the nose and forepaws of rats force fed for 7 days, diets deficient in methionine or histidine.

Grace and Steele (1954) found that when mice were fed a phenylalanine deficient diet, the amount of phenylalanine available for conversion to tyrosine was limited by the use of phenylalanine for protein synthesis. Body weights of mice receiving the phenylalanine deficient diets remained constant or decreased during the experimental period.

Similarly, Scott and Schwartz (1953) obtained severe weight loss in male rats fed a diet devoid of threonine. Singal et al. (1953) demonstrated a reduced rate of liver phosphatide synthesis in threonine deficient rats. Supplementary threonine produced normal values in 8 hours.

Totter and Day (1942) observed vascularization of the cornea of rats receiving tryptophan deficient diets and Cartwright (1945) observed lentricular opacity in pigs maintained on acid hydrolyzed casein or on zein. Shaeffer (1946) was able to promote regeneration of injured corneas of guinea pigs by direct application of amino acid solutions to the eye.

Vascularization of the cornea has been described by Totter and Day (1942), by Albanese (1945) and by Hock et al. (1945) in rats receiving lysine deficient diets. Sydenstricker et al. (1946) however, obtained the same abnormality with a diet low in protein, indicating that the effect may not necessarily be a specific action of a single amino acid deficiency.

Bothwell and Williams (1952) compared nitrogen excretion of rats fed a lysine deficient diet ad libitum with that of rats force fed the same diet by stomach tube. While the rats fed ad libitum developed anorexia and negative nitrogen balance, the force fed group lost weight but stayed in positive nitrogen balance even until death, which occurred in 9-14 days. There was a marked increase in urinary nitrogen during the first few days followed by a decrease as the deficiency symptoms progressed. This observation was in contrast to the results with methionine and histidine reported by Forbes and Vaughan (1954) in which a rather constant urinary and fecal loss of nitrogen was obtained throughout the experiment.

Mitchell and Block (1946) indicated that for rats methionine is the most limiting amino acid in soybean oil meal. Bell et al. (1950) studied the effects of protein from whole egg and from soybean oil meal on the nitrogen retention and growth of pigs. When a 10 percent protein ration was fed, the whole egg protein had a significantly higher biological value than the unsupplemented soybean protein, but the addition of methionine to equal the amount in the whole egg made soybean oil meal protein equal to whole egg protein.

Amino Acid Excesses:

More than thirty years ago Lewis (1925) observed albuminuria and death in fasting rabbits fed cystine for several days. A short time later Jackson et al. (1928) obtained 50 percent mortality and poor growth in rats fed a diet containing 35 percent gelatin. It is of interest to note that at this early date, an improper balance of amino acids was suspected of being responsible for the poor growth performance even though the classical work by Rose (1937) concerning the essential nature of certain amino acids was yet to be done.

Fishman and Artum (1942) described adverse effects of excess serine fed to rats receiving a casein diet. Feeding 100 milligrams of serine by stomach tube daily for 7 days resulted in growth cessation, anorexia, albuminuria and severe damage to the renal tubules.

Since that time several other amino acids have been shown to be harmful when provided in excess amounts. Niven et al. (1946) obtained growth retardation, vascularization of the cornea and edema in 50 percent of the rats fed a 10 percent casein ration containing 1 percent each of additional DL-phenylalanine and L-tyrosine.

Brown and Allison (1948) obtained reduced nitrogen retention in rats fed a 12 percent casein diet when 1.7 percent L-arginine was added.

The addition of this amount of arginine to a similar ration supplemented with 4.8 percent DL-methionine caused a less severe reduction in nitrogen retention than when no arginine was added. Kidney size was increased by excess methionine, but not by excess methionine plus arginine, further indicating that a relation exists between these amino acids under certain conditions.

Kade and Sheperd (1948) found that the addition of 1.0 percent DL-methionine to an 8 percent casein ration resulted in increased protein utilization by rats, but higher levels of 2.0, 2.5 and 3.0 percent added methionine severely inhibited growth and protein utilization. Grau and Kamei (1950) noted similar growth depressing effects of excess methionine but found that these adverse effects could be overcome by increasing the protein level of the diet. This finding substantiates the hypothesis advanced by Kade and Sheperd (1948) that the mixture of amino acids in which the proportion most nearly approaches that of the requirements of the animal will be the most efficiently utilized. Roth et al. (1950) found that practically all of the methionine fed to rats was absorbed from the gastrointestinal tract even when supplied in amounts up to 7 percent of the diet. Roth and Allison (1950) observed extensive tissue catabolism, weight loss and kidney hypertrophy in rats receiving this high level of methionine. When feeding a 12 percent casein diet containing 4.8 percent DL-methionine, they found that a level of 1.35 percent choline chloride was effective in preventing the adverse effects of the excess methionine. Other work involving the effects of excessive dietary methionine includes that of Van Pilsum and Berg (1950) who obtained retarded growth of rats by the addition of 1.8 percent DL-methionine to the ration. The growth retardation was removed by the simultaneous addition of 0.4 gram each of histidine, tryptophan and arginine and 1.4 grams of phenylalanine to the diet. Hardin and Hove (1951) presented evidence dealing with the toxicity of excess methionine which implicated several vitamins as well as other amino acids in the mechanism of action in the complicated picture of methionine metabolism. They found that the toxicity of 2 percent DL-methionine when added to a 10 percent casein diet, was more pronounced in the absence of vitamin E, folic acid and vitamin B_{12} than when these vitamins were included in the diet. Supplemental glycine and arginine gave significant protection even in the absence of the three vitamins, and complete protection when they were present. It was suggested that detoxification of excess methionine is accomplished either by supplying substances capable of utilizing the methyl groups, or by the high methionine level precipitating glycine and arginine deficiencies in the animal.

DeBey et al. (1952) showed that the growth of rats fed limiting amounts of vitamin B_6 could be depressed by levels of methionine only slightly above those required for maximum growth. This growth depressing effect was counteracted by vitamin B_6 , thereby demonstrating the necessity for vitamin B_6 in proper methionine metabolism.

Shahinian et al. (1952) hypothesized that excess threonine in a 9 percent casein ration caused growth inhibition by interference with the synthesis of tissue pyridine nucleotides (PN) from tryptophan via 3-hydroxy-anthranilic acid since threonine is structurally similar to that compound. However, ad libitum and forced feeding experiments in which a 9 percent casein diet was fed, produced insignificant differences in PN synthesis with high and normal levels of threonine. Ebisuzaki et al. (1952) could not produce amino acid imbalance using a free amino acid mixture simulating 9 percent casein, although definite growth inhibition occurred when threonine was added to a 9 percent intact casein ration. This was interpreted to mean that excess dietary threonine causes an imbalance by decreasing the availability of other amino acids in whole casein through an inhibitory effect on digestion.

Henderson et al. (1953) studied the niacin-tryptophan interrelationship in diets containing 10 percent casein hydrolysate. Threonine, lysine and valine were shown to be involved in causing growth inhibition when the casein diet was supplemented with tryptophan deficient proteins. It was postulated that tryptophan was spared for conversion to niacin when other amino acids limited protein synthesis. The addition of other amino acids caused tryptophan to be the most limiting, with the result that niacin formation was reduced and growth was depressed.

Koeppe and Henderson (1955) showed that rats receiving purified amino acid diets containing 0.10 - 0.11 percent tryptophan developed a niacin deficiency when all of the other essential amino acids were supplied at approximately the levels required for normal growth. When one of the essential amino acids was reduced to a level below the amount required, the growth was improved, indicating a less severe niacin deficiency. This finding further supports the view that tryptophan serves as a source of niacin more effectively when a relative deficiency of another amino acid limits its use for protein synthesis.

Amino Acids and Liver Fat:

As with amino acid deficiencies, the symptoms common to all excesses are anorexia and reduction or cessation of growth. In addition to these generalized effects, manifestations specific for certain amino acid imbalances have been described. Of these, the factors affecting liver fat levels has been studied extensively. Treadwell et al. (1948) and Beveridge et al. (1945), who demonstrated the effectiveness of methionine in reducing liver fat in rats fed choline deficient diets, found that the effect was reduced by the addition of other essential amino acids or by additional protein. They hypothesized that the growth stimulation created by extra amino acids was responsible for the reduction in lipotropic effect through increasing the demand for methionine in tissue protein synthesis.

Harper et al. (1954) obtained increased fat deposition in the liver of rats fed 9 percent casein diets containing choline by supplementing with 0.3 percent DL-methionine. In each case 0.36 percent DL-threonine in addition to methionine showed a lipotropic action. Harper et al. (1953) showed that 0.18 percent DL-threonine was effective in reducing the accumulation of liver fat in rats receiving a basal diet containing sucrose and 9 percent casein. The complete effectiveness of threonine suggests that it may play a specific role in liver fat deposition. The fact that Winje et al. (1954) showed that threonine is more effective in reducing liver fat in rats fed certain proteins than with other proteins, even though the threonine level of both diets is the same, indicates that other amino acids are also involved.

Singal et al. (1953) demonstrated that choline must be present to allow the lipotropic effect of threonine. The addition of niacin, tryptophan, valine or histidine did not prevent fatty livers when rats were fed a 9 percent casein diet. Fatty livers were completely prevented when rats were fed diets containing 20 percent casein. Benton et al. (1955) found that leucine, glutamic acid, aspartic acid and benzoic acid were effective in preventing excess liver fat in rats fed a low protein diet containing choline and methionine. The addition of glycine, serine and glutamic acid to this diet also resulted in lower liver fat. The addition of 0.36 percent DL-threonine to the basal ration was not completely effective in reducing liver fat. It was suggested that probably a different mode of action was involved in each cure and that threonine may be needed in liver enzyme or hormone synthesis involving liver lipid metabolism.

Arata et al. (1954) studied liver enzyme systems of rats fed diets known to produce fatty livers. The activities of the soluble cytoplasmic enzymes, xanthine oxidase and tyrosine oxidase were markedly increased when supplemental threonine was included in a 9 percent casein diet containing added methionine and tryptophan with or without choline. No significant differences were obtained in the activities of several mitochondrial enzymes studied. When tryptophan was removed from the ration, all differences in enzyme activity between threonine-deficient and threonine-supplemented rats disappeared, but no changes occurred in liver fat deposition. It was therefore suggested that the balance of amino acids in the diet is the primary factor responsible for the enzyme changes observed and that the accumulation of fat in the liver bears no direct relationship to those enzyme changes.

Amino Acids and Liver Enzymes:

Many liver enzyme systems have been shown to be extremely sensitive to deficiencies of one or more amino acids. Liver xanthine oxidase activity has been shown by Williams and Elvehjem (1949) to be drastically reduced during protein deficiency, and can be restored by increasing the dietary protein level. Bothwell and Williams (1951) have shown lysine, methionine and histidine deficiencies to be associated with reduced liver enzyme activity. Liver xanthine oxidase activity was reduced 50 percent in rats force fed a lysine deficient diet. Succinic oxidase, choline oxidase and endogenous respiration were reduced during lysine or methionine deficiency and succinic oxidase activity was reduced during histidine deficiency. The same workers (1954) have confirmed these earlier findings and point out that the pattern of liver enzyme activity is different for each amino acid deficiency.

Litwack et al. (1955) demonstrated that liver xanthine oxidase activity of rats receiving a purified diet containing only essential amino acids could be increased by the addition of ammonium citrate or urea. It appears that a valuable tool for determining amino acid adequacy of feedstuffs may be available in the future as more information is gathered concerning the sensitivity of various enzyme activities to variations in dietary protein and amino acid levels.

Protein Levels and Amino Acid Requirements:

Griminger et al. (1956) demonstrated that the tryptophan requirement of growing chicks increases as the protein level increases, although at a slower rate. Grau (1948) and Grau and Kamei (1950) had previously shown the same to be true for lysine and methionine. This relation between amino acid requirement and dietary protein level agrees with the concept of Almquist (1947) who stressed the desirability of expressing amino acid requirements as a percentage of the dietary protein.

The fact that zein is deficient in lysine and tryptophan is well recognized (Osborne and Mendel, 1914a and 1914b). Mitchell et al. (1952) confirmed previous work showing that the proportion of zein in corn increases as the total protein increases. Dobbins et al. (1950) and Eggert et al. (1953) have demonstrated the superiority of low protein to high protein corn in promoting growth of swine.

Sauberlich et al. (1953) reported results from growth trials with rats and chicks comparing the protein quality of low-protein (6.8-9.1 percent) and high-protein (9.5-13.6 percent) corn. The high-protein samples were superior to the low-protein samples in growth rate produced when fed on the basis of equal percentage of corn in the diet. When fed in this way the low-protein corn was deficient in lysine, tryptophan, isoleucine, threonine and valine, while the high-protein corn was deficient only in lysine and tryptophan. However, when the corn was fed on an equalized protein basis, the low-protein samples produced more rapid growth than the high-protein samples, reflecting the higher percentage of zein in the latter. Soybean oil meal was the best natural protein supplement to either high or low protein corn.

Effects of Processing on Amino Acid Availability:

Bell et al. (1950) reported that soybean oil meal is deficient in methionine. Curtin et al. (1952) were unable to demonstrate a practical need for methionine supplementation to a corn-soybean oil meal ration using solvent extracted soybean oil meal. Expeller processed soybean oil meal was used by Bell et al. (1950) and it was suggested that the high temperature used in processing reduced the availability of methionine. Clandinin et al. (1946) found that heating solvent extracted soybean flakes in an autoclave at 15 pounds pressure for more than 4 minutes adversely affected the nutritive value of the resulting meal. The detrimental effects of overheating were counteracted by the addition of known vitamins and amino acids. Other workers have also emphasized the importance of proper heating of soybean oil meal for maximum nutritive value. Only 48 percent as much lysine was liberated in a form available for microorganisms by hydrolysis with 2N HCl in the meal heated for 4 hours as by similar hydrolysis of raw meals or meals heated 4 minutes, in the work reported by Clandinin _et al_. (1947). Amino acid analyses of raw, properly heated (4 minutes) and overheated (4 hours) meals by Rieson _et al_. (1947) indicated no difference in essential amino acid content of raw meal and properly heated meal, but the recovery of lysine, arginine and tryptophan from the overheated meal was considerably less. Melnick _et al_. (1946) obtained an increase in biological value of soybean oil meal after autoclaving. It is clear from these studies that proper heating of soybean oil meal promotes the proper rate of release of amino acids from the protein but that excessive heating destroys certain amino acids. Clandinin (1949) studied the effects of methods of processing on the nutritive value of herring meals, and found that drying under a pressure of 10 inches of mercury for 10 hours, under 16 inches for 7.5 hours, or by the flame method at 185° F., all produced protein of equal quality as measured by feeding trials with chicks. However, flame drying at 220° F. produced protein of inferior quality. The value of a food as a source of protein therefore depends not only on its total protein content and its essential amino acid composition, but also on the availability of these amino acids for tissue synthesis.

Amino Acid Pattern of the Cereal Grains:

The poor amino acid balance of the cereal grains as a group has been demonstrated by several workers. Westerman _et al_. (1957) obtained significantly higher growth rate, greater feed efficiency and a healthier appearance in rats fed a diet containing 12 percent meat, milk and eggs and 37 percent flour than in those fed 44 percent flour and no meat. The addition of tryptophan or valine or both had no effect on rate of gain or feed efficiency in the rats fed the high cereal basal ration.

Pecora and Hundley (1951) obtained highly significant improvement in growth rate of rats fed rice supplemented with 2.0 percent DL-lysine and 1.2 percent DL-threonine. Neither lysine alone nor threonine alone produced growth above that obtained with the unsupplemented basal diet. Supplementation with several combinations of other essential amino acids was also without effect. It was therefore concluded that lysine and threonine are the most deficient amino acids in rice protein and are about equally limiting for rat growth.

Sure (1954) studied the relative nutritive values of proteins in wheat and rye and the effects of amino acid supplementation on growth. Whole rye flour was found to contain protein of superior nutritive value to that of whole wheat flour. Supplementation with 0.20 percent DL-threonine plus 0.25 percent L-lysine produced a significant increase in growth and protein efficiency of either grain, while 0.5 percent DL-valine produced the greater response in growth when added to rye flour.

In addition to the lysine and tryptophan deficiency of corn demonstrated by Eggert *et al*. (1953), Benton *et al*. (1955) have shown that 0.6 percent isoleucine will produce a marked increase in growth of rats fed an 89 percent corn diet supplemented with lysine, tryptophan, threonine and valine. Harper *et al*. (1955) presented data which indicates that an antagonism exists between isoleucine and leucine. When a purified diet containing a high amount of leucine was fed to rats, growth depression occurred which was corrected by the addition of isoleucine to the diet. If such a relationship exists between these two amino acids in natural feeds, the addition of isoleucine to diets containing large amounts of leucine might be expected to give a growth response.

Amino Acid Pattern of Milo Protein:

The average leucine content of milo as set forth by the National Research Council (1953) is 1.37 percent of the total grain. The estimated leucine requirement of the growing pig has been set by the National Research Council at 0.6 percent of the diet. This large excess of leucine above the requirement suggests that an inhibitory action of leucine on the isoleucine present in milo might well exist, in view of the work reported by Harper *et al.* (1955).

Shelton *et al.* (1951) reported that lysine is the most limiting amino acid in milo protein. In studying the protein quality of milo gluten meal, these workers concluded from digestibility determinations that the lysine present in the meal is poorly utilized. They calculated apparent digestibility of lysine by microbiological assay of the lysine in feed and feces. This method can be criticized in that any metabolic or bacterial lysine would be included in the feces, which would result in a calculated digestibility below the actual value.

Hillier *et al.* (1954) have reported the protein and lysine content of several varieties of milo grown in the Southwest. The addition of lysine to Martin, Redlan and Darset milo improved the growth of rats in each case. The wide range of values for percent protein in these varieties further complicates the problem of ascribing accurate protein characteristics to the grain sorghums as a group.

Hillier *et al.* (1953) presented data from a feeding trial with swine indicating that lysine is the first limiting amino acid in a milo-soybean oil meal type ration for growth of swine. The variety of milo used in that study was Kafir 44-14. This variety is widely grown in Oklahoma and has been used extensively as a livestock feed by virtue of its high palatability associated with a white seed coat low in tannic acid.

Heidebrecht _et_ _al_. (1948) obtained faster growth rate and greater efficiency of feed utilization in pigs fed a milo ration containing soybean oil meal than in pigs fed protein supplements of animal origin. Similar results were reported by Hillier _et_ _al_. (1955, 1956) in pigs fed Kafir 44-14 supplemented with soybean oil meal or animal protein. Therefore, it appears that the amino acid make-up of milo and of soybean oil meal are such that, when used in conjunction with each other, they provide a protein mixture of high quality.

Although values are available for average essential amino acid composition of several varieties of milo based on chemical or microbiological analyses, more information is needed concerning the biological availability of amino acids in these milo varieties for growth of swine.

A comparison of the average amino acid composition of the grain sorghums with the estimated amino acid requirements of growing pigs indicates that lysine, methionine, isoleucine and threonine are the most likely to be limiting for growth. Part of the experimental data presented in this thesis has been collected with the purpose of clarifying the amino acid inadequacies of milo protein.

<u>Urea Utilization by Growing Rats and Swine</u>:

The use of urea as a source of non-protein-nitrogen in rations for ruminants is a widely accepted practice. The efficacy of this practice, however, depends on the fact that rumen bacteria are able to synthesize protein from non-protein-nitrogen sources supplied to them. Experimental evidence concerning the utility of urea in rations for swine is limited, and the results of work published have been in disagreement as to the utilization of urea nitrogen by swine.

Early work reported by Grafe et al. (1913) and by Piepenbrock (1927) indicated that swine are capable of utilizing urea to replace a portion of the dietary protein.

English work reported by Braude and Foot (1942) in which urea was added to a low protein ration for growing pigs, indicated that no increases in protein synthesis occurred as a result of added nitrogen. Mean daily gains over a 21 week feeding period were not affected by the addition of approximately 1.75 percent urea to a 12.6 percent crude protein basal ration in which feed intake was equalized between treatments.

In studies on the adequacy of the essential amino acids for growth in the rat, Kinsey and Grant (1944) obtained consistant growth when a synthetic diet containing the 10 essential amino acids supplied the sole source of nitrogen. At 5.8 percent of the diet, the amino acid mixture produced slow growth, but when the diet contained 11 percent essential amino acids, growth was equal to that of pair fed mates receiving 18 percent casein. Lardy and Feldott (1950) have since shown that diammonium citrate can replace non-essential amino acids in the diet of growing rats fed a purified diet containing all the essential amino acids.

Finlayson and Baumann (1956) found that 5 percent urea in the diet of rats caused growth depression which was correlated with reduced feed intake and an elevated blood urea level. The daily feeding period was restricted to a 2 hour period each 24 hours. Other nitrogenous compounds such as L-leucine, ammonium carbonate, diammonium citrate and 2, 4-dinitrotoluene also depressed growth when the diet was fed in this manner.

Rose and Dekker (1956) used urea labelled with N^{15} to study the incorporation of urea nitrogen into tissue protein. They divided six weanling rats into two groups. One group was fed Diet A, a purified diet containing essential amino acids at the minimum requirement of each (8.82 percent of the diet) and no other nitrogen source. The other group was fed Diet B, a purified diet containing 18 percent casein. These diets were fed for 16 days in a paired feeding trial. Diet A was consumed ad libitum and the food intake of rats on Diet B was restricted to that of Diet A. Urea labelled with N^{15} was added to each ration at the rate of 0.577 grams of nitrogen per 100 grams of diet and the N^{15} of the tissues and excreta of all rats was measured at the end of the experiment. It was found that extensive utilization of urea nitrogen occurred in the rats fed Diet A, while very little N^{15} utilization occurred in rats fed Diet B. Cystine, glutamic acid and aspartic acid contained N^{15} in the highest concentration. This study clearly established that urea nitrogen can be utilized for synthesis of non-essential amino acids when they are excluded from the diet and when no other nitrogen source is provided. It was suggested that the urease activity provided by the microorganisms in the gastrointestinal tract is probably responsible for urea decomposition and utilization.

Lardy and Feldott (1950) obtained an increase in growth rate of rats when urea or ammonium citrate was added to diets containing 4, 8, or 12 percent essential amino acids as the only source of nitrogen. Since no increase occurred in fecal or urinary nitrogen it was assumed that the added nitrogen had been used for protein synthesis. However, no growth stimulation was obtained by adding ammonium salts to natural proteins such as fibrin or soybean oil meal when the diet contained 4-12 percent from these sources.

Brown et al. (1956) fed two-day-old dairy calves for 86 days on a limited milk-hay starter ration to study the value of urea as a nitrogen source for young calves. The calves in the group fed the ration containing urea and those in the group fed the linseed meal (both contained 15 percent protein equivalent) gained significantly faster than the calves fed the low protein (6.7 percent) basal ration. It was concluded that urea nitrogen can be successfully used by young dairy calves to meet part of their protein requirement.

Hanson and Ferrin (1955) compared the growth and feed efficiency of weanling pigs fed a 10.6 percent protein ration containing corn and small amounts of high quality protein supplement with and without 1.5 percent urea. The pigs fed urea required 6 percent more feed per pound of gain and grew at a rate of 1.45 pounds per day versus 1.46 pounds for the pigs receiving no urea. They concluded that under most conditions urea has no place in a ration for growing swine.

A series of experiments with growing swine were conducted by Hays et al. (1957) to study the effects of urea on the performance of pigs fed a low protein (8-10 percent) corn-soybean oil meal type ration. In the first experiment pigs weighing an average of 22.7 pounds initially were fed graded levels of urea to supply 0, 2.5, 5.0, 10.0, 20.0 and 40.0 percent of the protein equivalent (0, 0.16, 0.31, 0.62, 1.25 and 2.45 percent of the diet, respectively). Pigs fed the diet containing 2.45 percent urea weighed only 36 pounds after 84 days on test. The addition of 2.5 and 5.0 percent protein equivalent (0.16 and 0.31 percent urea, respectively) exerted no harmful effect on rate of gain. Feed required per pound of gain increased linearly as the urea level was increased from 0.0 to 1.25 percent of the diet. In another experiment a corn soybean oil meal basal diet containing 8 percent protein diluted with starch produced gains in 70 pound pigs equal to those obtained with the same diet plus 0.04 percent methionine and 0.26 percent lysine and/or with 0.65 percent urea (1.16, 1.07, 1.08 and 1.07 pounds average daily gain, respectively). The basal diet plus methionine, lysine and urea contained 10 percent protein equivalent. A 10 percent protein positive control ration composed of corn, soybean oil meal, vitamins, minerals and antibiotic produced an average daily gain of 1.69 pounds. In the third experiment weanling pigs weighing an average of 30.8 pounds initially were fed diets containing 8-10 percent protein in which 0, 10 or 20 percent of the protein equivalent was supplied by urea. In the basal diet 66 percent of the protein was supplied by dried skim milk and 34 percent by dried whole egg. The higher protein ration (10 percent) gave significantly greater gains and feed efficiency than the 8 percent protein diet. Urea produced a significant depression in growth rate, feed efficiency and feed intake at both protein levels. From these studies it was concluded that urea has no place in practical swine rations.

However, in view of the earlier work with rats already cited, it seems quite possible that pigs might use nitrogen from urea if they were fed a ration relatively low in protein but containing sufficient quantities of the essential amino acids.

With the exception of lysine (Shoenheimer and Weissman, 1941) and threonine (Elliot et al., 1949) and possibly valine and arginine (Borman et al., 1946) each of the essential amino acids can be replaced by the corresponding α-keto analog. Thus, it is the carbon chain that is essential, and on this basis it seems reasonable to suspect that non-protein-nitrogen could be of additional use to the animal for protein synthesis under certain conditions.

The average essential amino acid composition of milo and the estimated levels of each amino acid required by the growing pig appear in Figure 1.

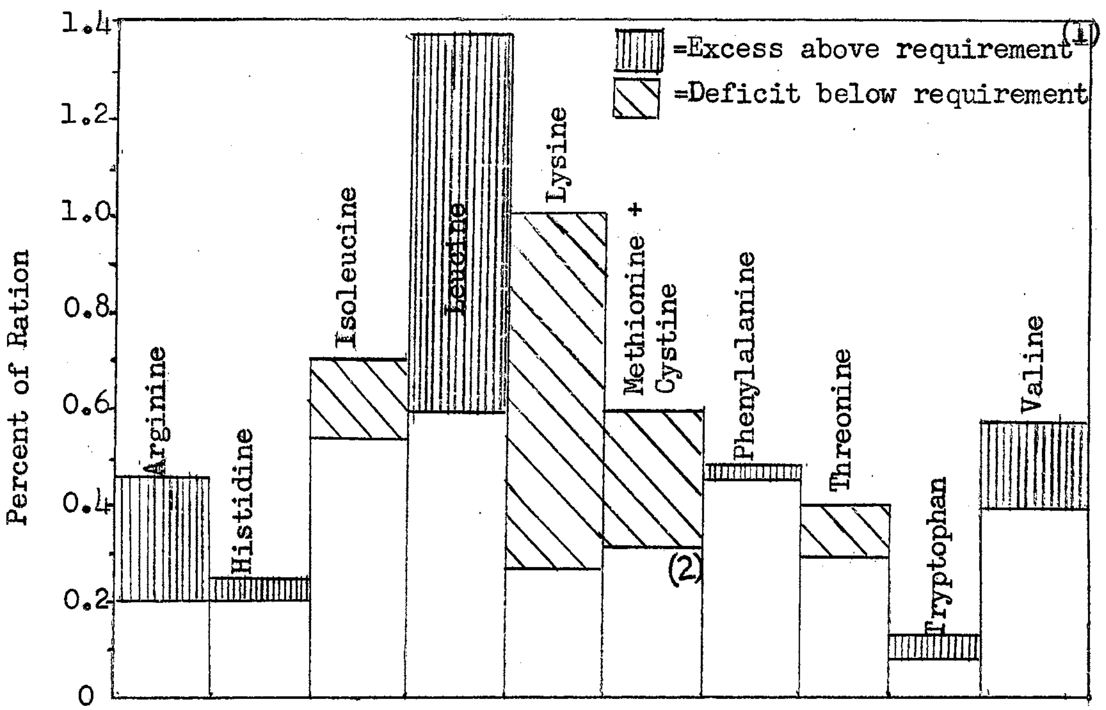

FIGURE 1 A COMPARISON OF THE AMINO ACID REQUIREMENTS OF GROWING PIGS WITH THE AVERAGE ESSENTIAL AMINO ACID COMPOSITION OF GRAIN SORGHUM (MILO)

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(1) N.R.C. requirements for the growing pig are, in percent of ration: arginine, 0.2; histidine, 0.20; isoleucine, 0.70; leucine, 0.60; lysine, 1.00; methionine plus cystine, 0.60; phenylalanine, 0.46; threonine, 0.40; tryptophan, 0.07; and valine, 0.40. Actual lysine and threonine analyses of milo used were 0.20 and 0.30 percent of the feed, respectively.

(2) Includes 0.11 percent methionine and 0.20 percent cystine.

EXPERIMENTAL

The studies reported herein have been conducted using growth rate as the primary criterion in interpreting results. The problem of studying the amino acid adequacy of milo protein was approached by attempting to correlate average essential amino acid composition of milo (National Research Council, 1953) with the quantitative level of each of the essential amino acids required by the pig for normal growth (National Research Council, 1953).

The average values for essential amino acid content of milo have been superimposed on the estimated requirement of the corresponding amino acid as shown in the bar graph in Figure 1. It appears that lysine is by far the most deficient amino acid, followed by methionine, isoleucine, and threonine. This value for methionine, however, is probably not a valid indication of the total sulfur amino acid content since some cystine is probably present in milo. Curtin et al. (1951) have shown that cystine can replace up to one-half of the methionine required by growing pigs. The fact that the leucine content of milo exceeds the requirement by such a wide margin increases the possibility that isoleucine might be deficient in milo, since Benton et al. (1955) and Harper et al. (1955) have shown that excess leucine increases the isoleucine needed to promote normal growth.

Therefore, the initial rat growth experiments were designed to test the theory that lysine, methionine, isoleucine and/or threonine are limiting for normal growth in rats fed milo as the sole source of protein.

The level of supplementation chosen for these amino acids was somewhat arbitrary, but an attempt was made to approximate the levels which had been shown by other workers to be beneficial in stimulating growth when added to low protein rations. For instance, Sure (1954) obtained a growth response in rats fed rye supplemented with 0.25 percent L-lysine and 0.2 percent DL-threonine.

Whitehair and MacVicar (1952) found that the addition of 0.2 percent DL-lysine and 0.2 percent DL-methionine to a corn-soybean oil meal ration caused a depression in growth rate of pigs below that obtained with only lysine. Hillier _et_ _al_. (1953) observed that levels of DL-lysine above 0.2 percent tended to depress growth rate of pigs fed low protein rations of the same type.

Therefore, it was considered important to keep the levels of supplementation low to avoid adverse effects from excess levels, which might not be recognized as such because of their non-specific effects.

RAT GROWTH STUDIES

General:

The rats used in all of the studies reported herein were albino males of the Holtzman strain. Unless otherwise indicated, they were weanling rats weighing approximately 55 grams when put on test.

The basal ration used in each experiment was prepared in a large batch at the beginning of the experiment, and appropriate portions of this total mixture were used in preparing the amino acid supplemented rations. This was done to minimize variations in ration composition due to mixing. Individual rations were mixed by hand. They were first spread in a thin layer on a large metal tray and the amino acids were then sprinkled over the whole surface to insure a uniform distribution of the amino acids throughout the rations. After mixing, the rations were stored in a refrigerator at +4° C to minimize vitamin destruction by the minerals of the salt mixture.

The rats were kept in individual wire-bottomed cages and were offered feed and water ad libitum from cylindrical metal containers.

Since feed wastage was extensive in all of the experiments, no attempt was made to measure individual feed consumption.

The statistical analyses of all results (Snedecor, 1956 and Duncan, 1955) are included in the appendix.

Growth Study 1.

Procedure:

Sixty-four rats were divided into eight groups of eight rats each and randomly assigned to the eight experimental treatments. The experiment was designed in a 2 x 2 x 2 factorial arrangement and consisted of a basal ration with all possible combinations of three supplemental amino acids comprising the eight treatments. The composition of the basal ration appears in Table 1. The variety of milo used in this and all succeeding studies was Kafir 44-14, a highly palatable variety grown in large acreage in the Southwest and used extensively as a livestock feed. The basal ration was calculated to be adequate in all known vitamins and minerals required by the rat for maximum growth. The amino acid supplements were, in percent: DL-isoleucine, 0.20; L-lysine, 0.10 (L-lysine·HCl, 0.125); and DL-threonine, 0.20. The lysine used in this and in all succeeding studies was L-lysine monohydrochloride. Each treatment is designated in Table 2.

The experimental period lasted 28 days. The body weight of each rat was recorded weekly during that time.

Results and Discussion:

The results have been summarized in Table 2.

It is apparent that the addition of 0.1 percent L-lysine to the basal ration had the largest effect on rate of growth, regardless of the amino acid combination in which it was contained. The addition of lysine alone produced a highly significant increase ($P < 0.01$) in growth rate, while the addition of 0.2 percent DL-threonine alone produced a significant reduction ($P < 0.05$) in growth rate below that obtained on the basal ration. There was a highly significant interaction ($P < 0.01$) between lysine and threonine in promoting growth above that obtained on the basal ration. Isoleucine had no significant effect on growth rate when added to the basal ration alone or in combination with lysine and/or

TABLE 1 BASAL RATION USED IN RAT GROWTH STUDIES

Ration Components(1)	Percent
Kafir 44-14	93.85
Salt mix(2)	4.00
B-vitamin mix(3)	2.00
Vitamin A and D Supplement(4)	0.15
	100.00

(1) The Kafir 44-14 contained 9.13 percent crude protein, 0.20 percent lysine and 0.30 percent threonine. The mixed ration, therefore, contained 8.57 percent crude protein, 0.19 percent lysine, 0.28 percent threonine, and, by calculation from average milo analysis, 0.51 percent isoleucine.

(2) Salts 4, Hegsted _et_ _al_. (1941).

(3) Composed of sucrose and the following vitamins to provide, in milligrams per 100 grams of ration: riboflavin, 3.0; calcium pantothenate, 5.0; thiamine-HCl, 0.2; niacin, 1.0; pyridoxine, 0.5; inositol, 10.0; para amino benzoic acid, 20.0; choline chloride, 50.0; folic acid, 0.2; vitamin B_{12}, 0.004; biotin, 0.10; and 2-methyl-1, 4-naphthoquinone, 0.50.

(4) Contains vitamins A and D to supply 1500 I.U. and 180 I. U. per gram of ration, respectively.

threonine. However, the presence of isoleucine in the ration containing lysine and threonine appeared to have a slight beneficial effect on growth rate (ration 8 vs. ration 7), even though the difference was not statistically significant.

TABLE 2 EFFECT OF ADDING ISOLEUCINE, LYSINE AND THREONINE ALONE AND IN COMBINATION TO THE BASAL RATION

	1	2	3	4	5	6	7	8
Treatment(1)	B	B+I	B+L	B+T	B+I+L	B+I+T	B+L+T	B+I+L+T
Percent L-isoleucine	0.51	0.61	0.51	0.51	0.61	0.61	0.51	0.61
Percent L-lysine	0.19	0.19	0.29	0.19	0.29	0.19	0.29	0.29
Percent L-threonine	0.28	0.28	0.28	0.38	0.28	0.38	0.38	0.38
No. of rats	8	8	8	8	8	8	8	8
Av. initial wt. (gms)	61.1	59.8	59.4	59.8	59.4	59.1	61.0	60.3
Av. final wt. (gms)	65.2	64.2	84.3	63.6	83.5	62.8	91.0	95.0
Av. gain/week (gms)	1.4 ±0.9(2)	1.1 ±1.0	6.2 ±1.9	1.0 ±0.7	6.0 ±1.8	1.0 ±0.7	7.5 ±1.5	8.7 ±2.3

(1) B = basal ration (Table 1), I = 0.2 percent DL-isoleucine, L = 0.1 percent L-lysine (1.25 percent L-lysine-HCl) and T = 0.2 percent DL-threonine.

(2) Standard deviation

It will be noted that the average weekly weight gains made by the rats in this experiment were very low as compared to the gains made by rats fed similar rations in later experiments. The reason for this poor performance was thought to be due to the fact that extremely high environmental temperatures prevailed in the mid-summer period during which this experiment was conducted. Heitman (1949) has shown with swine that feed consumption decreases as the air temperature is increased. This decrease in feed consumption would be reflected in slower weight gains for animals exposed to high temperatures.

Growth Study 1a

Procedure:

This experiment was conducted simultaneously with Rat Growth Study 1. Twenty rats from the same shipment as the rats used in Growth Study 1 were randomly divided into three groups of 4, 8 and 8 rats each and fed the basal diet (see Table 1) supplemented with 0.0, 0.05 or 0.10 percent DL-methionine, respectively. They were handled in the same way as the rats in the previous study. The experiment was terminated after 24 days.

Results and Discussion:

The summary of results appears in Table 3.

TABLE 3 EFFECT OF ADDING METHIONINE TO THE BASAL RATION

Treatment(1)	1 B	2 B+0.05 percent M	3 B+0.10 percent M
No. of rats	4	8	8
Av. initial wt. (gms)	66.4	66.6	66.8
Av. final wt. (gms)	72.5	74.8	72.0
Av. gain/week (gms)	1.8 ± 0.5	2.4 ± 1.2	1.5 ± 0.8

(1) B = basal ration (Table 1), M = DL-methionine

No significant differences were obtained among the three groups with respect to growth rate. The poor performance even on the methionine supplemented rations indicates that methionine is not the first limiting amino acid in milo grain.

Rat Growth Study 2

Procedure:

Ninety-six rats were used in this study. They were randomly divided into 12 groups of 8 rats each and assigned to the various treatments as shown in Table 4. It will be noted that the first eight treatments make up a 2 x 2 x 2 factorial arrangement, and this group of treatments was handled as such in the statistical analysis. It was the purpose of this study to test the possibility that some amino acid other than those suspected of being deficient (as indicated in Figure 1) might produce a growth response when added to the ration which contained lysine, isoleucine and threonine. This is the ration which had produced the most rapid gains in Rat Growth Study 1.

Methionine was again used in this study to determine whether it could produce a growth response if added in combination with other amino acids. Since tryptophan appeared to be borderline in adequacy as shown in Figure 1, a level of 0.1 percent DL-tryptophan was added to approximately double the level already present. Since Sure (1954) had shown valine to produce a growth response in rats under certain conditions when added to rye, 0.2 percent DL-valine was also used in this study.

The basal ration of the factorially arranged portion of this experiment was therefore identical with Ration 8 of Rat Growth Study 1. The composition of this basal ration and that of all other rations used in this study appears in Table 4.

The other four treatments included in the experiment were: the unsupplemented basal ration, identical to Ration 1 of Rat Growth Study 1; basal ration of Rat Growth Study 1 plus 0.1 percent L-lysine and 0.2 percent DL-threonine (Ration 7 of Growth Study 1); a purified diet containing 11 percent casein; and a purified diet containing 21 percent casein.

The 21 percent casein ration was included as a positive control capable of producing maximum growth of rats. The 11 percent casein ration was included since it contained approximately the same percentage protein as that supplied in the experimental rations and would, therefore, serve as an index for growth at that protein level. Since the value of isoleucine in promoting growth was questionable in Rat Growth Study 1, Ration 7 of that study was included here in an attempt to clarify this point.

The rats in this study were handled in the same way as in the previous experiments. Water and feed were available ad libitum. Feed was mixed and stored as described earlier.

The growth data obtained in Rat Growth Studies 1 and 1a indicated that the growth performance during the last two weeks of the trial did not differ materially from that observed during the first two weeks. Therefore, it was decided to adapt two weeks as the standard length of the experimental period in Rat Growth Study 2 and in all subsequent studies. Thus, after two weeks on test, all rats except those fed Rations 1, 7, 9, 10 and 11 were sacrificed. The surviving rats were continued on the experiment for an additional two week period. Rats fed Rations 9 and 11 were continued on their respective rations throughout. Those on 1, 7 and 10 were handled similarly except that one-half of each group was continued on the ration they had been on to this point, while the other half received the same ration plus an additional 0.1 percent L-lysine to make a total of 0.2 percent added L-lysine in the diet. This was done to test the effect of higher levels of supplemental lysine when added to various combinations of amino acids.

<u>Results and Discussion:</u>

The summary of results obtained during the first two week period appears in Table 4. There were no significant differences among the eight factorially arranged treatments in growth rate over the two weeks experimental period. This indicated that methionine, tryptophan and valine were not limiting in a milo ration supplemented with 0.2 percent DL-isoleucine, 0.1 percent L-lysine and 0.2 percent DL-threonine. Of further importance in these results was the fact that the omission of isoleucine from the ration containing lysine and threonine caused no reduction in rate of growth. This agrees with the results of the first study and increases the likelihood that isoleucine is not limiting in this variety of milo.

The unsupplemented basal ration (Ration 9) produced significantly slower growth ($P < 0.01$) than the amino acid supplemented rations. The ration containing 11 percent casein produced growth about equal to that obtained on the amino acid supplemented rations, indicating that the amino acid combination found in the supplemented milo rations is approximately equal to that of casein for promoting growth in rats.

The average growth rate obtained in rats fed 21 percent casein was approximately 50 percent higher than that obtained with 11 percent casein and with the amino acid supplemented rations. This demonstrates the need for higher protein levels than 10 percent for maximum growth in weanling rats when casein is the protein source.

Of the rats that were continued on the experimental rations for an additional two week period, those receiving the higher levels of supplemental lysine consistently outgained the rats continued on the lower level of lysine. The rats receiving 0.2 percent L-lysine plus isoleucine and threonine (Ration 1a) made highly significantly more rapid gains ($P < 0.01$) than those receiving 0.1 L-lysine plus isoleucine and threonine (Ration 1).

TABLE 4 EFFECT OF ADDING METHIONINE, TRYPTOPHAN AND VALINE ALONE AND IN COMBINATION TO THE BASAL RATION SUPPLEMENTED WITH ISOLEUCINE, LYSINE AND THREONINE

FIRST TWO WEEKS

Treatment(1)	BB	BB + M	BB + T	BB + V	BB M+T	BB M+V	BB T+V	BB+M T+V	B	BB minus isoleucine	11%(2) casein	21%(2) casein
Percent L-methionine	0.10	0.15	0.10	0.10	0.15	0.15	0.10	0.15	0.10	0.10	0.34	0.64
Percent L-tryptophan	0.10	0.10	0.15	0.10	0.15	0.10	0.15	0.15	0.10	0.10	0.17	0.32
Percent L-valine	0.53	0.53	0.53	0.63	0.53	0.63	0.63	0.63	0.53	0.53	0.65	1.25
No.of rats	8	8	8	8	8	8	8	8	8	8	8	8
Av.in.wt.(gms)	57.0	56.3	58.8	57.3	57.8	58.5	59.0	57.3	58.8	58.8	59.0	50.5
Av.final wt.(gms)	82.0	80.0	86.4	82.6	83.1	84.6	75.3	82.8	69.0	85.4	83.3	90.1
Av.gain/wk. (gms)	12.5 ±1.5	11.9 ±1.7	11.8 ±1.6	13.3 ±1.6	11.0 ±1.1	13.1 ±1.8	13.3 ±2.1	12.8 ±1.8	5.2 ±1.4	12.9 ±2.1	12.2 ±1.4	19.8 ±1.7

(1) BB = Ration 8 of Rat Growth Study 1, contained 0.2% DL-isoleucine + 0.1% L-lysine + 0.2%DL-threonine as added amino acids. BB contained a total of 0.61, 0.29 and 0.38 percent L-isoleucine, L-lysine and L-threonine, respectively. B = basal ration (Table 1); M = 0.1% DL-methionine; T = 0.1% DL-tryptophan and V = 0.2% DL-valine. Ration 11 contained 81.85 percent sucrose, 11.00 percent casein and 1.00 percent Mazola oil in addition to the mineral and vitamin mixtures which were identical to those of the other rations. Ration 12 contained 71.85 percent sucrose, 11.00 percent casein and 1.00 percent Mazola oil in addition to the other components.

(2) Since casein contains approximately 85 percent crude protein, the protein contents of the rations containing 11 and 21 percent casein were 9.4 and 17.9 percent, respectively. The 11 percent casein ration contained 0.61, 0.65 and 0.36 percent isoleucine, lysine and threonine, respectively, while the 21 percent casein ration contained 1.16, 1.23 and 0.69 percent of these three amino acids, respectively. (calculated from values given by Block and Bolling, 1951).

Furthermore, those receiving 0.2 percent L-lysine plus isoleucine, threonine, methionine, tryptophan and valine (Ration 8a) gained significantly faster ($P < 0.05$) than rats receiving the same ration at the 0.1 percent level of lysine (Ration 8). Although no statistically significant differences in growth rate were obtained between rats receiving high and low levels of lysine in the ration containing lysine and threonine (Ration 10 vs. 10a), there was a tendency for the rats receiving 0.2 percent L-lysine to gain faster than those receiving 0.1 percent. The summary of results appears in Table 5.

TABLE 5 A COMPARISON OF 0.1 AND 0.2 PERCENT SUPPLEMENTAL L-LYSINE ON THE GROWTH OF RATS FED THE BASAL RATION SUPPLEMENTED WITH VARIOUS AMINO ACIDS (Second Two Weeks)

Treatment(2)	1	1a	8	8a	9	10	10a	11
No. of rats	4	4	4	4	8	4	4	8
Av. in. wt. (gms)	82.5	81.5	82.5	83.0	69.0	87.0	82.0	83.3
Av. final wt. (gms)	120.0	131.3	118.0	127.5	82.3	123.0	126.0	127.8
Av. gain/wk. (gms)	18.8 ±1.3	22.9 ±1.7	17.8 ±3.3	22.3 ±1.0	6.7 ±1.2	18.0 ±1.8	22.0 ±3.2	23.3 ±2.6

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(2) "a" designates rations in which L-lysine was doubled (0.2 percent). Rats fed 9 and 11 were continued on the rations fed during the first two weeks.

It appeared that higher levels of lysine should be tried in conjunction with other amino acids to arrive at a proper level of supplementation, and that the value of isoleucine as a supplement to milo should be further clarified.

Rat Growth Study 3

Procedure:

This experiment was designed to test further the value of isoleucine in combination with lysine and threonine in promoting growth. Thirty-six rats were randomly divided into four groups of eight rats each and one group of four rats. The group containing four rats was fed the 11 percent casein diet (Table 4) calculated to contain approximately the same percentage protein as that contained in the experimental diets. The four remaining groups were each fed one of the following: basal ration (Table 1); basal plus 0.1 percent L-lysine; basal plus 0.1 percent L-lysine and 0.2 percent DL-threonine; or basal plus 0.2 percent DL-isoleucine, 0.1 percent L-lysine and 0.2 percent DL-threonine.

The rats were handled as described previously. The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appears in Table 6.

TABLE 6 EFFECT OF ADDING ISOLEUCINE AND/OR THREONINE TO THE BASAL RATION SUPPLEMENTED WITH 0.1 PERCENT L-LYSINE

Treatment(1)	1 B	2 B+L	3 B+L+T	4 B+I+L+T	5 11% casein
No.of rats	8	8	8	8	4
Av.in.wt.(gms)	56.3	57.0	55.9	57.0	56.5
Av.final wt.(gms)	62.9	77.4	76.8	79.1	75.3
Av.gain/wk.(gms)	3.3 ±1.0	10.2 ±1.3	10.5 ±1.4	11.1 ±2.3	19.4 ±1.4

(1) B = basal ration (see Table 1), L = 0.1% L-lysine, I = 0.2% DL-isoleucine and T = 0.2% DL-threonine. See Table 4 for composition of the 11 percent casein ration.

Highly significant differences ($P < 0.01$) were obtained among treatments.

However, separation of differences by the Multiple Range Test of Duncan (1955) showed that the three rations supplemented with amino acids were not significantly different from each other. The gains obtained on the unsupplemented basal ration were significantly less than those obtained by amino acid supplementation ($P < 0.01$), while the gains obtained in the casein ration were faster than those of the amino acid supplemented rations although statistical differences were not measured in this latter case.

This study again clearly demonstrated the importance of lysine as a supplement to milo for growth of rats. The reason for the superior performance of the rats fed casein in this experiment in contrast to the previous experiment is not clear. It cannot be explained on the basis of differences in initial weight since the weights of rats at the start of both experiments were almost identical.

The growth response obtained by the addition of threonine to the ration containing lysine is not in agreement with previous findings. However, it is possible, based on the results of Rat Growth Study 2,that the 0.1 percent level of added lysine was not high enough to allow a response from threonine. The desirability of testing this possibility further, as suggested in the previous experiment, is strengthened on the basis of the results obtained here.

Again in this trial, as previously found, no growth response was obtained from 0.2 percent DL-isoleucine. However, since no apparent inhibiting action resulted from its presence in the mixture, it seemed desirable to test it further in other combinations and at different levels, with the idea that some situation might be found in which it would be useful.

Rat Growth Study 4

Procedure:

This experiment was designed to study the effect on growth of rats of adding graded levels of L-lysine to the basal ration (Table 1) supplemented with 0.2 percent DL-isoleucine and 0.2 percent DL-threonine. Ninety-seven rats were randomly divided into 11 groups of 8 rats each and two groups of 5 and 4 rats each. The group containing 5 rats were fed the 11 percent casein ration while the group containing 4 rats served as the positive control and were fed the 21 percent casein ration (Table 4). One of the 11 other groups received the unsupplemented basal ration (Table 1) and served as the negative control, while the remaining ten groups received increments of added L-lysine at 0.1, 0.2, 0.3, 0.4 and 0.5 percent of the ration with and without 0.2 percent each of DL-isoleucine and DL-threonine.

The rats were handled the same as in all previous trials. Feed was mixed and stored as described earlier. Water and feed were provided ad libitum. The experimental period lasted two weeks.

Results and Discussion:

The results have been summarized in Table 7. The differences among treatments in this experiment were highly significant ($P < 0.01$). The addition of L-lysine to the unsupplemented basal ration produced an increase in growth rate above that obtained with the basal ration at all levels of lysine added. An important feature of these results is that the effect on growth obtained from supplemental isoleucine and threonine was related to the level of lysine present. At the 0.1 percent level of added L-lysine, growth rate was not significantly affected by the addition of 0.2 percent DL-isoleucine and 0.2 percent DL-threonine (9.5 vs.

TABLE 7 EFFECT OF SUPPLEMENTAL LYSINE LEVEL ON THE GROWTH OF RATS FED THE BASAL RATION SUPPLEMENTED WITH ISOLEUCINE AND THREONINE

Treatment (1)	No. of rats	Av. in. Wt. (gms)	Av. final Wt. (gms)	Av. gain/wk. (gms)
1. B	8	51.1	58.1	3.5 ± 1.2
2. B 0.1%L	8	50.4	69.7	9.5 ± 3.0
3. B 0.1%L I T	8	50.4	66.0	7.8 ± 2.8
4. B 0.2%L	7(2)	51.0	70.4	9.6 ± 3.7
5. B 0.2%L I T	8	50.8	84.4	17.5 ± 4.3
6. B 0.3%L	8	50.4	77.8	13.7 ± 4.2
7. B 0.3%L I T	8	51.1	92.4	21.3 ± 2.6
8. B 0.4%L	8	50.1	74.0	11.3 ± 3.0
9. B 0.4%L I T	8	50.3	97.0	23.4 ± 3.6
10. B 0.5%L	8	50.8	72.8	11.0 ± 4.0
11. B 0.5%L I T	8	50.3	100.0	24.9 ± 3.4
12. 11% casein	5	51.0	98.0	23.5 ± 2.4
13. 21% casein	4	52.0	120.5	34.5 ± 1.3

(1) B = basal ration; I = 0.2% DL-isoleucine; T = 0.2% DL-threonine. The basal ration (Ration 1) contained 0.51, 0.19 and 0.28 percent isoleucine, lysine and threonine, respectively. The supplemental levels of isoleucine and threonine supplied an additional 0.10 percent of the biologically active L-form of each.

(2) One rat died of unknown causes on day 12 and is not included in the data.

7.8 grams gain per rat per week). When differences were separated by the Multiple Range Test (Duncan, 1955) it was shown that the increased growth rate obtained in the presence of isoleucine and threonine was significantly higher ($P < 0.01$) at the 0.2, 0.3, 0.4 and 0.5 percent levels of supplemental L-lysine than in their absence. Although the average weekly gains of rats receiving isoleucine and threonine increased steadily with increasing levels of lysine, the differences in gains obtained at the 0.3, 0.4 and 0.5 percent levels of L-lysine were not statistically significant (21.25 vs. 23.37 vs. 24.87 grams gain per rat per week, respectively).

When comparing the growth rates obtained with added increments of lysine in the absence of supplemental isoleucine and threonine, it is clear that 0.3 percent supplemental L-lysine produced maximum growth response. The average weekly gain (13.68 grams) made by the rats fed that ration was higher than gains made by rats fed the 0.2 percent level of L-lysine (9.64 grams). However, this difference was not statistically significant. The 0.4 and 0.5 percent levels of added L-lysine produced slower, but not significantly different gains than the 0.3 percent level. Thus, it is apparent that a plateau was reached at the 0.3 percent level of L-lysine supplementation.

It is obvious from this experiment, that an interaction exists between certain essential amino acids at this level of protein. The presence of 0.2 percent each of DL-isoleucine and DL-threonine allowed approximately twice the rate of growth obtained with lysine alone, but this response was only obtained at supplemental L-lysine levels of 0.3 percent or more. The failure of the combination of all three amino acids to promote faster growth than lysine alone at the 0.1 percent level of

L-lysine agrees with the results of the previous experiment. These results support the suggestion that the first limiting amino acid (lysine) must be added in large enough amounts to cause another amino acid to be limiting before the other amino acid can exert an effect.

The 11 percent casein ration produced a growth rate approximately equal to that of the amino acid supplemented rations which produced the fastest growth. This again suggests that the balance of amino acids in the supplemented milo met the needs of the rats approximately as well as the ration containing 11 percent casein. However, the ration containing 21 percent casein again produced a considerably faster growth rate, emphasizing the importance of protein quantity as well as quality in obtaining maximum growth rate of young animals.

Rat Growth Study 5

Procedure:

In view of results obtained in previous experiments it seemed desirable to test the effect of varying levels and combinations of isoleucine, threonine and methionine on the growth of rats fed the basal ration (Table 1) supplemented with a higher level of lysine than used in the first three experiments. Since the 0.5 percent level of added L-lysine had produced the highest average weekly weight gains in Rat Growth Study 4, this level was used in all supplemented rations of this experiment. Ninety-six rats were randomly divided into 9 groups of eight rats and a series of other smaller groups. These smaller groups included the following treatments: basal ration (Table 1), 6 rats; basal plus 0.5 percent L-lysine and 0.05 percent DL-methionine, 5 rats; basal plus 0.5 percent L-lysine and 0.10 percent DL-methionine, 5 rats; a purified ration containing 11 percent casein, 4 rats; and a purified ration containing 21 percent casein, 4 rats.

The nine groups of eight rats each were fed the basal ration (Table 1) plus 0.5 percent L-lysine and other amino acids as follows: 0.2 percent DL-isoleucine and 0.2 percent DL-threonine, alone and in combination; 0.3 percent DL-isoleucine and 0.3 percent DL-threonine, alone and in combination; and 0.2 percent each of DL-isoleucine and DL-threonine plus 0.05 or 0.10 percent DL-methionine.

The rats were handled as described earlier. Water and feed were supplied ad libitum. Feed was mixed and stored as previously described. The experiment was terminated at the end of the second week.

Since work by Harper et al. (1953) and by Winje et al. (1954) showed that threonine was effective in reducing liver fat deposition in rats fed low protein diets under certain conditions, it was desired to determine whether a lipotropic action of threonine could be demonstrated in rats fed a milo ration. To study this effect the rats which had been fed the unsupplemented basal ration and those fed the ration supplemented with 0.5 percent L-lysine and 0.2 percent DL-threonine were sacrificed at the end of the two week experimental period for liver fat determination. The livers were removed, blotted free of blood, wrapped in wax paper and stored in the freezer at -4° C until analysis. Just before analysis, they were thawed, homogenized in a Potter-Elvehjem homogenizer, oven dried and ground for weighing. Since the dried weights were small, the entire liver was used for a single determination and no duplicate samples were available. The percentage fat present was determined by the difference in dried liver weight before and after a 12 hour ether extraction.

Results and Discussion:

The summary of growth results appears in Table 8. Analysis of variance of the growth rates obtained in Rations 1-9 revealed highly significant differences ($P < 0.01$) among treatments. However, the Multiple Range Test (Duncan, 1955) showed that no differences existed among the rations containing threonine, regardless of the level or the identity of other amino acids (isoleucine and methionine) present. All rations containing threonine, however, produced significantly faster weight gains than those containing no added threonine.

The addition of DL-isoleucine to the ration containing 0.5 percent L-lysine did not improve growth rate significantly, when added either at the 0.2 or 0.3 percent level. Furthermore, the ration containing lysine was not improved by the addition of 0.05 or 0.10 percent DL-methionine.

Therefore, it appears, from the results of this and previous studies, that threonine is unquestionably the second most limiting amino acid in milo. It is further indicated that 0.2 percent DL-threonine is as effective as 0.3 percent DL-threonine in promoting growth of rats fed milo basal diet supplemented with 0.5 percent L-lysine.

The 11 percent casein ration and 21 percent casein ration produced growth responses similar to those obtained in previous studies. It will be noted that the milo ration supplemented with 0.5 percent L-lysine and 0.2 percent DL-threonine produced growth equal to that obtained with 11 percent casein (27.7 vs. 26.2 grams gain per rat per week, respectively). This might be expected upon examination of Figure 1. The addition of 0.2 percent DL-threonine to the basal ration would increase the threonine content to 0.38 percent of the ration when expressed as L-threonine (the

TABLE 8 A COMPARISON OF VARIOUS LEVELS AND COMBINATIONS OF ISOLEUCINE, LYSINE, THREONINE AND METHIONINE WHEN ADDED TO THE BASAL RATION

Treatment(1)	No. of rats	Av. in. wt. (gms)	Av. final wt. (gms)	Av. gain/wk. (gms)
1. B	6	55.7	60.0	2.3 ± 1.9
2. LB	8	56.1	75.4	9.7 ± 2.6
3. LB .2I	8	56.0	76.5	10.3 ± 3.9
4. LB .2T	8	55.8	111.0	27.7 ± 3.6
5. LB .2I .2T	8	54.8	107.6	26.5 ± 2.0
6. LB .3I	8	55.9	73.8	8.8 ± 3.5
7. LB .3T	8	55.4	111.6	28.2 ± 6.4
8. LB .3I .3T	8	55.3	115.5	30.8 ± 3.9
9. LB .2I .2T .05M	8	55.4	112.5	28.6 ± 7.1
10. LB .2I .2T 0.1M	8	56.5	113.1	28.3 ± 5.5
11. LB .05M	5	55.8	72.0	8.1 ± 3.5
12. LB .1M	5	56.0	74.6	9.3 ± 6.9
13. 11% casein	4	54.8	107.0	26.2 ± 1.0
14. 21% casein	4	55.0	136.5	40.8 ± 1.7

(1) B = basal ration (Table 1); LB = basal ration plus 0.5 percent L-lysine; I = percent DL-isoleucine; T = percent DL-threonine and M = percent DL-methionine. See table 4 for composition of the casein rations. The basal ration (Ration 9) contained 0.51, 0.19, 0.10 and 0.28 percent isoleucine, lysine, methionine and threonine, respectively. The supplemental levels of methionine supplied an additional 0.025 or 0.05 percent of the biologically active L-form, while supplemental levels of isoleucine and threonine supplied 0.10 or 0.15 percent of the active L-form.

biologically active form). The addition of 0.5 percent L-lysine to the basal diet would increase the total lysine to 0.69 percent of the ration. While this level of lysine does not equal the estimated requirement for the pig (1.0 percent), it should be pointed out that this estimation was based on data obtained with a 20 percent protein ration. Grau (1948) and Grau and Kamei (1950) have shown that the lysine requirement increases as the protein level is increased. Therefore, it is possible that at a protein level of 10 percent, the lysine requirement for growth of rats does not exceed 0.69 percent. Hillier _et al._ (1953) obtained normal growth of weanling pigs fed a milo-soybean meal diet containing 14 percent protein and 0.58 percent L-lysine.

The lack of growth response from supplemental methionine in these studies is not explained. It is possible that the cystine level of milo is sufficiently high to make up for the low level of methionine.

The reason for the failure to obtain a growth response from isoleucine in this and in previous studies is not clear. Since no isoleucine analysis was available on this variety of milo, there is a possibility that the level present was higher than the values used in the calculations. In that case, no response would be expected by supplementation with isoleucine. Another possible reason for the lack of response is that some other amino acid not yet considered, is more limiting than isoleucine, and thereby preventing any response from isoleucine in these studies.

The liver fat content of rats fed the unsupplemented basal ration (Table 1) was significantly higher ($P < 0.01$) than that of rats fed the basal ration supplemented with 0.5 percent L-lysine and 0.2 percent DL-threonine. The livers of rats fed the basal ration contained 19.41 ±

2.44 percent fat on a dry matter basis, versus 9.54 ± 3.54 percent for the livers of those fed the lysine and threonine supplemented ration. This observation supports the findings of Harper et al. (1953) and Winje et al. (1954, who reported that threonine exerts a lipotropic effect in the livers of rats fed low protein diets.

Rat Growth Study 6

Procedure:

Since milo by-product feeds are available in relatively large quantities for use as protein supplements in livestock feeds, it seemed desirable to test the adequacy of milo gluten meal as a supplement to milo for growth of rats. Shelton et al. (1951) demonstrated that milo gluten meal is low in lysine. Therefore, it was expected that poor growth would result with no supplemental lysine present.

To test the effect of the addition of lysine to a ration composed of milo and milo gluten meal and adequate in vitamins and minerals, 14 rats were randomly divided into two equal groups. One group was fed a ration containing 84 percent Kafir 44-14, 10 percent milo gluten meal, and vitamin and mineral supplements. The other group was fed the same ration plus 0.3 percent L-lysine. The composition of these rations appears in Table 9.

The rats were handled the same as in all previous trials. Feed was mixed and stored as described earlier. The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appears in Table 9. A highly significant difference ($P < 0.01$) was obtained between the two groups in rate of growth. This was to be expected since microbiological assay of the milo and the milo gluten meal for lysine had shown these two feeds to contain only 0.20 and 0.46 percent lysine, respectively. The combination of feeds used in this experiment, therefore, supplied only 0.246 percent lysine. This combination supplied a total of 14.2 percent protein. It is significant to note that even though the total protein level of the basal ration was almost 50 percent higher than the basal ration of previous experiments, the growth rate obtained from the basal ration of this study was not appreciably higher than previously obtained values with only milo as the protein source.

TABLE 9 EFFECT OF THE ADDITION OF 0.3 PERCENT L-LYSINE TO A MILO RATION CONTAINING 10 PERCENT MILO GLUTEN MEAL

Treatment(1)	1 Milo gluten meal	2 Milo gluten meal + lysine
Percent crude protein	14.2	14.2
Percent L-lysine	0.25	0.55
Percent L-threonine	0.39	0.39
No. of rats	7	7
Av. in. wt. (gms)	55.6	55.0
Av. final wt. (gms)	64.6	97.7
Av. gain/wk. (gms)	4.5 ± 0.7	21.7 ± 6.7

(1) Ration 1 contained: Dwarf Kafir 44-14, 83.85 percent; milo gluten meal, 10.00 percent; salts, 4.00 percent; vitamin mix, 2.00 percent, and vitamin A and D supplement, 0.15 percent (see Table 1 for composition of the salt and vitamin mixtures). Ration 2 was identical to Ration 1, except for the addition of 0.3 percent L-lysine.

Moreover, the addition of 0.3 percent L-lysine to this 14.2 percent protein ration produced a more rapid average weekly growth than had been obtained earlier with the same level added to the nine percent protein milo basal ration (21.7 vs. 13.7 grams per rat per week). This indicates that other amino acids supplied by milo gluten meal allowed a higher growth response from supplemental lysine.

The extremely poor growth response obtained by the inclusion of milo gluten meal as a protein supplement in a milo ration for rats has been demonstrated. It is therefore unadvisable to use milo gluten meal to supply protein in appreciable amounts in rations where amino acid balance is important.

Rat Growth Study 7

Procedure:

This experiment was conducted to study the effects of processing on the availability of the amino acids in milo gluten meal for growth. Shelton *et al.* (1951) reported that the availability of the lysine present in milo gluten meal is very low. Melnick *et al.* (1946) obtained an improvement in nutritive value of soybean oil meal after autoclaving. Clandinin *et al.* (1946) showed that autoclaving soybean oil meal for longer than four minutes reduced the biological value, while Riesen *et al.* (1947) obtained less hydrolysis by pancreatin in overheated or in raw soybean oil meal. In view of these findings a growth study was designed to test the effects of heating and/or pancreatic hydrolysis of milo gluten meal on its nutritive value.

Sixty-eight rats were randomly divided into nine groups. One group contained four rats and was fed the unsupplemented milo basal ration used in all previous studies (Table 1). The other eight groups contained eight rats each and were fed rations containing 85 percent milo and 9 percent milo gluten meal plus vitamin and mineral supplements. The milo gluten meal used in this study was from the same lot as that used in the previous study. Two of these rations contained untreated milo gluten meal with and without 0.3 percent added L-lysine. The remaining six rations contained processed milo gluten meal. The processing consisted of autoclaving or pancreatic hydrolysis or both.

The meal to be autoclaved was heated for 4.0 minutes at 15 pounds pressure (121° C) plus time for attaining the desired pressure and reducing it after the four minute period. The meal was spread in a one-half inch layer on the surface of a sheet of heavy paper to insure complete exposure to the treatment.

Another portion of meal was handled in the following way. A 200 gram sample of milo gluten meal was dispersed in 5.0 liters of 0.2M disodium phosphate buffer (pH = 8.3) containing 10.0 grams of pancreatin. This mixture was shaken thoroughly and incubated for 12 hours at 37° C. At the end of this time the hydrolysate was filtered through fine meshed cloth. The solid material was saved for drying and the filtrate was discarded. Protein analysis of the dried material indicated that virtually no nitrogenous material had been lost in the filtrate, since the treated meal contained 63.00 percent protein versus 63.44 percent for the untreated meal.

A portion of the autoclaved meal as well as a sample of unheated meal were hydrolyzed in this manner making four types of meals for use in the experiment, namely: untreated, autoclaved, hydrolyzed, or autoclaved and hydrolyzed.

Each meal was fed in two rations, one with and one without 0.3 percent supplemental L-lysine. This made a total of eight rations containing milo gluten meal in addition to the all milo basal ration.

The rats used in this study were handled as in all previous experiments. Feed and water were supplied ad libitum. The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results obtained appears in Table 10.

TABLE 10 EFFECT OF PROCESSING ON THE NUTRITIVE VALUE OF MILO GLUTEN MEAL

Treatment[1]		No. of rats	Av. in. wt. (gms)	Av. final wt. (gms)	Av. gain/wk. (gms)
1. B		4	52.8	59.8	3.6 ± 1.9
2. B + MGM		8	55.8	63.4	3.8 ± 2.1
3. B + MGM	L	8	55.0	99.0	22.0 ± 4.5
4. B + MGM(autoclaved)		8	55.5	60.3	2.4 ± 1.8
5. B + MGM(autoclaved)	L	8	56.7	110.3	27.6 ± 8.8
6. B + MGM(hydrolyzed)		8	55.0	55.5	0.3 ± 1.5
7. B + MGM(hydrolyzed)	L	8	55.0	94.3	19.8 ± 3.9
8. B + MGM(auto. hyd.)		8	55.1	60.0	2.4 ± 1.6
9. B + MGM(auto. hyd.)	L	8	55.6	100.1	22.3 ± 1.9

(1) B = basal ration (Table 1), MGM = 9 percent milo gluten meal L = 0.3 percent L-lysine. Milo gluten meal was added at the expense of Kafir 44-14. The rations containing milo gluten meal supplied 13.47 percent crude protein, 0.21 percent lysine and 0.39 percent threonine.

It is apparent that the addition of 0.3 percent L-lysine produced a large growth response regardless of the ration to which it was added. There was a highly significant difference ($P<0.01$) among treatments in growth rate. The Multiple Range Test (Duncan, 1955) showed that no significant differences existed among any of the lysine supplemented rations in rate of gain produced. Similarly no significant differences were obtained among rations not supplemented with lysine. This indicates that neither heating nor hydrolyzing had an effect on the nutritive value of the milo gluten meal used in this study. However, it will be noted that the average weekly gain obtained in the ration containing autoclaved milo gluten meal and 0.3 percent added L-lysine was 5.3 grams greater (27.6 vs. 22.3) than the next highest growth rate, suggesting a tendency for this ration to be superior to any other.

The protein level of the mixed rations was 13.47 percent based on calculations from the individual feed components. This would provide a lysine content of 0.21 percent of the total ration. It is evident that an increase in lysine availability, even to 100 percent, could not be expected to produce satisfactory growth with such a low level present. Therefore, it is not surprising that no beneficial effects were demonstrated by the various processing methods. It is possible that the tendency for the autoclaved meal to produce faster growth than the other meals when supplemented with lysine as shown in this study, is a reflection of increased availability of lysine or of the second most limiting amino acid, probably threonine. However, since the differences among the lysine supplemented rations were not significant in this experiment, further studies should be conducted before any definite conclusions can be drawn.

In any case, this experiment served to further demonstrate the serious lysine deficiency existing in a ration containing only milo protein.

Rat Growth Study 8

Procedure:

Several workers have shown that non-protein-nitrogen can be utilized for growth of rats under certain dietary conditions. Lardy and Feldott

(1950) obtained an increase in growth of rats fed purified diets containing all the essential amino acids by adding diammonium citrate to the ration. Recently, Rose and Dekker (1956) demonstrated the incorporation of N^{15} into the body tissues of rats fed urea labelled with N^{15} as a supplement to a purified diet containing all of the essential amino acids as the sole nitrogen source.

Since the nitrogen content of milo is comparable to the levels contained in the purified diets used in the experiments cited above, it seemed desirable to know whether urea could be used as a nitrogen source for non-essential amino acid synthesis under any circumstances in a milo ration. To study this possibility, 32 male rats were used. These rats had all been used in a previous rat growth study. They were reallotted according to previous treatment and body weight into 4 groups of eight rats each and assigned to the following factorially arranged treatments: basal, basal plus 0.1 percent L-lysine, basal plus 0.5 percent urea, or basal plus 0.1 percent L-lysine and 0.5 percent urea. The composition of the basal ration is given in Table 11. This ration was composed of natural feedstuffs plus commercial sources of vitamins and antibiotic commonly used in practical rations for swine.

The rats were handled the same way as in previous studies. Feed and water were available ad libitum. The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appears in Table 11. Highly significant differences ($P < 0.01$) were obtained in growth rate among the treatments. Treatment breakdown revealed that the significance was due to lysine. The urea effect was not significant and there was no significant lysine

TABLE 11 EFFECT OF THE ADDITION OF LYSINE AND/OR UREA TO A MILO-ALFALFA MEAL RATION

Treatment(1)	1 B	2 B+L	3 B+U	4 B+L+U
No.of rats	8	8	8	8
Av.in.wt.(gms)	135.0	136.3	138.5	143.3
Av.final wt.(gms)	174.8	186.3	177.8	197.3
Av.gain/wk.(gms)	16.9 ± 3.9	25.0 ± 4.7	20.3 ± 4.2	27.0 ± 5.9

RAT GROWTH STUDY 9

Treatment(1)	1 B	2 B+L	3 B+U	4 B+L+U
No.of rats	8	8	8	8
Av.in.wt.(gms)	80.4	80.3	81.0	80.3
Av.final wt.(gms)	102.0	122.1	98.3	121.1
Av.gain/wk.(gms)	10.8 ± 7.1	19.7 ± 2.9	8.7 ± 2.6	21.0 ± 3.3

(1) B = Basal ration fed in swine experiments 1 and 2. Composed of Kafir 44-14, 92.1; dehydrated alfalfa meal, 5.0 percent; steamed bone meal, 2.0 percent; trace mineral salt, 0.5 percent; Aurofac, 0.3 percent; and Fortafeed, 0.1 percent. The mixed basal ration contained 9.79, 0.22 and 0.32 percent crude protein, lysine and threonine, respectively. The rations supplemented with urea therefore contained 11.10 percent protein equivalent.
L = 0.1 percent L-lysine (0.125 percent L-lysine HCl).
U = 0.5 percent urea 262 (This amount of urea 262 supplies 1.31 percent protein equivalent).

x urea interaction. It will be noted, however, that the average weekly gain was highest in the group receiving lysine plus urea. If any beneficial effect were to be obtained from urea, it would be expected, from published work, to occur in the ration most nearly balanced in essential amino acids. Since the addition of lysine resulted in increased growth rate, the balance of essential amino acids was apparently improved.

The urea used was a commercial product "Urea 262" which contained 2.62 percent protein equivalent. Therefore, the addition of 0.5 percent urea to the ration increased the protein equivalent by 1.31 percent. Since the mixed basal ration contained 9.79 percent protein by calculation from ration components, the total protein equivalent of the urea supplemented rations was 11.10 percent. The faster growth of the rats fed the 9.79 percent protein ration supplemented with lysine, again emphasizes the importance of amino acid balance in preference to quantity of nitrogen provided in a ration for simple stomached animals.

Rat Growth Study 9

Procedure:

This experiment was a repeat of Rat Growth Study 8. The rats were handled in the same way and exactly the same rations were fed. Thirty-two rats which had been used in a previous growth study were allotted on the basis of body weight and previous treatment to the experimental rations.

The only difference between this and the previous study was the difference between the two groups in average initial weight. The rats in this experiment averaged approximately 90 grams initial body weight versus approximately 138 grams for those used in the previous study.

The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appear in Table 11. In this trial as in the previous one, highly significant differences ($P < 0.01$) were obtained among treatments. Lysine was again shown to be responsible for the differences. No significant differences were attributable to urea or to lysine x urea interaction. A slight, though insignificant growth depression, occurred in the group receiving only urea as the supplement. This appears to be the only qualitative difference in results between this and the previous experiment. It is possible that the size of the rats fed urea is critical in determining the response obtained. If the reduction in growth rate indicated in this experiment is a real one, it appears that urea may be more toxic to young animals than to older ones. Rose and Dekker (1956) theorized that the urea utilization they obtained in a purified diet fed to rats was due to the urease activity of the microorganisms in the gastrointestinal tract. If this theory is correct, it seems likely that the ability of older animals to utilize urea would be greater than that of very young animals.

Rat Growth Study 10

Procedure:

In view of the fact that greater growth response was obtained in previous experiments by supplementation of milo with 0.3 percent L-lysine than with 0.1 percent, this experiment was conducted to determine whether this higher lysine level would have any effect on urea utilization.

Thirty-two rats weighing an average of approximately 137 grams were allotted on the basis of body weight and previous treatment to four groups of eight rats each.

One group received the basal milo ration used in the early rat growth studies (Table 1) while the other groups received the basal plus 0.3 percent L-lysine or the basal plus 0.4 percent urea or the basal plus both lysine and urea.

The rats were handled the same as in all previous studies. Feed and water were offered ad libitum. Feed was mixed and stored as described earlier.

The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appears in Table 12.

TABLE 12 EFFECT OF THE ADDITION OF LYSINE AND/OR UREA TO THE BASAL RATION

Treatment(1)	1 B	2 B+L	3 B+U	4 B+L+U
No.of rats	8	8	8	8
Av.in.wt.(gms)	137.1	137.3	137.9	137.0
Av.final wt.(gms)	128.9	156.8	143.8	163.9
Av.gain/wk.(gms)	-1.9 ± 3.7	9.8 ± 7.6	3.4 ± 3.9	13.5 ± 9.1

(1) B = basal ration (Table 1); L = 0.3 percent L-lysine; and U = 0.4 percent urea 262. This amount of urea supplies 1.05 percent protein equivalent. Since the basal ration contained 8.57 percent crude protein, the rations containing urea supplied 9.62 percent protein equivalent.

The differences obtained among treatments were again highly significant ($P < 0.01$). The addition of lysine was, as in the two previous trials, responsible for the significant difference, since treatment breakdown revealed the effect of lysine to be highly significant ($P < 0.01$), while the effect of urea and lysine x urea interaction were not significant.

However, the ration containing both lysine and urea again produced a higher average weekly gain than lysine alone (13.5 vs. 9.8 grams). Although this difference was not statistically significant, the trend is consistent with the trend of previous studies, for the ration containing both lysine and urea to produce the fastest growth.

The only explanation available for the poor growth performance of the rats in this study compared to those of the previous two studies is with reference to the treatment prior to the beginning of the experiment. The rats used in the two previous studies were transferred immediately from the first growth study to the second, with no time interval between. The rations of both experiments were of similar composition (milo), so no adaptation period to a new type of diet was necessary. In contrast, the rats used in this study were fed for 10 days previous to the experiment on Purina Laboratory Dog Chow. It is probable that the abrupt change in diet consistancy and composition was responsible for the poor performance, since the growth rate during the second week of the study was much greater than during the first week.

In any case, the relative differences among treatments in this experiment with respect to rate of gain were not materially different from those of the two previous trials. Therefore, it is indicated that the 0.3 percent level of L-lysine tended to have a beneficial effect on the utilization of urea.

Rat Growth Study 11

Procedure:

Since the inclusion of several amino acids as supplements to a milo ration contributes materially to the total nitrogen content of that ration, it seemed desirable to compare the effect of added isoleucine and threonine with that of an isonitrogenous amount of urea on the growth rate of rats. Levels of 0.2 percent DL-isoleucine and 0.2 percent DL-threonine were used, since this combination had been shown earlier to produce a growth response in weanling rats. Urea 262 was added to another ration at the level of 0.15 percent, which supplied 0.39 percent protein equivalent and was isonitrogenous with the amount supplied by the amino acids. These materials were added to the basal ration (Table 1) to which had been added 0.5 percent L-lysine.

Four other rations were included in the experiment. These were: basal ration (Table 1); basal plus 0.5 percent L-lysine; a purified diet containing 11 percent casein (Table 4); and a purified diet containing 21 percent casein (Table 4).

Forty-one rats were used in all. They were allotted to the treatments on the basis of body weight and previous treatment.

The 11 percent and 21 percent casein rations were assigned three and four rats, respectively, while the remaining four rations contained eight rats each. The rations are designated in Table 13.

The rats were handled as in all previous experiments. Feed was mixed and stored as described earlier. The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appears in Table 13. Highly significant differences ($P < 0.01$) were obtained in growth rate among the treatments. The Multiple Range Test (Duncan, 1955) showed that the basal ration produced significantly slower growth ($P < 0.01$) than the supplemented rations. The basal ration plus lysine and urea was not significantly different from the basal ration plus lysine, isoleucine and threonine in growth rate produced despite the fact that the average weekly gain obtained on the latter ration was 7.0 grams greater than on the former (29.9 versus 22.9). Therefore, it is indicated that the presence of urea in a milo ration containing 0.5 percent L-lysine is capable of producing some growth stimulation. However, it is not clear whether the mechanism of stimulation was the same as that of the isoleucine and threonine addition. The growth increase produced by the amino acids could conceivably be due partly to their nitrogen content, although this possibility seems unlikely in view of the results obtained in earlier experiments.

TABLE 13 A COMPARISON OF UREA WITH ISOLEUCINE AND THREONINE WHEN ADDED TO THE BASAL RATION SUPPLEMENTED WITH 0.5 PERCENT L-LYSINE

Treatment(1)	1 B	2 B+L	3 B+L+I +T	4 B+L+U	5 11 percent casein	6 21 percent casein
No. of rats	8	8	8	8	3	4
Av. in. wt. (gms)	84.4	85.4	84.6	85.9	77.5	78.3
Av. final wt. (gms)	108.8	126.5	144.4	131.6	126.0	142.5
Av. gain/wk. (gms)	7.2 ±4.5	20.0 ±4.7	29.9 ±4.3	22.9 ±6.5	23.4 ±1.1	32.2 ±4.2

(1) B = basal ration (Table 1); L = 0.5 percent L-lysine; I = 0.2 percent DL-isoleucine; T = 0.2 percent DL-threonine; U = 0.15 percent urea 262. This amount of urea 262 supplies 0.39 percent protein equivalent. Since the basal ration contained 8.57 percent crude protein, the ration containing urea would furnish 8.96 percent protein equivalent.

It will be noted that the group fed the diet containing lysine, threonine and isoleucine gained 29.9 grams per rat per week as compared to 32.2 grams gained by the group fed 21 percent casein. This difference is much smaller than obtained with earlier studies. The reason for this is not clear, but it is possible that the protein requirement of rats of the size used in this experiment was low enough so that a proper balance of amino acids such as that supplied by the amino acid fortified milo was able to promote nearly maximum growth.

Rat Growth Study 12

Procedure:

This experiment was designed to test the effect on growth of adding urea to the milo basal ration supplemented with isoleucine, lysine and threonine or with these three amino acids plus methionine. The addition of 0.2 percent DL-isoleucine, 0.5 percent L-lysine and 0.2 percent DL-threonine had produced the most rapid gains in previous studies. It was felt that this combination was probably sufficient to supply the requirements for all of the essential amino acids for growth. Therefore, it seemed that if the rat required additional nitrogen for non-essential amino acid synthesis, the addition of urea to this ration might serve this function. It was also desired to test once more the effect of adding methionine to the ration supplemented with the other three amino acids.

Thus, the experiment was designed in a 2 x 2 factorial arrangement as shown in Table 14. Thirty-two rats were allotted according to body weight and previous treatment to four groups of eight rats each and assigned to the various treatments. The rats were handled as in all previous experiments. Feed and water were supplied ad libitum. Feed was mixed and stored as described earlier. The experiment was terminated at the end of the second week.

Results and Discussion:

The summary of results appears in Table 14. No significant differences were obtained in growth rate produced among the treatments in this experiment. The fact that the addition of 0.1 percent methionine to either of the rations in this experiment caused no growth response verifies the results obtained earlier when methionine was used as a supplement.

TABLE 14 EFFECT OF METHIONINE AND/OR UREA ON THE GROWTH OF RATS FED THE BASAL RATION SUPPLEMENTED WITH ISOLEUCINE, LYSINE AND THREONINE

Treatment(1)	1 B+I+L+T	2 B+I+L+T+U	3 B+I+L+M	4 B+I+L+T+U+M
No.of rats	8	8	8	8
Av.in.wt.(gms)	113.6	110.0	112.1	111.8
Av.final wt.(gms)	176.0	177.0	183.4	179.4
Av.gain/wk.(gms)	32.6 ± 4.3	32.9 ± 6.2	34.4 ± 4.0	33.8 ± 3.2

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(1) B = basal ration (Table 1); I = 0.2 percent DL-isoleucine; L = 0.5 percent L-lysine; T = 0.2 percent DL-threonine; M = 0.1 percent DL-methionine; and U = 0.4 percent urea 262. This amount of urea 262 supplies 1.05 percent protein equivalent.

Since urea did not increase the growth rate of the rats in this study, it is possible that the non-essential amino acid content of the ration was sufficient to meet the needs of rats of this size or that the rat was unable to utilize the urea nitrogen for non-essential amino acid synthesis under these dietary conditions.

SWINE GROWTH STUDIES

Swine Growth Study 1 (Summer 1956)

Procedure:

The purpose of this study was to determine the effect on growth rate and feed efficiency of growing swine fed a milo ration supplemented with lysine and/or urea. Published data and the results of the initial growth study presented in this thesis indicated that lysine is the first limiting amino acid in milo. Since published findings concerning the value of urea in rations for swine are limited and somewhat in disagreement, it was desired to test urea in a low protein ration containing milo. Kafir 44-14 was used in this test. This is the same variety used in all of the rat growth studies.

Twenty-four Hampshire and Poland China pigs weighing an average of approximately 113 pounds were allotted on the basis of sex, breed and weight to the four treatments. The experiment was designed in a 2 x 2 factorial arrangement with six pigs on each of the four treatments. The basal ration contained, in percent: Kafir 44-14, 92.1; alfalfa meal, 5.0; bone meal, 2.0; trace mineral salt, 0.5; Aurofac, 0.3; Fortafeed, 0.1; vitamin A-D mix, 0.15; and zinc sulfate, 0.02. The supplemented rations were composed of: basal plus 0.1 percent L-lysine, basal plus 0.5 percent urea 262 and basal plus both lysine and urea 262.

These rations were identical to the corresponding rations in Rat Growth Studies 8 and 9.

The pigs were kept in individual pens with concrete floors in a well ventilated barn. Feed was supplied ad libitum in individual self feeders. Water was supplied in individual metal troughs which were filled twice daily.

All pigs were weighed every two weeks until they approached 180 pounds. They were then weighed more frequently to allow removal from the experiment at as near 180 pounds as possible.

Feed consumption was recorded for each pig to facilitate calculation of efficiency of feed utilization.

Results and Discussion:

The summary of results obtained appears in Table 15. There was a significant difference among treatments ($P < 0.05$) in growth rate and a highly significant difference ($P < 0.01$) in feed required per pound of gain. Treatment breakdown showed lysine to be responsible for the difference in rate of gain and in feed efficiency. No statistically significant effects were attributable to urea or to lysine x urea interaction, either with respect to growth rate or efficiency of feed utilization. However, there was a tendency for the ration supplemented with both lysine and urea to promote better performance.

On a practical basis, the performance of the pigs fed the ration containing both lysine and urea was quite satisfactory (1.37 pounds average daily gain and 4.59 pounds of feed required per pound of gain). The growth rate and feed efficiency obtained on the other three rations would not be considered an acceptable performance for pigs of that weight. (0.86, 1.06 and 0.92 pounds gain per day for Rations 1, 2 and 3, respectively).

No toxic symptoms were evident in pigs receiving urea in their rations. One pig in the group receiving the ration containing both ly-

TABLE 15 EFFECT OF LYSINE AND/OR UREA ON THE GROWTH AND FEED EFFICIENCY OF SWINE FED A MILO RATION

SWINE EXPERIMENT 1

Summer 1956

Treatment (1)	1 B	2 B+L	3 B+U	4 B+L+U
No.of pigs	6	6	6	5(2)
Av.in.wt.(lbs.)	115.3	113.0	112.8	113.0
Av.final wt.(lbs.)	176.7	179.7	183.8	187.4
Av.daily gain (lbs.)	0.86	1.06	0.92	1.37
Av.feed/lb.gain (lbs.)	7.16	5.31	6.32	4.59
Av. no.of days	76.5	66.0	78.8	55.4

SWINE EXPERIMENT 2

Winter 1956-57

Treatment	1	2	3	4
No.of pigs	8	8	8	8
Av.in.wt.(lbs.)	86.0	85.6	86.1	86.9
Av.final wt.(lbs.)	132.4	150.3	128.8	149.9
Av.daily gain (lbs.)	0.96	1.38	0.75	1.28
Av.feed/lb.gain (lbs.)	6.12	4.58	8.00	5.07
No. of days	56	56	56	56

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(1) B = basal ration. Composed of Kafir 44-14, 92.1; alfalfa meal, 5.0; bone meal, 2.0; salt, 0.50; Aurofac, 0.3; and Fortafeed, 0.1 percent. L = 0.1 percent L-lysine; U = 0.5 percent urea 262. This amount of urea supplies 1.31 percent protein equivalent.

(2) One pig developed cervical abscess and was removed midway through the trial.

sine and urea developed a large cervical abcess and was removed midway through the trial. The performance of this pig was not included in the final data.

Neither Hanson and Ferrin (1955) nor Hays et al. (1957) were able to demonstrate a beneficial effect from urea in low protein rations for swine. However, the former workers suggested that under conditions in which all of the essential amino acids are supplied in adequate amounts, a stimulation of growth rate might be expected from added urea.

It appeared from the results obtained in this study that urea was of no value when added alone to a low protein ration of poor protein quality. However, there was a tendency for faster growth and higher efficiency of feed utilization in pigs fed the ration containing the limiting amino acid, lysine, when urea was added.

Swine Growth Study 2 (Winter 1956-57)

Procedure:

In view of the results obtained in Swine Growth Study 1, it was considered desirable to repeat that study in an attempt to clarify the value of urea in a milo ration.

Thirty-two Poland China barrows weighing approximately 86 pounds were allotted on the basis of weight to the four treatments described in the previous treatment.

They were handled in the same manner as those in Swine Growth Study 1. They were kept in individual pens with concrete floors. Feed was supplied ad libitum in individual self feeders and water was supplied twice daily in metal troughs.

This study differed from the previous one in that the experiment was terminated at the end of 56 days. Information was therefore obtained on a time rather than on a weight constant basis.

Results and Discussion:

The summary of results appears in Table 15. In this experiment, highly significant differences ($P < 0.01$) were obtained among treatments both in growth rate and in efficiency of feed utilization. With regard to growth rate, lysine was responsible for the significant difference. The effects of urea and lysine x urea interaction were not statistically significant. There was no significant lysine x urea interaction in efficiency of feed utilization but the reduction in feed efficiency due to urea was significant ($P < 0.05$). Lysine caused a highly significant decrease ($P < 0.01$) in the pounds of feed required per pound of gain.

It will be noted from Table 15 that there was a tendency toward a reduction in growth rate in the rations containing urea. This is in contrast to the first swine growth study, since the tendency was toward an increase in growth rate in pigs fed the rations containing urea in that study. It is possible that the weight of the pigs fed rations containing urea is critical in determining the effect of urea on growth rate. The same tendency toward a growth depression due to urea in smaller animals was shown in the rat growth studies in which these same rations were fed. It was suggested in that discussion (see Rat Growth Studies 8 and 9) that possibly older animals would be expected to utilize urea more effectively than young animals. If urease activity of intestinal microorganisms is necessary for utilization of urea by the animal, as suggested by Rose and Dekker (1956), it seems reasonable to expect a greater response in older animals.

Since no clear cut effects of urea were obtained in these two growth studies with swine, it appears that any beneficial actions of urea in growth or efficiency of feed utilization would occur only in special cases. Therefore, it would not be justified to recommend the use of urea in rations for swine until more information is available about its effects under a variety of conditions.

The fact that no definite beneficial response was obtained in these studies does not, however, rule out the possibility that an advantage might exist for the addition of urea to milo rations under certain conditions. Possibly a higher level of lysine in these studies, or the addition of another amino acid would have provided the proper conditions for a beneficial response from urea.

Swine Growth Study 3

Procedure:

Earlier studies with rats and swine showed that lysine is the first limiting amino acid in milo. In the rat studies, supplementation of the basal ration with 0.1 percent L-lysine and other amino acids gave inconsistent effects on growth rate. Higher levels of added lysine gave more satisfactory results, and these higher levels in conjunction with supplemental threonine produced strikingly faster growth than when lysine was added alone.

Satisfactory growth had been obtained in work by Hillier *et al.* (1953, 1955, 1956) using small amounts of soybean oil meal as a supplement to milo. With this in mind it was desired to compare the growth and feed efficiency obtained by lysine supplementation with that obtained by soybean oil meal added in an amount sufficient to supply an equivalent total lysine content of the ration.

Forty-two weanling crossbred pigs (Duroc x Poland China - Landrace) weighing an average of approximately 43 pounds were allotted on the basis of sex and weight to seven groups of six pigs each. Each group was assigned at random to one of the treatments shown in Table 16.

One group received the unsupplemented basal ration. Three other groups received the basal ration supplemented with 0.1, 0.2 or 0.3 percent L-lysine. The three remaining groups received rations containing soybean oil meal in amounts to provide a protein mixture supplying approximately the same total lysine that was contained in the three lysine supplemented rations. Since it was necessary to start the experiment before actual amino acid analyses were available, calculations from average values from the literature resulted in rations which deviated somewhat from actual values (Table 16). However, these differences were probably not large enough to cause appreciable changes in the results obtained.

The animals were kept in individual pens as in the two previous trials. Feed was supplied ad libitum in individual self feeders and water was supplied twice daily in metal troughs.

The experiment was conducted for 28 days. At the end of this period all animals were weighed and the experiment was terminated.

Results and Discussion:

The summary of results appears in Table 17. Highly significant differences ($P < 0.01$) were obtained among treatments in the growth rate produced. The Multiple Range Test (Duncan, 1955) showed that the rations containing the two highest levels of soybean oil meal and the ration containing 0.3 percent supplemental L-lysine were not significantly different from each other with respect to rate of gain. These three rations produced significantly faster growth than the remaining four rations. The ration containing the highest level of soybean oil meal was signi-

TABLE 16 COMPOSITIONS OF RATIONS USED IN SWINE GROWTH STUDY 3

Ration Components[1]	RATIONS 1 Percent	 2 Percent	 3 Percent	 4 Percent	 5 Percent	 6 Percent	 7 Percent
Kafir 44-14	96.65	96.55	93.25	96.45	89.85	96.35	86.45
Soybean oil meal	----	----	3.40	----	6.80	----	10.20
Steamed bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Trace mineral salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Aurofac[2]	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Fortafeed[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine	----	0.10	----	0.20	----	0.30	----
	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Percent protein	8.82	8.82	10.07	8.81	11.33	8.80	12.58
Percent lysine	0.20	0.30	0.32	0.40	0.43	0.50	0.54
Percent threonine	0.29	0.29	0.34	0.29	0.39	0.29	0.44

(1) 0.02 percent zinc sulfate was added to all rations. Vitamin A concentrate was added to all rations to supply 4000 I U per pound of ration.

(2) Aurofac supplies 1.8 grams of aureomycin and 1.8 milligrams of vitamin B_{12} per pounds.

(3) Fortafeed supplies the following vitamins, in grams per pound: riboflavin, 2.0; pantothenic acid, 4.0; niacin, 9.0; and choline chloride, 90.0.

TABLE 17 A COMPARISON OF THE GROWTH AND FEED EFFICIENCY OF SWINE FED VARIOUS LEVELS OF LYSINE ADDED TO A MILO RATION IN THE PURE FORM OR AS A COMPONENT OF SOYBEAN OIL MEAL

Ration(1)	1 B	2 B+.1L	3 B+.1LSOM	4 B+.2L	5 B+.2LSOM	6 B+.3L	7 B+.3LSOM
No.of pigs	6	6	6	6	6	6	6
Av.in.wt.(lbs.)	43.0	43.2	43.8	43.3	43.7	42.5	41.0
Av.final wt.(lbs.)	59.3	59.3	58.5	60.0	66.8	70.0	71.2
Av.daily gain (lbs.)	0.58 ±0.17	0.58 ±0.18	0.52±0.10	0.60±0.30	0.83±0.39	0.98±0.21	1.08±0.23
Av.feed/pound gain (lbs.)	4.69 ±0.79	5.24 ±1.56	5.20±2.26	4.39±0.96	3.76±1.17	3.35±0.94	3.17±0.68

(1) B = Basal (Table 16), L = percent L-lsyine, LSOM = percent lysine from soybean oil meal.

ficantly higher, and the ration containing 0.1 percent lysine from soybean oil meal was significantly lower than the remaining five rations in growth rate produced.

With respect to feed efficiency significant differences ($P < 0.05$) among treatments were obtained. The general pattern was for the fastest gaining groups to have the highest efficiency of feed utilization. The four treatments which had produced the fastest growth were significantly superior to the remaining three treatments in promoting feed efficiency. These four treatments were, in ascending order of average feed per pound of gain: the ration containing the highest level of soybean oil meal; the basal ration supplemented with 0.3 percent L-lysine; the ration containing the intermediate level of soybean oil meal; and the basal ration supplemented with 0.2 percent L-lysine.

As shown in Table 17, none of the values for average daily gain were satisfactory. As would be expected, the ration containing the highest level of soybean oil meal produced the highest average daily gain and average feed efficiency. This ration contained 12.58 percent crude protein compared to 8.80 percent contained in the ration supplemented with 0.3 percent L-lysine. It is significant to note that despite this difference of 3.78 percent in protein content, the performance of pigs fed these two rations was not appreciably different.

As the percentage of lysine in the ration was reduced, the average daily gain and efficiency of feed utilization was also reduced. This is in agreement with the results obtained in the rat studies. Levels of lysine below 0.50 percent of the total ration were insufficient to support growth appreciably above that obtained with lower levels of lysine, when lysine was supplied either as added L-lysine or as a component of soybean oil meal. However, the tendency was for the rations containing soybean oil meal at the two highest levels to produce greater gain and feed efficiency than rations containing equal L-lysine supplied partly in the pure form. This tendency could be interpreted to indicate that the extra amino acids supplied by the soybean oil meal were responsible for the effect. In view of results obtained in previous studies, the effect was probably due primarily to the presence of the second limiting amino acid, namely threonine. The percentage of threonine supplied by the high protein ration (Ration 7) was 0.44 versus the 0.29 percent supplied by the rations containing no soybean oil meal. Obviously, the content of other amino acids were increased similarly in the rations containing soybean oil meal. Therefore, others could conceivably contribute to performance, but it is unlikely that the tendency toward faster gains and greater feed efficiency in the rations containing soybean oil meal was due to the non-specific effect of additional protein (Ration 6 versus Rations 5 and 7).

The experiment served to further demonstrate the importance of proper amino acid balance in rations for swine. The serious lysine deficiency of milo was again demonstrated. It is of value to note that moderate growth and efficiency of feed utilization can be obtained even in rations containing less than nine percent crude protein when the essential amino acid balance is improved by the addition of the most deficient amino acid.

NITROGEN BALANCE EXPERIMENT

Procedure:

Since Hillier et al. (1953) reported an increase in growth rate of swine fed a 14 percent protein milo ration by the addition of 0.1 percent DL-lysine, it was considered desirable to test the effect of lysine on the nitrogen balance of young pigs fed a similar ration. It was also indicated from previous work that the D-isomer of lysine tended to have an inhibitory effect on growth rate and feed efficiency when fed as DL-lysine.

To test the effect of lysine on nitrogen balance and at the same time, to compare the effects of the L-and DL-forms on nitrogen balance, the following experiment was performed. Nine weanling barrows (three Hampshires and six Chester Whites) were divided into 3 equal groups according to weight and breed, and assigned at random to the three experimental treatments. Ration 1 was the basal ration shown in Table 18. Rations 2 and 3 were composed of the basal ration plus 0.10 percent L-lysine and 0.20 percent DL-lysine, respectively. With this combination, the presence of the D-isomer could be observed with respect to its effect on nitrogen balance.

The pigs were kept in individual screen bottomed metal cages in an air-conditioned room. They were offered water ad libitum. Feed was supplied twice daily in a metal box attached to the side of the cage. Individual feed consumption during the first three days was recorded. During this period all animals were fed the basal ration. This orientation period was followed by a five day preliminary period. During this period daily feed consumption was restricted within each similar group of three animals to the amount eaten daily during the orientation period by the lightest eating animal of that trio.

At the end of the preliminary period daily collections of total feces and urine of each animal were begun. The feces for each animal were refrigerated in a jar containing toluene as a preservative. Daily urine volume was recorded and an aliquot was acidified and brought to an appropriate volume for storage under refrigeration.

Fecal and urinary collections from each animal during the nine day collection period were pooled for analysis of each component. At the end of the ninth day of collection all pigs were weighed and the experiment was terminated.

Results and Discussion:

The summary of the results obtained appears in Table 19. No significant differences were obtained among treatments, either in the absolute amount of N retained or in the percent of the nitrogen ingested that was absorbed. However, the average values for the amount of N retained, no matter how expressed, were larger for the pigs receiving the lysine supplemented rations, indicating an advantage from supplemental lysine. Comparison of the N retention of the pigs receiving 0.1 percent L-lysine with that of pigs receiving 0.2 percent DL-lysine (equivalent amounts of the biologically active form) indicates that the presence of the D-isomer in this study did not interfere with lysine metabolism. This, of course, does not rule out the possibility that such an interference could exist in many cases.

The apparent digestibility of protein was not significantly affected by the addition of lysine to the basal ration.

Any differences in nitrogen retention resulting from supplemental lysine would presumably be due to differences in protein utilization after absorption. A comparison of the fecal losses with urinary losses of nitrogen among treatments in this study tends to support this theory.

The results of this study provided evidence indicating that the increased gains obtained in swine fed rations supplemented with lysine are due, at least in part, to an increased nitrogen retention.

TABLE 18 BASAL RATION USED IN NITROGEN BALANCE STUDY WITH SWINE

Ration Components(1)	Percent
Milo	79.50
Soybean oil meal	10.25
Alfalfa meal (dehydrated)	7.00
Steamed Bone meal	2.00
Trace mineral salt	1.00
Aurofac 2A	0.25
	100.00

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(1) B vitamins were added in the following amounts: Thiamin -HCl, 0.5; riboflavin, 1.5; niacin, 6.0; calcium pantothenate, 4.0; pyridoxine, 0.6; and choline chloride, 200 milligrams per pound of mixed ration; vitamin B_{12}, 9.0 micrograms per pound of mixed ration; vitamin D_2, 8 grams per pound of mixed ration.

The mixed basal ration contained 15.15 percent crude protein and 0.48 percent lysine.

TABLE 19 - EFFECT OF SUPPLEMENTAL L-LYSINE OR DL-LYSINE ON THE NITROGEN BALANCE OF WEANLING PIGS FED A MILO-SOYBEAN OIL MEAL BASAL RATION

Treatment	Basal				Basal 0.1 percent L-lysine				Basal 0.2 percent DL-lysine			
No.of pigs		3		(Av.)		3		(Av.)		3		(Av.)
Initial Body Wt. (lbs.)	33.5	26.0	32.5	30.7	32.5	22.0	28.0	27.5	32.5	20.0	28.0	26.83
Final Body Wt. (lbs.)	45.0	33.5	44.5	41.0	42.5	30.5	40.0	37.7	42.5	30.0	42.0	38.16
Daily feed consumed (gms.)	900.0	616.6	900.0	----	900.0	616.6	900.0	----	900.0	616.6	900.0	----
Daily N intake (gms.)	21.60	14.80	21.60	19.3	21.60	14.80	21.60	19.30	21.60	14.80	21.60	19.30
Daily N in feces (gms.)	7.04	5.73	9.73	7.50	7.90	5.80	8.62	7.44	8.32	4.96	7.97	7.08
Daily N in urine (gms.)	5.10	3.34	4.55	4.33	3.41	2.67	3.65	3.24	3.72	3.55	4.01	3.76
Total Daily N excreted (gms.)	12.14	9.07	14.28	11.83	11.31	8.47	12.27	10.68	12.04	8.51	11.98	10.84
Total Daily N retained (gms.)	9.46	5.73	7.32	7.50	10.29	6.33	10.33	8.98	9.56	6.29	9.62	8.49
Apparent N digestibility (%)	67.4	61.3	54.9	61.2	63.4	60.8	60.1	61.4	61.5	66.3	63.1	63.7
N retention as percent of N intake	43.8	38.7	33.9	38.8	47.6	42.8	47.8	46.1	44.2	42.5	44.5	43.7

SUMMARY

A series of growth trials were conducted with rats and swine to study the amino acid adequacy of milo protein.

In the rat studies, supplemental lysine produced a significant growth response in all cases when added to a basal ration composed of Kafir 44-14 and purified sources of minerals and vitamins. No improvement in growth was obtained by the addition of 0.05 or 0.10 percent DL-methionine, 0.1 percent DL-tryptophan and/or 0.2 percent DL-valine to the basal ration in any of the studies.

Results obtained in the initial experiments indicated that threonine and isoleucine were the next limiting amino acids in milo protein. Subsequent studies showed that threonine is probably the second limiting amino acid and that the growth response obtained by supplemental threonine depends on the level of lysine present in the ration. When 0.1 percent L-lysine was added to the basal ration, the effects of supplemental threonine on growth rate were inconsistent. However, in the presence of 0.3 percent L-lysine, the addition of threonine resulted in more than a 50 percent increase in growth rate (13.7 grams vs. 21.3 grams gain per day). The most rapid growth was obtained when 0.5 percent L-lysine and 0.2 percent DL-threonine were added to the basal ration. No further improvement was made by increasing the DL-threonine level to 0.3 percent or by including isoleucine and/or methionine in the ration. The liver fat of rats receiving the basal ration was significantly higher (P 0.01) than that of rats receiving the basal plus 0.5 percent L-lysine and 0.2

percent DL-threonine. This growth rate was approximately equal to that obtained with a purified ration containing 11 percent casein, but inferior to that obtained with a 21 percent casein ration.

In the swine studies, the addition of 0.1 percent L-lysine to a basal ration containing Kafir 44-14, alfalfa meal, bone meal, salt and commercial sources of vitamins and an antibiotic produced highly significant increases in growth rate and efficiency of feed utilization in each of two studies with 85 and 113 pound pigs. In a third study, the addition of 0.3 percent L-lysine to a basal ration composed of Kafir 44-14, bone meal, salt, vitamins and an antibiotic produced growth approximately equal to that obtained on a similar ration containing enough soybean oil meal to supply 0.3 percent lysine. The addition of 0.1 or 0.2 percent L-lysine to the basal ration was ineffective in improving rate and efficiency of gain.

A nitrogen balance study was conducted with weanling pigs fed a 15 percent protein ration containing Westland milo and soybean oil meal with or without supplemental lysine. Although no statistically significant differences were obtained among treatments, the rations supplemented with 0.1 percent L-lysine or 0.2 percent DL-lysine produced a higher average nitrogen retention than was obtained from the basal ration.

Growth studies with rats demonstrated the serious lysine deficiency of milo gluten meal. No significant improvement in the nutritive value was obtained by autoclaving or by pancreatic hydrolysis.

The effect of urea on the growth of rats and on the growth and feed efficiency of swine fed a milo ration was also studied. No significant improvement by urea was obtained in either growth rate or feed efficiency in any of the studies. However, there was a tendency toward faster growth when urea was fed in conjunction with lysine. This tendency was greatest in rats weighing more than 100 grams initially and in swine weighing more than 100 pounds initially.

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APPENDIX

TABLE 1
PARTIAL COMPOSITION OF FEEDS

Feedstuff	Where used[(1)]	Percent Protein	Percent Lysine	Percent Threonine
Kafir 44-14	All growth studies	9.13	0.20	0.30
Milo gluten meal	RG 11, 12	63.44	0.46	1.40(2)
Alfalfa meal	RG 7, 8 SG 1, 2	18.70	0.73	0.70(2)
Soybean oil meal	SG 3	45.94	3.60	1.77
Westland milo	SNB	10.30	0.18	

(1) RG = Rat Growth Study, SG = Swine Growth Study, SNB = Swine Nitrogen Balance Study. Numbers refer to the study in which the feed was used.

(2) Average values from Morrison (1956).

TABLE 2
RAT GROWTH STUDY 1

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	63	87.8637	
Treatment	7	0.6104	40.92**
isoleucine	1	575.8800	0.28
lysine	1	11.6025	268.20**
threonine	1	1.4854	5.40*
isoleucine x lysine	1	2.5400	0.69
isoleucine x threonine	1	19.9697	1.18
lysine x threonine	1	2.9579	9.30**
isoleucine x lysine x threonine	1	2.1472	1.38
Experimental Error	56		

** Significant difference at the 1 percent level.
* Significant difference at the 5 percent level.

TABLE 3
RAT GROWTH STUDY 1a

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	19		
Treatment	2	0.9347	0.80
Experimental Error	17	1.1730	

** Significant difference at the 1 percent level.
* Significant difference at the 5 percent level.

TABLE 4
RAT GROWTH STUDY 2

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	63		
Treatment	7	2.7886	0.998
Experimental Error	56	2.7932	

TABLE 5
RAT GROWTH STUDY 2

ANALYSIS OF VARIANCE

Rations 1-9 (Includes unsupplemented basal ration of Rat Growth Study 1)

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Treatment	8	56.1125	20.87**
Experimental Error	63	2.6888	

** Significant difference at the 1 percent level.

TABLE 6
RAT GROWTH STUDY 2a

ANALYSIS OF VARIANCE

Ration 1 (0.1 percent L-lysine) vs. Ration 1a (0.2 percent L-lysine)

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	7		
Treatment	1	75.0300	34.79**
Experimental Error	6	2.1567	

ANALYSIS OF VARIANCE

Ration 8 (0.1 percent L-lysine) vs. Ration 8a (0.2 percent L-lysine)

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	7		
Treatment	1	40.5000	6.85*
Experimental Error	6	5.9167	

ANALYSIS OF VARIANCE

Ration 10 (0.1 percent L-lysine) vs. Ration 10a (0.2 percent L-lysine)

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	7		
Treatment	1	32.0000	4.80
Experimental Error	6	6.6667	

** Significant difference at 1 percent level.
* Significant difference at 5 percent level.

TABLE 7
RAT GROWTH STUDY 3

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	106.2100	44.14**
Experimental Error	28	2.4061	

** Significant difference at the 1 percent level.

MULTIPLE RANGE TEST(1)

Standard error of the mean = 0.548

n = 28

Basal	Basal + 0.1%L	Basal + 0.1%L + 0.2%T	Basal + 0.1%L + 0.2%T + 0.2%I
3.31	10.19	10.44	11.06

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(1) L = L-lysine, T = DL-threonine, I = DL-isoleucine.

TABLE 8
RAT GROWTH STUDY 4

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	86		
Treatment	10	377.8020	33.75**
Experimental Error	76	11.1940	

** Significant difference at the 1 percent level.

MULTIPLE RANGE TEST(1)

Standard error of the mean = 1.18

n = 76

B	B+.1%L +I+T	B+.1%L	B+.2%L	B+.5%L	B+.4%L	B+.3%L	B+.2%L +I+T	B+.3%L +I+T	B+.4%L +I+T	B+.5%I +I+T
3.50	7.81	9.50	9.64	11.00	11.31	13.68	17.43	21.25	23.37	24.87

(1) B = Basal, L = L-lysine, I = 0.2 percent DL-isoleucine, T = 0.2 percent DL-threonine.

TABLE 9
RAT GROWTH STUDY 5

ANALYSIS OF VARIANCE
(Rations 1 - 9)

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	71		
Treatment	8	680.1238	27.16**
Experimental Error	63	25.0371	

** Significant difference at the 1 percent level.

MULTIPLE RANGE TEST(1)

Standard error of the mean = 1.77

n = 63

B+L	B+L+.3I	B+L+.2I	B+L+.2I +.2T	B+L+.2T	B+L+.3T	B+L+.2I +.2T+.1M	B+L+.2I +.2T +.05M	B+L+.3I +.3T
9.69	10.01	10.25	26.44	27.63	28.13	28.31	28.56	30.75

(1) B = Basal, L = 0.5 percent L-lysine, I = percent DL-isoleucine M = percent DL-methionine and T = percent DL-threonine.

TABLE 10
RAT GROWTH STUDY 5

ANALYSIS OF VARIANCE

Liver Fat Content
Basal versus Basal Lysine and Threonine

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	12		
Treatment	1	299.7388	30.05**
Experimental Error	11	9.9732	

** Significant difference at the 1 percent level.

TABLE 11
RAT GROWTH STUDY 6

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	13		
Treatment	1	1028.5700	45.15**
Experimental Error	12	28.7800	

** Significant difference at the 1 percent level.

TABLE 12
RAT GROWTH STUDY 7

ANALYSIS OF VARIANCE
(Rations 2 - 9)

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	63		
Treatment	7	400.0000	4.18**
Experimental Error	56	95.6148	

** Significant difference at the 1 percent level.

MULTIPLE RANGE TEST(1)

MGM (hyd)	MGM (aut)	MGM (hyd+aut)	MGM	MGM (hyd)+L	MGM L	MGM (hyd+aut) + L	MGM (aut)+L
0.25	2.38	2.44	3.81	19.75	22.00	22.25	27.56

(1) MGM = Milo gluten meal, L = 0.3 percent L-lysine

TABLE 13
RAT GROWTH STUDY 8

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	104.9480	4.71**
lysine	1	294.0313	13.20**
urea	1	13.7813	0.62
lysine x urea	1	7.0313	0.32
Experimental Error	28	22.2811	

** Significant difference at the 1 percent level.

TABLE 14
RAT GROWTH STUDY 9

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean-Square	F Value
Total	31		
Treatment	3	342.8425	18.31**
lysine	1	1010.2513	53.95**
urea	1	9.2450	0.49
lysine x urea	1	9.0313	0.48
Experimental Error	28	18.7272	

** Significant difference at the 1 percent level.

TABLE 15
RAT GROWTH STUDY 10

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	309.6120	7.26**
lysine	1	835.3828	19.58**
urea	1	92.8203	2.18
lysine x urea	1	0.6328	0.02
Experimental Error	28	42.6751	

** Significant difference at the 1 percent level.

TABLE 16
RAT GROWTH STUDY 11

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	693.1767	25.19**
Experimental Error	28	27.5157	

** Significant difference at the 1 percent level.

MULTIPLE RANGE TEST(1)

Standard error of the mean = 1.86

n = 28

B	B + L	B + L + U	B + L + I + T
7.19	19.93	22.88	29.88

(1) B = Basal, L = 0.5 percent L-lysine, U = 0.15 percent urea 262, I = 0.2 percent DL-isoleucine and T = 0.2 percent DL-threonine.

TABLE 17
RAT GROWTH STUDY 12

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	15.1767	0.73
urea	1	4.5000	0.22
methionine	1	32.0000	1.53
urea x methionine	1	9.0300	0.43
Experimental Error	28	20.8907	

TABLE 18
SWINE GROWTH STUDY 1

ANALYSIS OF VARIANCE
Average Daily Gain

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	23		
Treatment	3	0.3180	4.43**
lysine	1	0.6567	9.06**
urea	1	0.2072	2.86
lysine x urea	1	0.0900	1.24
Experimental Error	20	0.0725	

** Significant difference at the 1 percent level.

TABLE 19
SWINE GROWTH STUDY 1

ANALYSIS OF VARIANCE
Feed per Pound of Gain

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	23		
Treatment	3	7.6410	6.69**
lysine	1	19.2425	16.84**
urea	1	3.6582	3.20
lysine x urea	1	0.0222	0.02
Experimental Error	20	1.1429	

** Significant difference at the 1 percent level.

TABLE 20
SWINE GROWTH STUDY 2

ANALYSIS OF VARIANCE
Average Daily Gain

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	0.6837	11.10**
lysine	1	1.8336	29.98**
urea	1	0.1953	3.17
lysine x urea	1	0.0221	0.36
Experimental Error	28	0.0616	

** Significant difference at the 1 percent level.

TABLE 21
SWINE GROWTH STUDY 2

ANALYSIS OF VARIANCE
Feed per Pound of Gain

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	31		
Treatment	3	18.3040	8.29**
lysine	1	39.8278	18.03**
urea	1	11.2338	5.09*
lysine x urea	1	3.8503	1.74
Experimental Error	28	2.2087	

** Significant difference at the 1 percent level.
* Significant difference at the 5 percent level.

TABLE 22
SWINE GROWTH STUDY 3

ANALYSIS OF VARIANCE
Average Daily Gain

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	41		
Treatment	6	0.3002	4.71**
Experimental Error	35	0.0637	

** Significant difference at the 1 percent level.

MULTIPLE RANGE TEST(1)
Average Daily Gain

Standard error of the mean = 0.103

n = 35

B+.1L from SBOM	B	B+.1L	B+.2L	B+.2L from SBOM	B+.3L	B+.3L from SBOM
0.52	0.58	0.58	0.60	0.83	0.98	1.08

(1) B = Basal, L = percent L-lysine, SBOM = soybean oil meal.

TABLE 23
SWINE GROWTH STUDY 3

ANALYSIS OF VARIANCE
Feed per Pound of Gain

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	41		
Treatment	6	4.3160	2.72*
Experimental Error	35	1.5845	

* Significant difference at 5 percent level.

MULTIPLE RANGE TEST(1)

Standard error of the mean = 0.514

B+.3L from SBOM	B+.3L	B+.2L from SBOM	B+.2L	B	B+.1L from SBOM	B+.1L
3.17	3.35	3.76	4.39	4.69	5.20	5.24

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(1) B = Basal, L = percent L-lysine, SBOM = soybean oil meal.

TABLE 24
NITROGEN BALANCE STUDY

ANALYSIS OF VARIANCE
Nitrogen Retained as a Percent of Nitrogen Ingested

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	8		
Block	2		
Treatment	2	41.2933	3.93
Experimental Error	4	10.5167	

TABLE 25
NITROGEN BALANCE STUDY

ANALYSIS OF VARIANCE
Grams Nitrogen Retained per Day

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	8		
Block	2		
Treatment	2	1.7037	0.56
Experimental Error	4	3.0450	

TABLE 26
NITROGEN BALANCE STUDY

ANALYSIS OF VARIANCE
Apparent Digestibility of Nitrogen

Source of Variation	Degrees of Freedom	Mean Square	F Value
Total	8		
Block	1	18.100	
Treatment	1	5.7500	0.38
Experimental Error	6	15.2450	

VITA

Wilson Gideon Pond

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES ON THE AMINO ACID ADEQUACY OF MILO PROTEIN FOR GROWTH OF RATS AND SWINE

Major Field: Animal Nutrition

Biographical:

Born: Minneapolis, Minnesota, February 16, 1930

Education: Bachelor of Science Degree in Animal Husbandry from University of Minnesota, June, 1952. Master of Science Degree in Animal Husbandry from Oklahoma A. & M. College, May, 1954.

Experience: Active duty in United States Army, June, 1954 - March, 1956. Graduate Assistant, Animal Husbandry Department, Oklahoma A. & M. College, September, 1952- May, 1954; September, 1956 - May, 1957.

Member of Alpha Zeta and American Society of Animal Production
Associate Member of Sigma Xi.

Date of Final Examination: July, 1957