MONTANA STATE UNIVERSITY

Effect of addition of amino acids to barley rations for rats and swine
by Richard M Davidson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University
© Copyright by Richard M Davidson (1962)

Abstract:
The trials reported herein were initiated to study the effect of adding amino acids to barley rations.

The results of Rat Trial I indicated average daily gains were slightly greater when feeding rations containing a low protein barley when compared to rations containing a high protein barley. All rations were corrected to 10 percent protein before the addition of amino acids. P.E.R. values were increased when lysine was added to the rations containing either the high or the low protein barley. Supplemental methionine appeared to have little effect on P.E.R. values.

When rats were fed rations containing 17.0 percent protein barley (rations corrected to 15.9 percent protein) added lysine increased the P.E.R. value. Lysine and methionine added together gave slightly greater P.E.R. values than when lysine alone was added. Little response was observed when adding only methionine.

Seventeen percent protein barley rations (rations corrected to 15.9 percent) with lysine added at the 0.4 or 0.6 percent levels and methionine added at the 0.3, 0.4, 0.5 and 0.6 percent levels, all resulted in similar P.E.R. values. All rations compared favorably with rations containing casein as the sole source of protein in gains.

The results of Swine Experiment I indicated pigs fed L-lysine HCl gained slightly more than pigs fed Lyamine. The addition of lysine to barley rations indicated trends for increased gains and feed efficiency. Source or levels of lysine did not appear to affect the fat content of the carcass. Results, however, indicated adding lysine to the barley rations increased the ribeye area and the loin weights of the carcasses. Gilt carcasses contained a heavier ham, shoulder, loin, butt, lean trim and had a greater ribeye area than barrows.

Results of Swine Experiment II indicated pigs fed rations containing a low protein barley (13.3 percent protein) resulted in greater gains than pigs fed rations containing a high protein barley (17.0 percent protein). The grower rations, using both barley sources, contained approximately 15.0 percent protein. The fattening rations using the 13.3 percent protein barley contained approximately 12 percent protein and those having the 17.0 percent protein barley had an approximate protein content of 15 percent.

The pigs fed the low protein rations (12.0 percent) had greater gains and increased feed efficiency in the fattening phase when compared to the pigs receiving the greater protein rations (15.0 percent). Average daily gain and feed efficiency were slightly greater when adding lysine, especially to the rations containing the low protein barley. Results indicated supplementary lysine produced greater effect on gain and feed efficiency in the growing phase than in the fattening period.

EFFECT OF ADDITION OF AMINO ACIDS TO BARLEY RATIONS FOR RATS AND SWINE

by

RICHARD M. DAVIDSON

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science

Approved:

Fred S. Willson

Head, Major Department

L. G. Young

Chairman, Examining Committee

Leon H. Johnson

Dean, Graduate Division

MONTANA STATE COLLEGE
Bozeman, Montana

June, 1962

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. L. G. Young for assistance and council throughout the graduate program and especially for helpful suggestions while conducting the experimental work and preparation of the manuscript; to Dr. O. O. Thomas for suggestions and assistance throughout the graduate program; to Dr. D. W. Blackmore for helpful suggestions in the preparation of the manuscript; to Dr. E. P. Smith for assistance in the statistical analyses of the data and to Professor J. R. Dynes for assistance with the carcass work.

Appreciation is also extended to all members of the staff and employees of the Animal Industry and Range Management Department for their cooperation and assistance throughout the graduate program. Special thanks, also, to Mr. Donald R. McCarl, the swine herdsman, for his cooperation while conducting the experimental work.

Appreciation is also expressed to Merck and Company who contributed to the support of the trials and who furnished the lysine.

TABLE OF CONTENTS

INDEX TO TABLES

INDEX TO APPENDIX

ABSTRACT

The trials reported herein were initiated to study the effect of adding amino acids to barley rations.

The results of Rat Trial I indicated average daily gains were slightly greater when feeding rations containing a low protein barley when compared to rations containing a high protein barley. All rations were corrected to 10 percent protein before the addition of amino acids. P.E.R. values were increased when lysine was added to the rations containing either the high or the low protein barley. Supplemental methionine appeared to have little effect on P.E.R. values.

When rats were fed rations containing 17.0 percent protein barley (rations corrected to 15.9 percent protein) added lysine increased the P.E.R. value. Lysine and methionine added together gave slightly greater P.E.R. values than when lysine alone was added. Little response was observed when adding only methionine.

Seventeen percent protein barley rations (rations corrected to 15.9 percent) with lysine added at the 0.4 or 0.6 percent levels and methionine added at the 0.3, 0.4, 0.5 and 0.6 percent levels, all resulted in similar P.E.R. values. All rations compared favorably with rations containing casein as the sole source of protein in gains.

The results of Swine Experiment I indicated pigs fed L-lysine HCl gained slightly more than pigs fed Lyamine. The addition of lysine to barley rations indicated trends for increased gains and feed efficiency. Source or levels of lysine did not appear to affect the fat content of the carcass. Results, however, indicated adding lysine to the barley rations increased the ribeye area and the loin weights of the carcasses. Gilt carcasses contained a heavier ham, shoulder, loin, butt, lean trim and had a greater ribeye area than barrows.

Results of Swine Experiment II indicated pigs fed rations containing a low protein barley (13.3 percent protein) resulted in greater gains than pigs fed rations containing a high protein barley (17.0 percent protein). The grower rations, using both barley sources, contained approximately 15.0 percent protein. The fattening rations using the 13.3 percent protein barley contained approximately 12 percent protein and those having the 17.0 percent protein barley had an approximate protein content of 15 percent. The pigs fed the low protein rations (12.0 percent) had greater gains and increased feed efficiency in the fattening phase when compared to the pigs receiving the greater protein rations (15.0 percent). Average daily gain and feed efficiency were slightly greater when adding lysine, especially to the rations containing the low protein barley. Results indicated supplementary lysine produced greater effect on gain and feed efficiency in the growing phase than in the fattening period.

INTRODUCTION

Barley production is increasing in the United States and especially in the western states. To maintain a desirable market for this increase in production the use of barley must also be expanded.

Barley is an excellent grain for swine feeding and produces pork of high quality. The protein content of barley varies from approximately 10 to 17 percent. Barley supplies only slightly less total digestible nutrients than corn. In several experiments, pelleted barley, fed in properly balanced rations, has produced nearly as rapid gains as corn.

Barley, however, is deficient in calcium, vitamin D and vitamin A. Also, the protein of barley may be deficient in certain of the essential amino acids. In feeding swine efficient results cannot, therefore, be realized unless protein supplements of good quality are fed in addition to barley so sufficient amounts of these amino acids will be supplied. Some of the higher quality protein feeds that have been used to supplement barley are fish meal, meat scrap, tankage and soybean oil meal.

Today's nutritional and industrial technology makes possible the manufacturing of barley rations, formulated to include those ingredients which have been found to be deficient in swine rations. With improved methods of obtaining pure amino acids the addition of certain limiting amino acids to these formulated rations may have economic advantages.

Little research has been conducted to determine the effects of adding amino acids to barley rations for swine. Therefore, it appeared important to conduct additional experimental work to determine the desirability of adding amino acids to barley rations as a means of increasing rate of gain, feed efficiency and carcass quality of swine.

Additional experimental work was conducted with rats to determine the effect of adding amino acids to barley rations.

LITERATURE REVIEW

The nutritive importance of proteins and the dependence of animals on plants for these substances were first pointed out by G. J. Mulder around 1840 (Encyclopedia Americana, 1960). A few years later Boussingault, writing in the Economie Rurale (Encyclopedia Americana, 1960) said, "The alimentary virtues of plants reside above all in the nitrogenous substances, and consequently their nutritive potency is proportional to the quantity of nitrogen entering into their composition."

McCollum, as quoted by Mendel (1923, p. 121), remarked that the investigations carried out during the period between 1910 and 1920 on protein foods of plant origin "leave no room for doubt that all the amino acids necessary for the nutrition of an animal are contained in the proteins found in each of these foods. Certain of these are, however, present in such limited amounts as to restrict the extent to which the remaining ones, which are more abundant, can be utilized."

Flodin (1953) states, "the quantity and quality of protein supplied by the diet are of vital importance to health at every portion of the life span. Wherever total quantity or average quality of the protein consumed fall significantly below accepted standards for good nutrition, the signs and symptoms of protein deficiency (hypoproteinosis) appear, involving various degrees of retardation or failure of tissue synthesis." The discovery that many of the amino acids composing body proteins must be supplied as such by food protein explains why different foods and rations of the same protein content have different protein values in nutrition. They differ in protein quality. It must be kept in mind that there are certain qualitative differences as to the essential amino acids required by different species and for different functions in the same species. There are also quantitative differences per unit of body weight or of growth tissue formed. These considerations mean that one cannot generalize from one species to another or one function to another as to either qualitative or quantitative requirements.

MEASUREMENT OF PROTEIN QUALITY

One of the most common methods of determining the quality of protein utilizes the criterion adapted by Osborne and Mendell, viz-the gain in weight per gram of protein ingested or protein efficiency ratio (P.E.R.). From theoretical consideration, the maximal utilization of absorbed protein for the synthesis of body protein is the most valid expression of the growth promoting quality of dietary protein, according to Barnes _et al_. (1945). They go on to state, "The establishment of the maximal ratio of body weight gained to protein consumed is the most useful of the methods of measuring nutritive value of proteins for growth that do not involve fecal and carcass nitrogen analysis, but it does not necessarily provide wholly accurate indices of protein values." Chapman _et al_. (1959) have standardized this procedure, by using rats of certain age, correcting protein of diet to 10 percent, maintaining the trial for a four week period, and adjusting results to a constant value of 2.5 for casein. Morrison and Campbell (1960) using this procedure found that female rats tended to give maximal P.E.R. values at lower dietary protein levels than did males. It was also found that differences between casein and a plant protein mixture were greatest during the early stages of the experiment in both sexes.

Hegsted (1947) found a very high correlation between weight gain and protein efficiency. He also found that protein efficiency is a function of gain in weight rather than a characteristic of protein fed. He concludes that, in studies on the relative nutritive value of various proteins using growing rats fed ad libitum, little additional information is gained by taking into account the amount of protein eaten, i.e., the calculation of protein efficiency.

McHenry <u>et al</u>. (1961) employed the liver-N method with rats to determine the nutritive value of a number of proteins. The liver-N method is based on the fact that, for relatively small protein intakes, the values of liver-N [(mg) per 100 g. initial body weight] varied linearly with the amount of protein eaten, provided the nutritive value of the protein was not better than that of casein. When they used casein as the standard of reference for a series of proteins there was good agreement between values obtained by the liver-N and balance sheet methods for proteins with nutritive values equal to or less than that of casein.

A method to determine protein quality with respect only to lysine has been described by Carpenter (1960) employing the Sanger reaction with 1 fluoro-2:4 dinitro benzene for the determination of the free ε-amino groups of lysine units in purified proteins. Baliga <u>et al</u>. (1959) in using this method in cottonseed meal found a relationship between the content of lysine with the free ε-amino groups and protein quality as determined in rat protein repletion tests.

Mitchell (1924) used a method based upon nitrogen balance data involving direct determination of the amount of nitrogen in the feces and in the urine and indirect determinations of the fractions of the fecal nitrogen and of the urinary nitrogen that were of dietary origin. The biological value of the protein is taken as the percentage of the absorbed nitrogen (nitrogen intake minus fecal nitrogen of dietary origin) that is not eliminated in the urine.

McLaughlan _et al._ (1959) based their determination on the content of lysine and methionine or methionine and cystine and developed a simplified chemical score. Because the simplified chemical score method is relatively rapid, yields reproducible results, and correlates with animal assays, it was proposed as a rapid screening procedure for the evaluation of protein in food, but was not intended to replace the rat bio-assay method.

Physico-chemical methods of amino-acid analysis by isotope dilution may also be employed (Foster 1945). This procedure, which appears to be the most accurate method now available for the determination of amino-acids in protein hydrolyzates, is limited only by the availability of the equipment and the material.

There has been considerable use of biological methods employing micro-organisms and specific enzyme systems for the routine estimation of all the known amino acids.

The results of the microbiological assay may be affected by many factors such as oxygen (Bohonos _et al._, 1942), carbon dioxide (Lascelles _et al._, 1954), sparing of amino acids by the addition of other amino acids or compounds (McClure _et al._, 1954), interactions with other amino acids (Fildes 1953), and the relative proportions of various amino acids and other compounds (Brickson _et al._, 1948) and (Sirny _et al._, 1951).

However, Stokes *et al*. (1945) found that, in general the microbiological values for purified and impure proteins are in reasonably good agreement with those obtained by the more recent improved chemical methods. Block and Mitchell (1947) indicated a higher degree of reproducibility than was noted in the work conducted by Stokes.

The evaluation of bacteriological methods for the determination of protein quality by comparisons with protein efficiency ratio (P.E.R.) values determined by standardized rat growth assay was conducted by Rogers et al. (1959). Results with enzyme hydrolyzates correlated poorly with P.E.R. values, whereas with acid hydrolyzates, a good correlation was obtained for cereal proteins.

Bayne *et al*. (1961) reports on evaluation of 130 samples of seven different types of protein concentrates, which were evaluated by the Gross Protein Value (G.P.V.) procedure as supplements to cereal protein for chicks. In addition Net Protein Utilization (N.P.U.), with the samples as the sole source of protein for rats, was determined for a limited number. Microbiological procedure correlated well with these methods.

THE QUALITY OF PROTEIN IN CEREALS

Maynard and Loosli (1956) states, "Cereal grains are deficient in lysine." Morrison (1956) also concludes "when fed as the only source of protein, the grains all fall decidedly below such a food as milk in quality of protein." In fact, it has been concluded by Morrison and Campbell (1960) that P.E.R. values for bread and flour diets were a direct function of the lysine content of the protein. McLaughlan and Morrison (1960) found that for mixtures of foods in which cereal products contribute approximately half or more of the protein, the lysine content is a reliable guide to the nutritional value of the protein mixture.

Carroll and Krider (1956) states, "The proteins of all cereal grains are deficient in certain essential amino acids. For this reason protein supplements must provide not only more protein but protein having a good balance of the essential amino acids."

The results obtained by McElroy et al. (1948) agreed with the established fact that grain protein is lacking in quality for the promotion of efficient growth in swine.

Morrison (1956), and the National Research Council Publications 648 and 659 (1959) show barley as deficient in some amino acids for swine and rats, especially lysine.

THE EFFECT OF PROTEIN CONTENT ON THE BIOLOGICAL VALUE OF THE PROTEIN

Marked differences in the growth response of both rats and pigs attributable to variation in the protein content of the grain was observed by McElroy et al. (1949). Mitchell (1924) found biological values were smaller at the higher protein content of corn. Mitchell et al. (1952) observed the proportion of tryptophan and of lysine in the total protein of corn decreased with increasing content of protein. However, Miller et al. (1950) found that amino acid content of corn varied directly with protein content and there was no change in protein quality with increase in the amount of protein within the range from 8.49 percent to 14.12 percent.

Esh et al. (1960) working with Bengal gram of different protein levels found the P.E.R. with the high protein gram was slightly higher than with the low protein sample.

Sure (1957) observed that order of the rations, based on their protein efficiency ratios, varied at different planes of protein intake. For example, at the 15 percent level of intake the P.E.R. of defatted soybean flour and cottonseed meal are far superior to that of corn gluten meal, whereas at 25 and 30 percent planes of intake, the P.E.R. of the corn gluten meal is appreciably higher than that of either the soybean flour or cottonseed meal.

Bressani _et al_. (1958) determined lysine requirements for rats at 4 percent increments from 8 to 24 percent and at 32 and 40 percent crude protein. The maximum lysine requirements expressed as a percentage of the diet remained essentially constant in the protein range of 16 to 40 percent. Expressed as a percentage of the total protein, the lysine requirements were 6.7, 5.6, 4.2, 3.6, 2.6 and 2.2 percent with 8, 12, 16, 20, 24, 32 and 40 percent of total protein (N X 6.25) respectively.

Graw (1948) found that, as the protein level was increased, the lysine requirement for maximum growth at a particular protein level increased.

In a somewhat different approach Bruneger _et al_. (1950a) found that a ration containing 10.6 percent protein, the lysine requirement was 0.6 percent of the ration. When rations were fed containing approximately 22 percent protein, the lysine requirement increased to 1.2 percent of the ration. The difference in these requirements is largely eliminated if they are expressed in terms of their proportion to the protein in the ration. The lysine requirements of 0.6 percent and 1.2 percent of the ration correspond to 5.7 and 5.5 percent of the protein in the 10.6 and 22 percent protein rations respectively.

Almquist (1952) also indicated the amino acid requirements increase as the protein level in the diet increases. However, amino acid requirements expressed as a percentage of the dietary protein appeared to decrease as the protein level increased. However, Graw and Kamei (1950) found that, as the protein level of the chickens' diet is increased, the lysine and methionine plus cystine requirements also increase, but at a slower rate.

EFFECT OF AMINO ACID IMBALANCES IN RATS AND SWINE

Working with amino acid imbalances in rats Sauberlich (1952) found that such imbalances resulted in depressed growth. It was found that this condition could be corrected by the addition of the deficient amino acid or acids to the diet.

Harris et al. (1943) found that a deficiency of lysine in a diet produced cessation of growth and hypoproteinaemia in young rats. The changes observed were assumed to be due to general inhibition of protein formation. This resulted in a reduced growth of some organs which developed at the expense of others and protein was transferred according to a fixed system of growth priorites.

Gillespie et al. (1945) found a loss of protein from the liver and a hypoproteinaemia, while the body protein content seemed to be unchanged. The importance of the liver for protein metabolism and its possible role in connection with the synthesis of serum proteins was postulated.

Conducting experimental work with baby pigs Mertz et al. (1949) showed that lysine is indispensable for growth and development. Lack of lysine resulted in cessation of growth, decreased feed consumption and decreased feed efficiency. Lysine deficient pigs manifested a depraved appetite, rough hair coat, emaciated look and inanition.

The findings of Elvehjem (1956) show that excess quantities of amino acids also affect growth. He found that the addition of 0.4 percent of methionine to an 18 percent casein diet caused growth depression. He also found an amino acid-vitamin relationship in which pyridoxine will counteract the effect of moderate excess amounts of methionine.

Hanks _et al_. (1949) found the addition of 0.2 percent DL-methionine in place of 0.2 percent L-cystine in a 9 percent casein ration for rats gave the same growth effect as 0.2 percent L-cystine in the presence of either 0.078 percent DL-threonine or 2 percent acid hydrolyzed casein. They postulated that the growth inhibitions obtained by adding the various combinations of amino acids appeared to be due to the increased requirements of the limiting amino acid when all others were supplied in adequate or generous amounts.

By raising the levels of certain essential amino acids in diets containing marginal levels of trypotophan Henderson _et al_. (1953) induced a niacin deficiency in rats. It was found that levels of lysine above approximately 0.5 percent and valine above 0.7 percent caused a growth suppression which was corrected by an addition of niacin.

A relationship between methionine and vitamin B_6 was found by DeBey _et al_. (1952). They found that levels of methionine only slightly above those necessary for growth depressed the growth of rats fed limited amounts of vitamin B_6. Vitamin B_6 counteracted the effect of moderate by excess amounts of methionine although, when the diet contained 3.5 percent of methionine, high levels of the vitamin failed to restore growth.

Rose (1937) emphasized that in determining amino acid imbalances many factors such as proportion of fat and carbohydrates in the ration must be taken into consideration and that the age, weight and sex of the animals may play important roles in determining the minimum level of a given component.

THE ESSENTIAL AMINO ACIDS

Classifying the essential amino acids for the pig Mertz et al. (1952) found that arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine must be present in the diet.

Beeson (1951) states, "If any one of the essential amino acids is dropped out of the ration the growth of the pig will stop immediately."

SUPPLEMENTATION WITH NATURAL PROTEIN TO IMPROVE PROTEIN QUALITY

Hoagland and Snider (1927) conducted experiments to determine the value of beef protein as a supplement to the proteins in certain vegetable products. These tests showed that the rations containing equal parts of beef and cereal proteins were practically of the same value im promoting growth in rats as rations containing only meat protein.

Animal proteins have also been used effectively to supplement chicken rations, Almquist et al. (1935).

Carpenter et al. (1957) used dehydrated fish products as supplementary proteins to cereals. They found that addition of lysine to a commercial fish meal raised its value.

Morrison (1956) recommends that cereal grains be supplemented with good quality protein such as fish meal, meat scrap, tankage or peanut oil meal.

Gupta et al. (1958) found a considerable difference in values for biological availability of the lysine in different purified protein.

SUPPLEMENTING WITH PURIFIED AMINO ACIDS

Rama Rao et al. (1960) found that rats grew normally when fed a complete L-amino acid diet containing all the amino acids at their minimal requirement levels in a 10 percent conventional protein (N X 6.25) ration.

Findings of Bressani et al. (1960) showed that when a cereal diet was supplemented with all of the limiting amino acids according to the pattern of the F.A.O. reference protein, a sustained nitrogen retention sometimes similar to that obtained with milk feeding was observed.

Rosenberg and Rohdenberg (1952) obtained significant growth responses in weanling rats with the addition of increasing amounts of lysine to diets of dried bread supplemented with fat, salt and vitamins. They found a supplement of 0.5 percent DL-lysine HCl, corresponding to a 0.2 percent L-lysine, to a bread diet improved the average gain in weight after 5 weeks from 32 percent to about 75 percent of the average gain on the stock diet. If sufficient lysine were added to bring the total L-lysine content of the diet to about 0.8 percent, or more, a growth response similar to that obtained with the stock diet was observed.

Brunegar et al. (1949) fed experimental diets containing 0.34 percent, 0.42 percent, 0.50 percent, 0.58 percent and 0.74 percent pure L-lysine. The first four levels of lysine were fed to 3 pigs each and the 0.74 level was fed to 2 pigs. The pigs weighed 10 Kg. each. The averages of the grams weight gain per gram of protein consumed were 2.60, 2.85, 3.12, 3.47 and 3.49 for each of the respective lysine levels. The average biological values for the corresponding lysine levels were: 52, 51, 61, 73 and 72.

Another experiment was conducted by Brunegar et al. (1960) using a basal diet of corn and barley. The diet, consisting of 21.1 percent protein, contained 0.57 percent lysine, and was supplemented with histidine and methionine. This diet was fed to weanling pigs for four weeks. Experimental diets were made to contain 0.57, 0.75, 0.97, 1.07, 1.32 and 1.63 percent pure lysine. Each increased lysine level up to 1.07 percent improved the growth rate and feed efficiency. In another trial rations containing 21.3 percent protein were supplemented with methionine, histidine and tryptophan. Lysine levels of 0.96, 1.00, 1.20 and 1.40 percent were each fed to five pigs. Increases in growth rate and feed efficiency were noted up to the 1.20 percent lysine level. The data of these two experiments show that with diets containing approximately 22 percent protein weanling pigs require approximately 1.20 percent L-lysine in the ration. Lyman et al. (1956) found the lysine requirements of the young pig to be 3.45-3.65 percent of the crude protein by microbiological assay.

An experiment supplementing Teff with 0.4 percent lysine monohydrochloride (LMH) was conducted by Jansen et al. (1957). Their findings indicated that adding LMH to Teff raised the 4 week weight gain and P.E.R. from 50.3 grams and 1.95 to 125 grams and 3.27 respectively. Similarly, supplementation of pear millet with 0.50 percent of LMH increased weight gain and P.E.R. from 3.62 grams and 1.83 to 118 grams and 3.28, respectively.

Hale and Lyman (1961) added 0.62 percent lysine to sorghum grain-cottonseed meal rations for growing-fattening pigs. Their results showed pigs in all groups receiving the ration containing added lysine made significantly greater ($P<0.01$) daily gain. Their findings also showed that lysine additions to the basal rations significantly improved feed efficiency.

Pond et al. (1953) supplemented corn and milo rations with amino acids for growing pigs. They obtained a significant improvement in growth rate and feed efficiency by adding lysine to the basal diet in one trial and the improvement approached significance in another trial.

Larson et al. (1960) used lysine supplementation of oat rations for weanling pigs. Findings in the first trial showed the younger and smaller pigs (20 lb.) responded to 0.3 percent supplemental lysine whereas for the heavier pigs (28 lb.) the 0.1 percent level of lysine was most beneficial. In both trials, the best rate of gain obtained on the lysine supplemented rations was similar to that obtained on the 10 percent soybean meal rations. In the second trial, the lower level of lysine supplementation (0.1 percent) seemed to be the most desirable.

Sure (1955) supplemented pearled barley with amino acids. Supplementing the protein in pearled barley, fed at an 8 percent level of protein, with 0.4 percent L-lysine, resulted in 57.2 percent increased growth and 50.0 percent increase in P.E.R. The further addition of 0.5 percent D-L threonine was followed by a 78.6 percent additional gain in body weight and 118.4 percent further increase in protein efficiency. The supplementation of pearled barley with L-lysine, D-L threonine and 0.5 percent D-L methionine resulted in 15.3 percent additional growth and 56.3 percent increase in protein utilization.

When supplementing barley rations with lysine Dinnuson et al. (1958) found no difference in final feed conversion, however, large differences were noted before the pigs reached 100 pounds. The addition of lysine, at all levels studied and in all trials, gave beneficial results in average daily gain.

Reisen et al. (1946) fed rats diets containing 8, 18 and 50 percent casein. They found the growth of rats receiving 8 percent casein was increased with additional intake of methionine or cystine. Their results further showed that an increased intake of both methionine and cystine resulted in retarded growth when rats received 8 or 50 percent casein, but not with those receiving 18 percent casein.

When studying the effect of methionine supplementation of a soybean oil meal-purified ration for growing pigs, fed at the 10 percent level of protein, Bell et al. (1950) found that the protein from soybean oil meal was less efficiently utilized by growing pigs and had significantly lower biological value than whole egg protein. The addition of methionine to the soybean oil meal protein to equal the amount in the whole egg protein made the two proteins equal.

Kade et al. (1948) found that better growth was obtained when using an 8 percent casein diet supplemented with 1.5 percent D-L methionine than when using the basal diet without additional methionine. Methionine added at levels of 2, 2.5 and 3 percent of the diet definitely inhibited growth and protein utilization.

Methionine or lysine was found to be the first limiting essential amino acid in commercial mixed feeds for swine by Rosenberg (1957). He further found that successful supplementation of a feed consisted of adding the first limiting essential amino acid to the feed in such a manner as to achieve a balance with the second limiting essential amino acid as any amount in excess of that needed for proper balance was lost.

Lewis (1962) conducted a feeding trial with pigs using high nitrogen barley as the sole major constituent of the diet. The pigs were divided into four groups: a control group receiving a typical standard ration, a basal group given only barley, a basal barley group with the addition of two amino acids and a basal barley group with the addition of 5 amino acids. A batch of barley of lower total nitrogen (equivalent to about 11 percent protein) was used for the finisher phase. When the pigs were given the ration of barley only, supplemented with amino acids and minor constituents, the performance was equivalent to that with a good standard ration. Assessment was made in terms of growth, feed conversion ratios, nitrogen retention, and carcass composition.

UTILIZATION OF D AND L FORMS OF AMINO ACIDS

When supplementing with purified amino acids some factors must be taken into consideration in relation to availability. One of these factors is the utilization of D and L forms of the amino acids. Jackson and Block (1953) found that D methionine, as well as the naturally occurring L methionine, stimulated growth in rats ingesting a cystine-methionine deficient diet.

Berg (1936) found D lysine unable to promote growth when fed to rats as a supplement in a lysine deficient diet. Van Pulsum et al. (1950) found rats fed the L forms of the ten essential amino acids as components of a D-L mixture constituting 22.4 percent of the diet grew less well than control rats fed only the L isomers at a dietary level of 11.2 percent protein. When allowance was made for the growth promoting capacities of the D components of the D-L mixture, and only half as much D-L phenylalanine, tryptophan, methionine, and arginine and an intermediate level of D-L histidine were included, the resulting 18.6 percent of D-L amino acids promoted as good growth as that attained on the L mixture. The growth retardation was traced to excess methionine. Comparative tests showed that the growth retardation produced by the natural L isomer of methionine was greater than that produced by either the D-L or the D modification.

TIME FACTOR

Another consideration is the influence of time of ingestion of essential amino acids upon utilization in tissue synthesis. Cannon et al. (1947) working on this problem found that for effective tissue synthesis all essential amino acids must be available to the tissues practically simultaneously; otherwise the first group absorbed is not stored long enough to enable its essential amino acids to combine with those of the second group for the synthesis of complete tissue proteins. This occurred even when the two incomplete rations were offered at alternate hours over a 14 hour period followed by the non-protein basal ration for the remainder of the 24 hour period. The two incomplete rations combined contained all of the ten essential amino acids.

A report by Geiger (1947) supports the view that "incomplete" amino acid mixtures are not stored in the body but are irreversibly further metabolized. It was shown that with delayed supplementation of the lacking amino acids the missing tryptophan, methionine or lysine, when fed several hours after feeding the "incomplete" mixture did not promote growth.

Elman (1947) found the injection of tryptophan (and methionine) 6 hours after an injection of an incomplete mixture of amino acids, lacking only tryptophan, failed to induce positive nitrogen balance, whereas the injection of tryptophan (and methionine) simultaneously succeeded in doing so. He concluded that retention of nitrogen is facilitated when all of the complete mixture of amino acids is present to the tissues at the same time.

Yang et al. (1961), however, found growth data and the biological value obtained with the lysine supplement administered apart from the diet, either immediately or 4, 8, 12 or 16 hours after the 4-hour feeding period, were not different from those observed with the lysine supplement incorporated in the diet.

EFFECT OF LYSINE SUPPLEMENTATION ON CARCASS QUALITY

Vipperman et al. (1961) found an increase of total muscle mass with lysine supplementation of swine rations. The carcass specific gravity increased reaching a maximum at the 0.9 percent lysine level. The yield of skinned ham, Boston butt, picnic, and trim loin increased, and the total lean yield increased ($P<0.01$).

Seerley (1962) supplemented milo rations for weanling pigs with 0.1, 0.2, and 0.3 percent L-lysine. Slaughter data collected were average backfat thickness, carcass length, loin eye area and percent lean cuts.

Results of slaughter data showed that carcasses may be improved by lysine supplementation. As the level of lysine increased backfat thickness decreased and the loin eye area and percent lean cuts increased. Comparison of carcasses from pigs fed rations without lysine and 0.3 percent lysine were 1.64 vs. 1.46 inches backfat, 3.35 vs. 3.78 square inches loin eye, and 50.24 vs. 52.84 percent lean cuts, respectively.

RAT EXPERIMENTS

EXPERIMENTAL ENVIRONMENT

Experimental animals

Both male and female rats were used in all studies and were approximately 21 days of age at the beginning of the trials. Rats were housed in the Animal Industry small animal research room in the Medical Science Building. This room was heated by a central heating system and, as a result, the temperature varied considerably. Variations as great as 20° F were observed during the trials. The room was also inadequate in ventilation, becoming very stuffy at times. No artificial light was provided at any time during the trials except when someone was working in the room.

General care of the animals

Rats were weighed and earmarked at the initiation of the trial so each rat could be identified. Rats were fed and watered in individual cages. Feed and water were supplied ad libitum. The feeders were refilled twice weekly and fresh water was provided as needed. The feeders were placed in crocks to minimize the spilling of feed and facilitated a reasonably accurate weigh back of feed. The experimental period lasted 28 days. The animals were weighed at weekly intervals.

Basal ration

The basal ration consisted of 80 percent corn starch, 10 percent corn oil, 5 percent non-nutritive cellulose, 4 percent U.S.P. #14 salt mix, and 1 percent vitamin diet fortification mixture from Nutritional Biochemical Corporation. The barley was substituted for the corn starch in the various trials to obtain the desired protein content for the ration. The rations were not analyzed chemically.

Lotting

The rats were allotted to the various treatments maintaining an equal litter distribution. A uniform sex ratio was also maintained throughout the various treatments.

Protein efficiency ratio (P.E.R.)

P.E.R. values were calculated according to the method of Chapman *et al*. (1959) by dividing the weight gained in grams by the grams of protein consumed. A correction factor was obtained by using the formula $\frac{2.5}{\text{P.E.R. for casein}}$. The figure 2.5 is a determined constant P.E.R. of reference standard casein. 1/ The denominator is the P.E.R. actually received from reference standard casein diet for the trial being considered.

The P.E.R. values of all except the casein diet were multiplied by the correction factor to convert each to a common basis for comparison with the standard casein diet.

1/ A.N.R.C. Reference Casein. Sheffield Chemical. Norwich, N. Y.

METHODS AND PROCEDURES

Rat Trial I

Trial I was conducted to determine the effect of supplementing rations containing high and low protein barley (13.3 and 17.0 percent respectively), with lysine and/or methionine. The composition of the rations is shown in Table I.

In this trial 6 rats (3 males and 3 females) were allotted to each treatment. The lots with their respective treatment are shown in Table II.

Table I. Rat Trial I. Composition of the Rations.1/

Rations	I 2/	II 3/	III 4/
Ingredients			
Casein	---	---	11.13%
Barley	58.80%	75.20	---
Corn oil	10.00	10.00	10.00
Cellulose	5.00	5.00	5.00
Salt Mix #14	4.00	4.00	4.00
Vitamins	1.00	1.00	1.00
Corn starch	21.20	4.80	68.87

1/ All rations corrected to 10 percent protein before the addition of amino acids.
2/ 17.0 percent protein barley.
3/ 13.3 percent protein barley.
4/ Reference casein.

Table II. Rat Trial I. Experimental Treatments. 1/

Lot 1	Ration I 2/
Lot 2	Ration II 3/
Lot 3	Ration I plus Lysine 4/
Lot 4	Ration II plus Lysine 4/
Lot 5	Ration I plus Methionine 4/
Lot 6	Ration II plus Methionine 4/
Lot 7	Ration I plus Lysine and Methionine 4/
Lot 8	Ration II plus Lysine and Methionine 4/
Lot 9	Ration III

1/ All rations corrected to 10 percent protein before addition of amino acids.
2/ 17.0 percent protein barley.
3/ 13.3 percent protein barley.
4/ L-lysine HCl and/or D-L Methionine.

Rat Trial II

In Trial II the procedures outlined by Chapman et al. (1959) were altered so the protein of the various rations were corrected to a 15.9 percent level. As a result, the composition of the barley ration, with respect to corn oil and cellulose, was altered somewhat to facilitate the 15.9 percent protein level. Methionine, lysine or the combination of the two were added to the basal rations. The composition of the rations is shown in Table III.

Six rats (3 males and 3 females) were used per treatment. The lots with their respective treatments are shown in Table IV.

Table III. Rat Trial II. Composition of the Rations.

Ration	I	II 1/
Ingredients		
Casein	17.4%	---
Barley	---	93.0%
Corn oil	2.0	2.0
Cellulose	5.0	---
Salt mix #14	4.0	4.0
Vitamins	1.0	1.0
Corn starch	70.6	---

1/ 17.0 percent protein barley used in the ration.

Table IV. Rat Trial II. Experimental Treatments.

Lot 1	Ration I
Lot 2	Ration II 1/
Lot 3	Ration II plus 0.44 percent D-L Methionine
Lot 4	Ration II plus 0.52 percent L-lysine HCl
Lot 5	Ration II plus 0.44 percent D-L Methionine and 0.52 percent L-lysine HCl

1/ 17.0 percent protein barley used in the ration.

Rat Trial III

In Trial III a regimen was devised to approach the problem of finding the optimum levels of lysine and methionine which should be added to a 17.0 percent protein barley ration. Two supplemental levels of lysine were used with 4 different levels of methionine added to each lysine level.

The rations in this trial were also corrected to 15.9 percent protein rather than the 10 percent protein correction used by Chapman. The rations are shown in Table V.

Six rats (3 males and 3 females) were allotted to each treatment. The lots with their respective treatments are shown in Table VI.

Table V. Rat Trial III. Composition of Rations.

Ration	I	II 1/
Ingredients		
Barley	---	93.0%
Casein	17.4%	---
Corn oil	2.0	2.0
Cellulose	5.0	---
Salt mix #14	4.0	4.0
Vitamins	1.0	1.0
Corn starch	70.6	---

1/ 17.0 percent protein barley

Table VI. Rat Trial III. Experimental Treatments. 1/

Levels of Methionine	0.4% Lysine	0.6% Lysine
0.3%	Lot II	Lot VI
0.4	Lot III	Lot VII
0.5	Lot IV	Lot VIII
0.6	Lot V	Lot IX

1/ All rations contain 17.0 percent protein barley.
Lot I fed the casein ration.

RESULTS AND DISCUSSION

Rat Trial I

The average daily gain, feed per gram gained, P.E.R. and corrected P.E.R. are shown in Table VII. The P.E.R. values were the only result analyzed statistically. The casein ration was fed to obtain a correction factor for the P.E.R. This was calculated by using the formula $\frac{2.5}{\text{P.E.R. for casein}}$. The correction factor obtained for this experiment was 0.86. All rations were corrected to 10 percent protein before the addition of the amino acids.

Table VII. Results of Rat Experiment I.

Lot		A.D.G.	Feed/ Gm. Gain	P.E.R.	Corrected P.E.R.
		Grams	Grams		
I	Ration I 1/	1.75	6.12	1.64	1.41
II	Ration II + Methionine 2/	1.86	5.83	1.74	1.50
III	Ration I + Lysine 3/	2.56	4.58	2.24	1.91
IV	Ration II + Lysine	2.56	4.55	2.21	1.90
V	Ration I + Methionine	1.58	6.32	1.59	1.37
VI	Ration II + Methionine	1.70	5.70	1.78	1.53
VII	Ration I + Lysine and Methionine	2.68	4.55	2.23	1.92
VIII	Ration II + Lysine and Methionine	2.68	4.57	2.20	1.89
IX	Reference Casein	3.32	3.49	2.92	----

1/ 17 percent protein barley.
2/ 13.3 percent protein barley.
3/ L-lysine HCl and D-L Methionine both added at 0.2 percent of the ration.

There was a variation of approximately 8 grams between lots in average initial weights when the rats were placed on experiment. All animals were within 3 days of the same age.

The rats in lots receiving lysine supplementation definitely had improved P.E.R.'s. The addition of methionine had no appreciable effect. The two sources of barley, containing 13.3 and 17.0 percent protein, respectively, responded equally well with lysine and gave about equal P.E.R. values when supplemented. This is not in agreement with Mitchell (1924) and Unpublished Data (Montana State College) where findings showed that biological values were lower at higher protein contents of the feed.

The analysis of variance showed a highly significant difference ($P<0.01$) due to rations. When Duncan's New Multiple Range Test (Duncan, 1955) was applied to the results of this trial, a highly significant difference ($P<0.01$) was found between the rations containing lysine (Lot III, IV, VII, VIII) and those not receiving supplemental lysine (Lot I, III, V, and VI).

Rat Trial II

The average daily gains, feed per gram gain, P.E.R. and corrected P.E.R. are shown in Table VIII. Only the Protein Efficiency Ratios were used for statistical analysis. The correction factor used in this trial was 1.26.

The rations in this trial contained 15.9 percent protein before the addition of the purified amino acids.

There was a variation of 2 grams in average lot weights when the rats were placed on experiment. The rats were approximately the same age