AMINO ACID SUPPLEMENTATION OF PEACE RIVER BARLEY FOR GROWING-FINISHING PIGS

by

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B.S.A., Kon-kuk University, 1966

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in the Department

of

ANIMAL SCIENCE

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

March, 1973

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ABSTRACT

The work reported in this thesis comprised two sections, each of which compared six rations in pigs in the body weight range 16-85 kg. All rations were based on barley. A supplement of L-lysine HCl was added to provide a total lysine content of 0.90% and 0.75%, with the latter level also tested with 0.05% added L-threonine, alone or with 0.10% DL-methionine or 0.10% DL-methionine plus 0.10% L-isoleucine. A control ration consisted essentially of barley and soybean meal to provide 0.75% total lysine. These rations were used for growth and nitrogen balance experiments.

The supplementation of barley with 0.50% L-lysine HCl (0.75% total lysine) tended to improve nitrogen utilization more than a supplemental level of 0.69% L-lysine HCl (0.90% total lysine). The biological value of the ration containing the lower level of lysine was significantly higher than that of the ration containing the higher level of lysine, which was reflected in a trend indicating that pigs fed the lower level of lysine were leaner and had larger eye muscle areas than those fed the higher level of lysine.

Adding 0.05% L-threonine to the lower level of lysine improved growth performance but not carcass quality. The addition of L-lysine and L-threonine to the barley tended to improve nitrogen balance above that obtained by the addition of L-lysine alone. The further addition of 0.10% DL-methionine with or without 0.10% L-isoleucine did not improve growth performance but gave a further slight non-significant improvement in nitrogen metabolism measurements. Therefore, both the feeding experiment

and nitrogen balance experiment indicated that threonine was the second limiting amino acid after lysine.

Supplementation of barley with amino acids regardless of levels or combinations, gave significantly poorer daily gain, feed efficiency and carcass quality results but significantly higher biological value figures than the barley-soybean control ration. Thus, it would appear that the amino acid-supplemented rations still were deficient in some way, although the biological value data would tend to contradict the suggestion that this is associated with an amino acid imbalance.

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ACKNOWLEDGEMENT

The writer wishes to express his gratitude to Dr. R.M. Beames of the Department of Animal Science for his constant encouragement and guidance throughout the course of this study.

Thanks are also extended to Dr. W.D. Kitts, Chairman of the Department of Animal Science, for the use of necessary facilities for this study.

I also wish to express my gratitude to Mrs. L. Mather for her assistance in carrying out the amino acid analyses.

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I. INTRODUCTION

It is well known that cereal protein has a low nutritional value, primarily due to a relatively low level of the essential amino acids, lysine, methionine, threonine, tryptophan and isoleucine. Pig rations are normally based on these cereals. It is therefore necessary that a supplement provide adequate levels of these limiting amino acids.

Traditionally, the protein of grain is supplemented with high quality protein meal of plant or animal origin to increase the total protein content and to counteract any essential amino acid deficiency in the basal ration of the growing pig. The total or partial replacement of protein supplement by synthetic amino acids could reduce the cost of a ration.

The importance of the lysine content of the diet is well established and there are many examples in the literature of the improvement in performance that can be brought about by lysine supplementation of cereal rations. It has been reported that supplementing cereal mixtures with lysine, threonine, tryptophan and in some experiments also with isoleucine results in performance equal to that obtained with rations of grain plus protein concentrates.

The present study was undertaken to examine the effects of adding two levels of lysine with or without threonine, methionine and isoleucine to a basal ration of Peace River barley in terms of daily gain, feed conversion effeciency, carcass quality and nitrogen balance.

II. REVIEW OF LITERATURE

A. ESSENTIAL AMINO ACIDS AND NONESSENTIAL AMINO ACIDS

Amino acid supplementation of feeds is based on the results of over sixty years of research on the nutritional quality of proteins and their constituent amino acids. Much of the fundamental work was carried out by Osborne and Mendel (1919, 1920) who studied the growth-promoting qualities of isolated proteins and recognized that specific amino acids were missing or present in low concentrations in those proteins which did not support adequate growth of the animal. Rose (1938) divided the amino acids found in proteins into two main categories, the essential and the nonessential amino acids. Essential amino acids are those which cannot be synthesized by the organism at a rate adequate to meet metabolic requirements and must be supplied in the diet. Nonessential is the term applied to those amino acids which the body can synthesize from normal food constituents via transamination reactions. It should be emphasized that this classification is based on growth studies in the rat (Rose 1938). Classification of the amino acids with respect to their growth effect in the white rat are given below. Glycine is not essential for the growing pig but is essential for the growing chick. The requirement for arginine and glycine appears more acute in the more rapidly feathering breeds of birds (Hegsted _et al_. 1941).

ESSENTIAL		NONESSENTIAL	
Arginine*	Methionine	Alanine	Hydroxy glutamic acid
Histidine	Phenylalanine	Aspartic acid	Hydroxy proline
Isoleucine	Threonine	Citrulline	Norleucine
Leucine	Tryptophan	Cystine	Proline
Lysine	Valine	Glutamic acid	Serine
		Glycine	Tyrosine

* Arginine can be synthesized by the rat but not at a sufficiently rapid rate to meet the demands of normal growth.

The proportion of essential amino acids provided by various proteins is the major factor influencing the biological value. The failure of young animals to grow on a diet deficient in one or more of the essential amino acids is a reflection of its inability to synthesize adequate quantities of body protein under these experimental conditions. The result of an amino acid deficiency in the diet is that the tissues do not synthesize protein requiring that particular amino acid.

The terms essential and nonessential relate mainly to dietary requirements and have little meaning with respect to the relative importance which amino acids may have in metabolism. The amino acids which are essential in the diet are compounds with carbon skeletons which can not readily be synthesized by the body. In a real sense, the so-called nonessential amino acids are of equal significance for the economy of the organism which is participating in diverse cellular reactions and functions, and provide precursors for the synthesis of many important cellular constituents. Indeed, certain of the nonessential amino acids, e.g., glutamic acid, have so many important metabolic roles that, were a mammal to lose suddenly its capacity to synthesize glutamic acid, serious disorganization of key reactions of metabolism might result as the animal may be unable to wait until the next meal to replenish its supply.

B. AVAILABILITY OF AMINO ACID IN THE RATION

It has been stressed that the content of amino acids in a protein may not accurately reflect its nutritive value, since some of the amino acids may not be available to synthesize the tissue of the animal. Problems involved in determining availability of amino acids have been discussed by Grau and Carroll (1958). A considerable number of biological and a few chemical procedures have been developed. A satisfactory chemical procedure for measuring lysine availability has been described by Carpenter (1960) which depends upon the reaction of dinitroflurobenzene with the ε-amino group of lysine. The reaction produces a colored lysine derivative and it can, therefore, be used to estimate the lysine with the free ε-amino group. The results obtained with animal proteins correlate closely with gross protein value determined in the chick under conditions where the lysine content of the materials is emphasized. With vegetable protein and high carbohydrate diets, the method is not as satisfactory, the figures obtained being too low because of the destruction of the colored lysine derivative. Moran et al. (1963) used the reaction of the dye Orange G with basic amino acids to determine the availability of free amino, amidazole, and guanidyl group of proteins. The dye binding capacity of soybean meal heated for varying periods of time was closely related to growth of chicks fed the meal.

The most widely used biological procedures for determining availability of amino acid are those based on growth of rats (Calhoun et al. 1960). In this method, increments of the pure amino acid to be tested are added to a basal diet deficient in the amino acid and a growth response curve is obtained. The response found with protein-bound amino acids is then compared with that obtained with the pure amino acid to determine availability. Oh et al. (1972) determined biological availability of methionine in various protein supplements. Four procedures of bioassay were studied on the basis of slope-ratio technique: (a) weight gain versus levels of dietary methionine; (b) efficiency of feed utilization versus levels of dietary methionine; (c) weight gain versus total available methionine consumed; and (d) gain in weight attributable to intake of test protein methionine versus methionine intake from the test protein.

Method (d) was the most precise and reliable method as judged by the usual statistical criteria. Using this method,the available methionine content of meat, fish, blood, rapeseed, soybean and feather meal was estimated to be 91.1, 89.9, 66.3, 87.2, 94.7 and 35.0% respectively.

Nielson (1971) showed that the digestibility of amino acids from different balanced rations was related to the digestibility of nitrogen in growing pigs. The results indicated that the digestibility of lysine was not significantly different from the digestibility of nitrogen. Lower digestibility coefficients were found for methionine, threonine and alanine, whereas the digestibility coefficients for cystine, valine, leucine, isoleucine, phenylalanine, tyrosine, histidine, arginine, glutamic acid and aspartic acid were higher than for nitrogen.

There is much variation in amino acid availability from various protein supplements. Amino acid contents should be listed as available amino acid rather than total. However, it is difficult to show available figures because availability of amino acids in many ration components varies from batch to batch, and the method of determining amino acid availability is difficult and time consuming.

C. AMINO ACID DEFICIENCY, EXCESSES AND INTERACTION

Deficiencies, interactions and excesses can reduce growth rate although excesses generally have to be at higher levels than those found in normal rations before they interfere with growth.

The question of amino acid imbalance and antagonism has arisen fairly recently. To explain the term imbalance it might be appropriate to give the definition of a well-balanced protein, which is "a protein in which the essential amino acids occur in ideal proportions, and are thus required in minimal quantities to satisfy the amino acid requirements of the animal". Amino acid imbalance may be defined as a depression in growth (or any other adverse effect) which results from an alteration of amino acid balance of the ration, produced by adding a mixture of amino acids or a protein that lacks one of the essential amino acids. The adverse effect can be counteracted by feeding a small supplement of the most limiting amino acid. For example, Harper (1959) mentioned that when gelatin is added to a casein diet, growth may decrease unless tryptothan is also added. It is typical of an imbalance that the requirement for the most limiting amino acid is increased. Antagonism is described as an excess of a single amino acid that may cause a growth depression and result in an increased need for one or more of the other amino acids. An excess of leucine causes an increase in the requirement for isoleucine. This recognition is based upon the observation that dietary additions of incomplete mixtures of amino acids cause a severe growth depression which is prevented by supplementation of the diet with the amino acids that are most limiting (Harper 1958; Deshpande et al. 1958).

In three papers by D'Mello and Lewis (1970 a, b, c), evidence has been presented to demonstrate the unique interaction between lysine and arginine, between leucine, isoleucine and valine, and between threonine and tryptophan in the chick. The results indicate that excess dietary lysine increases the arginine requirement of the young chick, and excess threonine increases tryptophan requirement quantitatively, while excess leucine increases the quantities of isoleucine and valine required to sustain normal growth. The results of these experiments permit the general conclusion that the requirements of amino acids are interdependent. The unique interaction between lysine and arginine in chick nutrition supports the observations of other authors (O'Dell et al. 1958; Jones 1964; Smith and Lewis 1966). The interactions between leucine, isoleucine and valine also have been observed in experiments with rats (Harper et al. 1954; Benton et al. 1956; Rogers et al. 1967). The interaction or interrelationship between threonine and tryptophan has been known to occur in the rat (Salmon 1954; Morrison et al. 1960; Florentino et al. 1962).

D. METHOD OF DETERMINING AMINO ACID REQUIREMENT

Determination of the nutritional value of protein is inherently non-specific. In the case of vitamins or essential minerals, one can evaluate a single essential nutrient at a time. Conversely, in the assay of protein value, one must be concerned with the qualitative and quantitative adequacy of at least nine amino acids. There are also problems of amino acid balance and utilization to be considered.

It is inevitable that different assay methods will differ in the classification of proteins as to nutritive value. The different assay methods have differing parameters of measurement. Despite the vagaries inherent in assay procedures, it is of interest to note that most methods have classified most food proteins in the same general order of adequacy. The balanced proteins of meat, milk and eggs have been used as standards of excellence. The major grain proteins are low in one or more essential amino acids and are generally improved in feeding value by appropriate amino acid fortification. Balance can also be achieved, as in the case of manufactured feeds for poultry and swine, by fortification with a single protein source such as soybean meal.

The approximate amino acid needs of several species are known. By a knowledge of the essential amino acid composition of a protein, one can predict its feeding value with a fair degree of accuracy. One cannot predict, however, inefficiencies in digestibility or the effects of processing, which are not generally revealed by composition analyses. Protein evaluation methods, which reflect true feeding value, continue, therefore, to provide the ultimate in biological evaluation. Indirect methods of protein evaluation confirm and complement the direct feeding methods.

Most of the experimental work referred to in this review has been carried out on the growing animal, mostly with an ad libitum feeding regime. Growth rate, feed conversion and in many cases carcass quality have acted as the criteria in pig production for the evaluation of ration adequacy. The performance and carcass quality of pigs are influenced not only by the quality of the diet, but also by the system of feeding. Clausen et al. (1959-1964) in trials with amino acids, have used both ad libitum feeding and restricted feeding. An interpretation of their results is that the response to both treatments has been about the same.

It is recommended that any program for the evaluation of amino amino acid supplementation include an evaluation of the effect on the growth of an animal. Carcass measurements have in some cases added valuable information in growth experiments. Backfat thickness, eye muscle area and fat-lean ratio are all correlated to the percentage of lean (or protein) in the carcass, with a correlation coefficient of about 0.7 (Clausen et al. 1959-1964; Marcum et al. 1961).

Although performance is important in practical animal production, weight gain is an inaccurate criterion of nitrogen retention. Nitrogen balance values are of much greater value in assessing protein quality. The concept of "biological value" was first introduced by Thomas (1909) in terms of percent of digestible nitrogen from a test food which was retained by the human adult. Mitchell et al. (1945) brought the method to a greater state of precision by using the growing rat. Biological value is a direct measure of the proportion of dietary protein which can be utilized by body tissues and may be defined as the percentage of the absorbed nitrogen which is retained by the animal. A balance trial is conducted in which nitrogen intake and urinary and fecal excretions of nitrogen are measured. The results are used to calculate the biological value as follows:

$$BV = \frac{\text{N intake-(Fecal N + Urinary N)}}{\text{N intake-Fecal N}} \times 100$$

The metabolic fecal nitrogen and the endogenous urinary nitrogen are not directly derived from immediate dietary nitrogen. The existence in both feces and urine of nitrogen fractions, the excretion of which is independent of dietary nitrogen, is most convincingly demonstrated by the fact that some nitrogen is excreted when the animal is given a nitrogen-free diet. The revised formula is

$$BV = \frac{\text{N intake-(Fecal N-MFN)-(Urinary N-EUN)}}{\text{N intake-(Fecal N-MFN)}} \times 100$$

where MFN = metabolic fecal nitrogen and EUN = endogenous urinary nitrogen.

In determining biological value, the greatest amount possible of the dietary protein should be provided by the protein under test. Protein intake must be sufficient to allow adequate nitrogen retention, but must not be in excess of that required for maximum retention; if the latter level were exceeded, the resulting general amino acid catabolism would depress the estimate of biological value. For this reason, sufficient non-nitrogenous nutrients must be given to prevent catabolism of protein to provide energy.

The amino acid mixture absorbed by the animal is required for the synthesis of body proteins. The efficiency with which this synthesis can be effected depends partly on how closely the amino acid proportions of the absorbed mixture resemble those of body proteins, and partly on the extent to which these proportions can be modified. The biological value of a food protein depends upon the number and kind of amino acids present in the molecule. Since essential amino acids cannot be effecrively synthesized in the animal body, an imbalance of these in the diet leads to a wastage. Food protein with a deficiency of any particular essential amino acid will tend to have a low biological value.

Since biological value is dependent primarily upon essential amino acid make-up, it would seem logical to assess the nutritive value of a protein by a quantitative estimation of its essential amino acid constitution and then compare this with the known amino acid requirement of a particular class of animal.

To overcome the economic problems associated with biological methods for describing protein quality, two schemes have been proposed, both of which arrive at a numerical value for quality by considering the relative amount of the amino acids present in the protein as determined by chemical (or in some cases biological) analysis.

Block and Mitchell (1946) suggested that poor protein quality was caused primarily by a relative shortage of some one essential amino acid. They took the composition of whole egg protein as the standard or ideal, and determined the percentage by which the amino acid in greatest deficit was considered limiting. The complement of the percentage deficit of this amino acid was the chemical score given to that protein. Such values correlate well with the biological values for the rat and human beings but not for poultry. They are useful for grouping proteins into categories, but suffer a serious disadvantage in that no account is taken of the deficiencies of amino acids other than the amino acid in greatest deficit.

Oser (1951), although approving the general principle of chemical score as outlined by Block and Mitchell (1946), indicated that all essential amino acids should be considered rather than the one which is most deficient with respect to some standard. He developed the essential amino acid index which may be defined as the geometric mean of the essential amino acids when each is expressed as a percentage of the level of the same amino acid in egg protein and is calculated as

$$\text{EAAI} = \sqrt[n]{\frac{100a}{ae} + \frac{100b}{be} + \frac{100c}{ce} + \qquad + \frac{100j}{je}},$$

where a, b, c, ... j = percentage of the essential amino acids in the food protein, ae, be, ce, ... je = percentage of the same essential amino acids in egg protein, and n = the number of amino acids entering into the calculation.

The index has the advantage of predicting the effects of supplementation in combinations of proteins. It has the disadvantage that proteins of very different amino acid composition may have the same or a very similar index.

E. THE USE OF AMINO ACIDS IN PIG NUTRITION

In the 1930's the ten amino acids essential for promoting normal growth in the rat were verified (Rose 1938), and in the 1940's the quantitative requirements of the 12 amino acids needed in chick growth were virtually established. The first serious attempt to find the requirements for amino acids in pig nutrition was probably made by Beeson _et al_. (1948, 1949), who used a purified ration in combination with hydrolyzed fish protein for young pigs. The ration was practically tryptophan-free (0.01%). Addition of 0.4% DL-tryptophan caused a marked response in growth rate, as the control pigs had no weight gain and pigs fed tryptophan gained 634 g a day during a four-week period. This clearly demonstrates the significance of tryptophan in the ration of the growing pig. Work was continued by Beeson and his associates at Purdue. More precise than the first attempt was the next experiment, where graded levels of DL-tryptophan from 0.1 to 0.4 were added to the basal diet containing 0.01% tryptophan (Shelton 1951a). In this case zein and gelatin were used in the basal diet, which contained 24.5% protein. Although there were only two pigs per treatment, the results were very consistant, 0.2% DL-tryptophan being sufficient for normal growth. At the same time as the Purdue group (Beeson et al. 1948, Mertz et al. 1949; Beeson et al. 1953, Mertz et al. 1955) were investigating the requirements for other amino acids, Loosli and his co-workers at Cornell (Bell et al. 1950; Brinegar et al. 1950a and 1950b; Kroening et al. 1962) performed extensive studies along the same lines. The third group of workers that has done much work in evaluating the quantitative needs of amino acids in the growing pigs, is Becker and his associates at Illinois (Becker et al. 1955; Becker, 1959 and Becker et al. 1963). Several other scientist have also carried out such studies. In recent years, for instance, comprehensive experiments have been undertaken at Cambridge (Evans, 1958, 1960, 1962, 1963) and in Copenhagen (Clausen et al. 1959-1964).

Most experiments have been done on weanling pigs (eight weeks old), but suckling pigs have also been used to some extent. The method frequently used is to make up a basal ration of purified carbohydrate-rich ingredients plus a protein that is deficient in one or more of the naturally occurring amino acids. It has not been too difficult to find protein low in tryptophan (hydrolyzed feather meal, zein, gelatin), lysine (zein, linseed meal, wheat protein), isoleucine (blood flour), methionine (expeller soybean meal) and threonine (isolated soybean protein, rice protein). If the protein used is a poor source of other amino acids in addition to the one in question, a proper supplement of the others must be given. The scarcity of proteins deficient in the amino acids not mentioned above has limited the use of this technique.

Consequently, a diet with all ten essential amino acids (for the rat) as the sole source of nitrogen, except ammonium citrate, was successfully developed as early as 1950 by Shelton et al. for weanling pigs. Experiments with addition of one or more amino acids to practical type diets have also been beneficial to our present knowledge of the quantitative requirement of the different amino acids (Clausen et al. 1959-1964). Another approach to the problem was proposed by Curtin et al.(1952c). This was based on amino acid composition of the carcasses of the pigs. The lysine requirement, established earlier, was used as the basis while the requirement of the other essential amino acids was established in proportion to the content of lysine in the body. Studying the amino acid content of pigs at various ages, Durtin et al. (1952c) found a surprisingly constant level of the individual amino acids, when they were expressed in percentages of the total protein in the body. In 1952 Mertz et al. concluded, on the basis of their own experiments as well as those of others, that the growing pig, like the growing rat, needs ten amino acids. The pig also resembles the rat in the ability to synthesize part, but not all of the arginine required for normal growth and performance.

F. AMINO ACID REQUIREMENT FOR GROWING-FINISHING PIGS

It has been known for many years that the protein requirement of animals can be stated more precisely in terms of amino acid requirements. Pigs require for normal growth ten essential amino acids. Also they should be given additional nitrogen so that their bodies can synthesize the nonessential amino acids. Amino acid needs increase as protein level and caloric density increase (Bowland 1962; Becker et al. 1963; VanLoen 1966). Hence, some calculations and interpolations are required (Becker et al. 1963; VanLoen 1966).

a. The Requirement for Lysine.

Mertz et al. (1949) were the first to offer experimental evidence that lysine was an essential factor in the growing pig. Brinegar et al. (1949) concluded that 0.58% lysine was the requirement of weanling pigs when the protein content of the ration was 10.6%. In later studies (Brinegar et al. 1950a,b), a basal ration containing 22% protein was fed to weanling pigs and the growth data showed a requirement of 1.20% lysine. According to these studies there seemed to exist a proportionality between the lysine requirement of 5.5% - 5.7% of the dietary protein when the protein content ranged from 10.6 - 22%, meaning that the growing animal's lysine requirement could be stated as a percentage of the total dietary protein. McWard et al. (1959) found that the requirement of lysine in a 12.8% protein diet was 0.71% and in the 21.7% protein ration, 0.95%.

Change et al. (1958) failed to achieve consistent results. In one experiment the lysine requirement, expressed as a percentage of the diet, was not influenced by the protein level, while in a second experiment they found a lysine requirement of 0.7% when the ration contained 10 or 15% protein, and 0.9% with a ration of 20% protein. Becker (1959) arrived at the conclusion, based on chick and pig experiments, that the requirement of each amino acid is linear, but not proportionally related to the protein content of the ration. His recommended allowances of amino acids in the nutrition of weanling pigs are given for seven different protein levels, varying from 12 to 24% protein in the dry ration. The need for each amino acid, expressed as a percentage of the total dietary protein, is assumed to decrease by a certain percentage as the protein level of ration is increased. Most of the lysine work mentioned above points toward a requirement in weanling pigs of about 0.6 - 0.7% of the ration when the total dietary protein content is 12 - 15%. Becker (1963) recommends 0.74% lysine for weanling pigs fed a 16% protein ration.

It is suggested by the Illinois workers that caloric density of the ration may also influence the amino acid requirement. This can partly be hidden by the fact that the pigs eat more of a diet low in calories than of a diet of high caloric density (McWard et al. 1959). This point has earlier been stressed by Sewell et al. (1956) and Abernathy et al. (1958), but is hardly clear enough to justify a definite conclusion. Bell et al. (1968) concluded that the lysine requirement for 23 to 57 kg pigs fed a ration containing 3,330 kcal. digestible energy/kg was about 0.7%. Evidence concerning the lysine and protein requirement of finishing pigs (57 - 90 kg approximately) likewise remains controversial. Bell (1965) demonstrated that by increasing the dietary lysine level from 0.55 to 0.67% was as effective as increasing the protein level from 13 to 16%, when the response criteria were growth rate and efficiency of feed utilization. The reverse was the case when indices of lean meat content were assessed.

The British A.R.C. publication (1967) suggested that young pigs would give satisfactory performance if, up to about 50 kg liveweight, the diet contains about 0.75 - 0.8% lysine and thereafter about 0.6 - 0.65% (0.9 - 0.95 and 0.7 - 0.75% of the dry matter in the diet respectively) compared with the N.A.S. - N.R.C. (1968) recommendation of 0.7% to 20 - 35 kg pigs and 0.5% for finishing pigs.

b. The Requirement for Methionine.

Bell et al. (1950) demonstrated the need for methionine in swine rations. Curtin (1952a, 1952b, 1952c) using soybean plus purified diets containing 22% protein as feed for weanling pigs, found no improvement in growth rate, feed conversion or nitrogen balance by adding DL-methionine. He stated that the requirement did not exceed 0.31% methionine, when the cystine content was 0.38%. Shelton (1951a) used oxidized casein, gelatin and tryptophan as amino acid sources in a purified diet to which an animal-protein-factor-supplement, liver extract, and choline were also added. Using weanling pigs in growth studies, they concluded that the methionine requirement in the absence of cystine amounted to 0.60%, but the need of S-containing amino acids could also be met by using 0.30% methionine plus 0.30% cystine. Using weanling pigs, graded levels of DL-methionine and L-cytine were added to a purified ration containing 12% isolated soybean protein. Becker et al. (1955) concluded that a level of 0.25% methionine in the presence of 0.17% cystine supported a satisfactory rate of growth. Expressed as a percentage of the dietary protein, the combined methionine + cystine requirment was calculated to be 3.33%. Cystine can apparently provide about 40% of the need for S-bearing amino acids.

A considerable proportion of the trials conducted to determine the methionine requirement of pigs have been carried out with corn-soybean meal rations. However, the method for extracting the oil meal is relevant as the heat treatment will influence protein quality (Becker et al. 1953). This may at least be one of the explanations for the experiments on supplementation of methionine to soybean meal rations having failed to give consistent results. There may be other factors. Berry et al. (1962) have shown that methionine is definitely the first limiting amino acid in soybean meal, as might be expected from the amino acid composition. In their experiment with soybean meal added to a purified ration, a significant growth-promoting value was found by adding methionine. As the grains are also relatively poor sources of methionine, one could expect a response in the growth rate of pigs when adding methionine to a grain-soybean meal ration. Experimental evidence for this has been produced by some workers (Bell et al. 1950; Pfander et al. 1953; Bayley et al. 1968), while others have reported no growth response (Catron et al. 1953; Sewell et al. 1958; Arcker et al. 1959; Change et al. 1960; Beames et al. 1969; Oestemer et al. 1970).

Other rather commonly used protein feeds, such as peanut meal, cottonseed meal and sesame meal, have about the same methionine content as soybean meal. In some cases (Whitehair et al. 1952), addition of DL-methionine to rations containing these meals as protein concentrates has improved feed utilization.

Becker et al. (1966) indicated a requirement of 0.50% methionine + cystine for growing pigs with an initial weight of 20 kg fed a diet containing 16% protein; they also indicated that cystine could supply 40% of the total need for the sulfur bearing amino acids. The N.A.S.-N.R.C. (1968) indicated a methionine requirement of 0.50% of the diet for pigs in the weight range 20 - 30 kg. Oestemer et al. (1970) suggested that the methionine + cystine requirement of growing pigs from 21 to about 40 kg was somewhat less than the figure of 0.42% to 0.50% of the diet as reported by investigators previous cited. These trials were conducted in a series of experiments with growing swine to determine the capacity of opaque-2 corn to provide adequate dietary methionine. The corn rations (crude protein 10.85%) contained 0.275, 0.279, 0.227% methionine + cystine. Neither rate of gain, gain/feed nor protein evaluation ratio (PER) was significantly improved by supplementing the basal corn diet with 0.07, 0.14, 0.21 or 0.28% DL-methionine.

Keith et al. (1972) indicated that when Yorkshire gilts averaging 18 kg body weight were fed a semipurified diet containing six graded levels of methionine during six 4-day feeding periods, plotting serum methionine concentration against dietary methionine intake showed that the methionine requirement was 0.46% of the ration. This estimate was substantiated by animal performance data (protein 18.39%).

Hill (1965) has pointed out that methionine and cystine are largely destroyed by hydrochloric acid during acid hydrolysis and must, therefore, be converted to stable derivatives. If methionine and cystine were under-estimated in the basal ration or cereal diet as the result of destruction by acid, their calculated requirement would be variable. Therefore, the method used in determining the amino acid contents in various experiments should be taken into account when interpreting results.

c. The Requirement of Tryptophan.

Beeson et al. (1948, 1949) were the first to investigate the need for tryptophan. Shelton et al. (1951b) working with weanling pigs, used a purified 24.5% protein ration containing zein and gelatin plus some amino acids. They concluded that the growing pig needed 0.2% tryptophan in the diet or 0.8% of the total dietary protein. By adding graded levels to a basal ration with 0.09% tryptophan (17% protein), the Purdue workers found an optimum supplement of 0.06% DL-tryptophan. The requirement was set at 0.12% L-tryptophan, considering L-tryptophan as the only form to be utilized. The finding that tryptophan can be converted into niacin in pigs (Luecke et al. 1946) and confirmed by Powick et al. (1948) is considered important in corn rations, as the corn is a very poor source of available niacin. Becker et al. (1954a) suggested 0.13% tryptophan to be adequate for weanling pigs. This agrees with nitrogen balance studies made by Meade (1956), starting with pigs of 32 kg liveweight and using a 15.9% protein corn-soybean meal ration. In this trial niacin was added to the basal ration.

The A.R.C. (1967) suggests that 0.15% to 0.20% L-tryptophan in the dry matter in the diet should be adequate in the presence of adequate amounts of nicotinic acid. The N.A.S.-N.R.C. (1968) recommends that 0.13% tryptophan is adequate for growing pigs weighing 20 - 35 kg and that the level can be reduced to 0.09% for finishing pigs.

d. The Requirement for Threonine.

The threonine requirement has been studied in weanling pigs by Shelton et al. (1950c), and in suckling pigs by Sewell et al. (1952), using purified or semi-purified diets including casein and washed isolated soybean protein. In a report from Illinois (Berry et al. 1962) it is suggested that the protein in soybean meal has threonine as the third limiting amino acid (after methionine and lysine). Experimental evidence (Berry et al. 1962) with rats and pigs indicates that threonine might be second limiting and lysine third. Beeson et al. (1953) gave young pigs a diet based on corn and including nine essential amino acids. The diet provided 13.2% protein and 0.2% threonine. When a supplement of L-threonine was given, maximum weight gain and best feed conversion efficiency were with 0.4% dietary threonine (3.0% of the dietary crude protein). Mertz et al. (1952) also estimated the requirement of L-threonine to be 0.4% of the diet. With providing 12% C.P. based on dried skim milk, Becker et al. (1954b) calculated that 0.61% L-threonine was a satisfactory dietary concentration. These workers calculated the threonine content of a diet which had produced satisfactory growth, so that their estimate of requirement might well be excessive.

In a similar way, Evans (1958) calculated the threonine content of diets which had been proven to give satisfactory performance, namely diets containing either 7% fish meal, 20% ground nut meal, or 15% soybean meal, to be 0.52, 0.55 and 0.55% respectively. In further experimental work, the same author (1963) gave a diet containing 6% soybean meal, supplemented with lysine and methionine which provided 0.43% threonine to pigs up to 36 kg liveweight and obtained a significant response in terms of growth rate and feed conversion efficiency when a supplement of 0.15% L-threonine was added.

Robinson et al. (1963) fed an all-cereal diet supplemented with lysine and methionine, and providing 0.46% threonine was supplemented with isoleucine, threonine and tryptophan and a significant improvement in performance was noted. An appraisal of these data, however, indicates that the response was more probably due to the isoleucine and/or the tryptophan, since these amino acids were more severely deficient with respect to assumed requirements.

The A.R.C. (1967) suggests that the requirement for L-threonine for weanling pigs is about 0.45 - 0.5% of the ration (0.5 - 0.6% of the dry matter in the ration). This compares well with the level of 0.45% for growing pigs given by the N.A.S.-N.R.C. (1968).

e. The Requirement for Isoleucine.

Brinegar et al. (1950c) fed a semi-purified diet of starch, glucose, blood flour and DL-methionine to growing pigs. Maximum weight gain (630 g/day) was obtained when L-isoleucine was added to give a level of 0.7% of the ration equal to 3.2% of the total dietary protein. Becker et al. (1963) reported that pigs weighing 5 kg required 0.76% dietary isoleucine, 3.45% of the dietary protein, while pigs weighing 45 kg required 0.35% dietary isoleucine, 2.63% of the dietary protein.

Bravo et al. (1970) reported that pigs weighing from 20 - 40 kg require from 0.27 to 0.32% isoleucine in the diet when average daily gain, feed conversion ratio and levels of plasma free isoleucine were the response criteria. Using practical type rations, Evans (1962) found no improvement either in growth or nitrogen retention from the addition of isoleucine to a diet containing 0.59% isoleucine.

The A.R.C. (1967) suggests that 0.65% L-isoleucine in the diet (0.75% of the dry matter in the diet) is adequate for pigs between 14 and 45 kg liveweight and that the requirement might well be less for pigs of over 45 kg liveweight. This compares with a recommendation of 0.5% for pigs weighing 20 - 35 kg by N.A.S.-N.R.C. (1968).

G. SUPPLEMENTATION WITH AMINO ACID OF GRAIN-PROTEIN CONCENTRATE RATIONS AND GRAIN ONLY.

For pig diets normally based on cereals, protein concentrates are added to increase the total protein content and to counteract any essential amino acid deficiencies in the basal ration. The importance of the lysine content of the ration is well established and there are many examples in the literature showing the improvement in performance that can be brought about by lysine supplementation of rations containing poor-quality protein (Evans 1960; Jones et al. 1962; Rozman et al. 1968; Ostrowski 1969; Braude et al. 1972). It has also been reported that a small improvement in performance can be obtained when lysine is added to diets containing white fish meal, but economic appraisal of the results showed that this was not a viable proposition at prevailing prices (Braude and Lerman 1970). However, the greatest use of lysine and other amino acids as supplements may lie in the possible replacement of protein concentrates, since the latter are by far the most expensive dietary components. It has been reported that the feeding of cereal mixtures supplemented with lysine, threonine, tryptophan, methionine and in some experiments also isoleucine, results in performance equal to that obtained with rations containing protein concentrates (Robinson and Lewis 1963; Müller et al. 1967b).

Weanling pigs were fed a cereal diet with different supplements of amino acids. The cereal diet consisted of wheat, barley and oats. The trial was conducted from 14 - 18 kg body weight to 50 kg body weight. The combination of lysine and threonine had a marked effect upon gains and feed conversion. Supplementing with lysine alone enhanced gains in one case by 19% and in another case by 21%. The combination of lysine and threonine increased gains by 59% in the first trial and by 92% in the second trial. Feed conversion improved by 31 - 34%. In the combination of lysine and tryptophan, 0.02% added tryptophan showed a positive influence, whereas a tryptophan addition of 0.04% caused a depression in all trials. The supplement of lysine alone increased weight gain by 26 - 28%, whereas the combination of lysine and tryptophan increased gain by 35 - 42%. In trials with a combination of two methionine levels and lysine, a slight increase was recorded in comparison with a supplement of lysine alone. However, differences were not statistically significant. A supplement of 0.03% methionine gave better results than a supplement of 0.05% methionine (Müller et al. 1967a). It appears that threonine and tryptophan are both equal as the second limiting amino acids.

Since the report by Jensen et al. (1965) on grain plus amino acid diets, there has been considerable effort put into finding nutritionally adequate and econimical rations using these two ingredients as the major components. From a series of papers (Müller and Malék 1967a, 1967b, 1967c; Müller et al. 1967a, 1967b, 1967c; Müller and Rozman 1968; Rozman et al. 1968) it is evident that Czechoslovakian scientists are greatly interested in this type of ration.

In the paper by Jensen et al. (1965) it was reasoned on the basis of the University of Illinois feeding standards (Becker et al. 1963) that lysine and possibly methionine were all that were required as a supplement to sorghum for pigs from 45 to 90 kg body weight, whereas lysine and tryptophan were the required amino acids to supplement corn. Their results showed a growth response to the addition of 0.25% lysine to sorghum grain but no response to the further addition of methionine. In a similar experiment reported by Beames et al. (1968) up to 0.2% lysine improved growth rate and feed efficiency of fattening pigs, but the improvement in carcass quality was slight. Ericson et al. (1962) also improved growth rate and feed efficiency in a wheat barley-amino acid ration fed from 32 kg body weight to 90 kg with lysine added to the grain to a level of 0.86% of the ration.

In the supplementation of several grain-protein concentrate rations with amino acids, responses have been obtained to incremental increases of lysine up to levels in excess of most of the suggested requirements (Ericson et al. 1962), but even though such supplemented diets have often given sub-optimal performance, the further addition of methionine generally gives no response (Jensen et al. 1965; Beames and Pepper 1969). Optimal growth and feed efficiency, however, were obtained by Müller and

Rozman (1968) with maize, barley and wheat when each was supplemented with lysine (0.41 - 0.61%), threonine (0.1 - 0.27%), tryptophan (0.04 - 0.15%) and methionine (0.14 - 0.25%) from weanling pigs of 30 kg body weight and with reduced levels thereafter.

Cystine can satisfy 40 percent of the need for methionine (N.A.S.-N.R.C. 1968). Although the methionine + cystine requirements of growing pigs are widely taken as being 0.5 - 0.6% of the ration, there are some experiments which indicate that 0.3 - 0.42% is adequate for optimal growth (Beames and Pepper 1969; Oestemer et al. 1970). It could be suggested that lack of response to methionine supplementation under some circumstances was due to other factors in the ration being limiting. However, the result of Oestemer et al. (1970) would tend to refute this as they obtained good performance with a total methionine + cystine level of 0.23 - 0.28% of the ration.

Berry et al. (1966), using corn-soybean and corn-sugar-soybean diets, found that the response to supplementation with lysine and methionine depended on the addition of threonine, indicating that this amino acid is limiting in low protein, corn-soybean meal diets.

Pecora and Hundley (1951) obtained a large improvement in growth rate of rats fed rice by supplementing the diet with lysine and threonine. Sure (1954) demonstrated that wheat and rye were deficient in lysine and threonine and further, that rye was deficient in valine. Pond et al. (1958) indicated that lysine and threonine were probably the most limiting amino acids in milo for growth and that the liver fat content of rats receiving the basal ration was significantly reduced by addition of 0.5% L-lysine and 0.2% DL-threonine. Rosenberg et al. (1959) established lysine and threonine as the first and second limiting amino acids in rice protein for the growth of the weanling rat. Methionine, isoleucine and tryptophan were next limiting amino acids in this order.

Pick et al. (1971) showed that excellent daily gain, gain/feed and protein efficiency ratio (nitrogen retention) could be obtained when a diet containing 89.5% opaque-2 was supplemented with 0.1% L-isoleucine, 0.18 to 0.35% L-lysine, 0.2% DL-methionine, 0.12% L-phenylalanine, 0.14% L-threonine and 0.08% L-valine. Final dietary levels of 0.36% isoleucine, 0.7% lysine or less, 0.40% methionine + cystine, 0.59 to 0.65% phenylalanine + tyrosine, 0.43% threonine and 0.54% valine appeared to be adequate for the growing rat.

H. FACTORS AFFECTING AMINO ACIDS SUPPLEMENTATION OF FEEDS

a. Energy Content of the Ration.

It has long been realized that a protein can be utilized at maximum efficiency only if there is sufficient energy in the diet from nonprotein sources to satisfy the requirement of the organism for calories.

That the available energy in a diet can be of critical importance for the successful amino acid supplementation of animal feeds was not realized until a direct relationship between the energy and protein content was demonstrated in broiler feeds. The system for expressing the energy value is that digestible or metabolizable energy are used almost universally for pigs and poultry. When protein-energy relationships are considered, ideally only the completely balanced portion of the protein should be calculated as protein and the rest as energy. Using a corn-soybean meal diet (Rosenberg et al. 1955) little or no response was obtained in the chick when a small amount of methionine was added to the ration in spite of the fact that the first limiting amino acid in the diet was considered to be methionine. However, when fat was added to the diet the chicks responded to the supplemental dietary methionine with improved growth and feed efficiency. This effect was also shown when carbohydrates were used to replace the fat (Baldini and Rosenberg 1957). These studies suggest a direct relation between caloric density and the level of amino acids necessary in the diet.

The combined results of two such experiments (Baldini and Rosenberg 1955; Baldini and Rosenberg 1957) indicated that three different calorie levels, 1960, 1985 and 2205 calories of productive energy per kg, required different levels of methionine for optimum performance of chicks. In poultry, the energy-methionine relationship has been explained on the basis that birds eat primarily to satisfy their energy requirements, although their food intake is governed also by a number of other factors including protein concentration and composition of the protein. When the energy content of a diet is increased, birds eat less per unit of gain. An increase in energy content of the diet results, therefore, in an increase in the efficiency of feed utilization. An increase of the fat content of the diet increases the caloric density and may cause a drop in food intake. If protein content is not increased in a ration with added fat, the level of protein per calorie is decreased, resulting in a fall in protein intake even though the calorie intake may be unchanged. In broiler rations containing different levels of added fat, this question is an important one, and has been stressed by Waddel (1959). Baldini et al. (1957), Rosenberg and Culik (1955), and Williams and Grau (1956) showed that the caloric density is one of the factors governing the amino acid requirements of growing turkeys, rats and chicks.

Sewell et al. (1956), Abernathy et al. (1958) and McWard et al. (1959) observed that pigs eat more of a diet low in calories than of a diet of high calroic density. Whether or not all of the supplemented amino acids - lysine, threonine, methionine, etc. - needed to effect amino acid balance can be utilized will depend largely upon the energy content of the diet. This concept of amino acid utilization, of course, does affect the way in which the amino acid requirements are formulated, but naturally this calls for proper knowledge of the requirement of both essential amino acids and energy.

Henry (1968) found that ad libitum fed growing-finishing pigs achieved maximum energy intake on diets having 3.25 Mcal/kg digestible energy.

Robinson et al. (1964) concluded that the best growth performance and carcass lean content of pigs receiving a diet of low energy content was recorded when the ratio of Kcal D.E. to kg dietary lysine was approximately 3500. At the higher dietary energy level the best performance in terms of carcass quality was also recorded at a similar ratio of energy to lysine. Overall carcass quality was adversely affected by high energy levels in the finishing diets but was improved as the protein level was raised. The response of gilts to increasing protein and lysine levels in the diet continue beyond the level where the response in barrows creases. Robinson's experiment showed no significant sex differences in terms of liveweight gain or efficiency of feed utilization but gilts were much superior to barrows in terms of carcass quality. With pigs averaging 16 to 22 kg liveweight initially, the rate of gain and gain/feed ratio were influenced by the dietary lysine levels. There was no evidence of interaction between lysine level and energy level of the diet on rate of gain, but there was a significant interaction for gain feed ratio in one experiment. The calculated lysine needs, expressed as percentage of the diet, were 0.65, 0.77 and 0.80 for maximum rate of gain and 0.66, 0.71 and 0.85 for maximum feed efficiency in diets containing 2926, 3267 and 3718 kcal. ME. per kg respectively. These values were equivalent to an average lysine need in the diet equal to 0.23% per 1000 kcal. ME. (Mitchell et al.1965).

b. Protein Content of the Ration.

One of the major points of disagreement in establishing the amino acid requirement is whether it should be given as a percentage of the diet or as a percentage of the total dietary protein. Arguments have been produced for the validity of the concept of a certain percentage of the dietary protein at all levels of protein in the ration (Brinegar et al. 1950a) while other research work has produced results in disagreement with this finding (Hutchinson et al. 1957). Altogether, much evidence has been produced in favor of the concept that the requirement should be stated as a percentage of the diet, but in order to interpret these data it is necessary that the protein level of the diets be known.

Harper (1959), and Harper and Kumta (1959) are of the opinion that the increase in the requirement of an amino acid with increasing protein percentage is rather small and of doubtful significance over the normal range of protein levels. If the requirement of an amino acid is expressed in percentage of the total dietary protein, this percentage will decrease as the protein level of the ration is raised. Barnes and Bosshardt (1946) have emphasized that the ideal evaluation of protein should be made at the level of protein that gives maximum efficiency in converting food protein to body protein. This protein level will be somewhat lower than the protein allowance recommended for farm animals. The frequently used technique to test the supplementation by a certain amino acid of a practical type diet at two or three different protein levels around the recommended standard may be a useful means of detecting responses both as to protein level and amino acid supplementation at the same time

The report of Grau (1948) is frequently referred to in order to illustrate the connection between the requirements of lysine and protein. As the protein percentage of the ration was increased from 10 to 30, the calculated lysine requirement was doubled. Becker (1963) states that isoleucine and lysine requirements of pigs, expressed as a percentage of the total dietary protein, decreases with increasing protein level in the ration. Harper (1959) mentions that if there are two limiting factors such as protein and lysine, the lysine need will increase until protein, as such, becomes limiting. This is not actually an increase in the requirement. Harper (1959) adds; "Nevertheless, there does come a point at which it is possible to demonstrate that an adequate diet is made less adequate if the overall protein is increased while the level of one amino acid is held constant".

Boomgaardt and Baker (1970, 1971) indicated that the lysine and tryptophan requirements of young chicks are a constant percentage of dietary crude protein. Expressed as a percentage of diet, the requirement increased linearly as dietary crude protein level increased. It was established that the lysine requirement remained constant at 4.59% of the protein, and tryptophan requirement was 0.87% of the protein. These results with tryptophan and lysine do not support the generally-accepted concept that the requirement for an essentail amino acid decreases linearly as a percentage of the protein and increases curvilinearly as a percentage of the diet as dietary protein level increases from deficiency to adequacy. This experimentally established relationship may be expressed in a different manner when the ratio of calories to protein is held constant, the amount of amino acids required by the body for best performance and expressed as percent of protein remains relatively constant when the concentration of protein in the diet is increased. When the ratio of calories to protein is increased, the amino acid requirement as a percentage of protein increases also. When the ratio of calories to protein decreases, the amino acid requirement, as a percentage of protein, decreases (Rosenberg 1959).

The effect of change in protein level is a problem of considerable practical importance as feeds for domestic animals range from 10% protein for the fattening pig to 30% protein for the turkey poult (Rosenberg 1959). Percent protein in the diet is plotted against percent of amino acid in the protein for lysine, methionine, and the combination of methionine and cystine in corn-soy diets. As the level of protein is increased, the relative amount of lysine in the diet increases, while the relative amounts of the sulfur-bearing amino acids decrease. Therefore, as the protein level increases, methionine deficiency is likely to occur. Conversely, as the protein level decreases, lysine deficiency is likely to occur (Rosenberg et al. 1959). Lawrence (1971) indicated that barley diets, compared with maize diets, gave better growth rates and energetic conversion efficiencies, lighter carcasses, lower killing out percentage and smaller eye muscles. The narrow calorie protein/lysine (CPL) ratio diets[1] when compared with wide CPL ratio diets[2] gave better growth rates and energetic conversion efficiencies, lighter cold carcass weights, smaller backfat deposits and greater percentage of lean and bone but smaller percentage of fat in the carcass.

1. Digestible energy: crude protein = 198:1, Crude protein: lysine = 19.8:1.

2. Digestible energy: crude protein = 263:1 or 273:1, crude protein: lysine = 27.6:1 or 26.9:1.

c. Age and Sex of the Species Consuming the Rations.

The unique functions in the animal body served by the amino acids resulting from protein digestion are all anabolic in character. They relate to the replacement of essential tissue constituents that have been degraded in catabolic reactions, or to the formation of new tissue constituents in growth. In the rapidly growing animal, the latter functions dominate the body's requirements for amino acids. In the mature animal, the replacement functions may dominate the amino acid requirements, but the growth functions still persist, since some tissues continue to grow throughout life (Mitchell 1949). Among adult animals of different species, the relative importance of the growth functions in determinging amino acid requirement will depend mainly on the rate of growth of the epidermal structures, such as hair, nails and claws.

Species differences in amino acid requirements during growth would be expected not so much on the basis of differences in the nature of the anabolic reactions, as on the basis of differences in the rate of growth of new tissue in comparison with the weight of tissue to be maintained, that is, the percentage rate of growth. The higher the percentage growth rate, the greater the extent to which the growth of new tissue dominates the total amino acid requirements (Mitchell 1959). It seems clear that as far as growth rate and feed conversion efficiency in pigs are concerned, the crude protein requirements as a percentage of the diet falls with increasing liveweight. Considering the period up to about 45 - 50 kg liveweight, the requirement as a percentage of the dry matter in the diet appears to be about 16 - 17% crude protein, and from 50 kg to 90 kg liveweight about 13 - 14% crude protein. A.R.C. (1967) indicated that the presence of antibiotics reduced the requirement for crude protein, at least as far as growth rate is concerned.

Less attention has been paid to a difference between sexes with respect to their amino acid requirements. Actually, there is a difference although this is relatively small. Maximum growth was obtained with about 0.65% lysine in the diet of male rats while only about 0.57% lysine was needed for maximum growth of the females. Interestingly enough, this difference in the requirement disappears if the amount of lysine required per gram of gain is calculated (Rosenberg and Rohdenberg, 1952). A similar difference has been observed in the growing chick (Rosenber 1959). Accordingly, it has been proposed to feed male and female broilers and poults separate diets.

Bayley et al. (1968) indicated that there was a signficiant interaction between the strain of pig and protein level. Lacombe and Yorkshire pigs responded to an increase in the dietary protein level from 13 to 16% by increasing gain, whereas the crossbred and Landrace pigs did not.

Smith et al. (1967) showed that gilts were more responsive to an improvement in the level and type of protein than barrows, while Bayley et al. (1968) indicated that boars were even more responsive than gilts to the adequacy of the protein content of the diet. Robinson et al. (1964) indicated that the response of gilts continued with increasing protein and lysine levels in the diet while that of barrows ceased at a much lower level. This will influence the level of lysine or other amino acids to be recommended and will be different for barrows and gilts. Robinson (1964) therefore concluded no significant sex differences in terms of liveweight gain or efficiency of feed utilization but gilts were superior to barrows in terms of carcass quality.

III. EXPERIMENT I

A. EXPERIMENTAL PROCEDURE

a. General.

Protein is one of the more costly nutrients in practical swine rations. Barley is the most common energy source for Western Canadian swine growers, while soybean meal is widely used as a protein source. Consequently, swine rations generally include these two feedstuffs as major ingredients. In this experiment, barley and soybean meal were the sole major ingredients in the control ration.

Swine do not require protein per se but require the contained amino acids for the synthesis of body tissue. The essential amino acids for optimum growth of swine which are generally deficient in barley according to N.A.S.-N.R.C. (1968) recommendations are lysine, threonine, methionine and isoleucine. The following experiment was designed to study the effect of supplementation of Peace River barley with two levels of lysine in combination with threonine, methionine and isoleucine on the performance of growing-finishing pigs.

The experimental design consisted of six treatments with three replications. Each of the six treatments within replicates comprised a pen of six pigs (three male castrates and three females) which were group-fed.

b. Animals.

A total of 108 Yorkshire and Yorkshire x Landrace pigs were used in this experiment. Pigs were commenced on trial at an average body weight of 16.9 kg, weighed weekly and sent for slaughter at a body weight of 84 kg or over. The two last pigs in the pen were withdrawn from trial when the body weight of the heavier one exceeded 84 kg. Rations and pens were allocated on a random basis.

c. Rations.

The ingredients for the six rations are shown in Table I. The trial consisted of six treatments as follows:

1. Barley-soybean meal to supply 0.75% total lysine (control ration)
2. Barley + .69% L-lysine HCl to supply 0.90% total lysine
3. Barley + .50% L-lysine HCl to supply 0.75% total lysine
4. Barley + .50% L-lysine HCl to supply 0.75% total lysine + 0.05% L-threonine
5. Barley + .50% L-lysine HCl to supply 0.75% total lysine + 0.05% L-threonine + 0.10% DL-methionine
6. Barley + .50% L-lysine HCl to supply 0.75% total lysine + 0.05% L-threonine + 0.10% DL-methionine + 0.10% L-isoleucine.

The amino acid content and chemical composition of the barley and the soybean meal are shown in Table II. The amino acid composition of each ration is listed in Table III. All rations were computed to contain adequate amounts of all nutrients known to be required for growing-finishing pigs except total protein and amino acid content.

d. Management.

(i) Housing.

The experiment was conducted at the Swine Research Unit, the University of British Columbia. The building was insulated, with air exhaust fans fitted with thermostatic controls set at 18.5°C. The housing consisted of concrete floored partially slatted pens with an area per pig of 1.2 m^2.

(ii) Feeding and Watering.

Pigs were fed their rations twice daily at 8:00 a.m. and 1:00 p.m. Feeding was in troughs on an ad libitum basis with maximum allowance limited to 2.7 kg/day. Daily records of feed consumption were kept. Water was supplied ad libitum by nipple.

(iii) Feed Storage and Mixing.

All feed ingredients were purchased and stored prior to the commencement of the experiment to ensure a uniform ration composition throughout. The barley was stored in the whole form. As required, 450 kg batches of each ration were prepared by hammer milling the barley and then mixing it with the other ingredients in a vertical mixer* for 4 - 5 minutes. On the first replicate the grain was ground in a medium screen (10 mm diameter) but a fine screen (7 mm diameter) was used for replicates two and three to reduce scattering of feed which was a problem with the coarser grind.

e. Records.

All pigs were weighed initially and thereafter at weekly intervals throughout the experiment. Weekly feed consumption, corrected for spilt feed, was also recorded.

f. Chemical Analysis.

All feed was analyzed for moisture, ash, crude protein and ether

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* Kelly Duplex Mill Machinary, size 220, serial 57364. The Duplex Mill Mfg. Co., Springfield, Ohio.

extract according to A.O.A.C. methods (1960) and acid detergent fibre was determined according to VanSoest method (1963). Hydrolyzates of the barley and the soybean meal were analyzed for all essential amino acids except tryptophan on a Phoenix* model 7800 amino acid analyzer equipped with a VARIPUMP according to the method described by Piez and Morris (1960). The hydrolyzates were prepared by acid hydrolysis (Kohler et al. 1967) except for cystine, methionine and tryptophan analysis. Cystine was determined as cysteic acid and methionine as methionine sulfone by oxidative hydrolysis (moore 1963). The foregoing analysis was done because of the losses of methionine and cystine associated with acid hydrolysis. For tryptophan analysis, the barley were hydrolyzed with the enzyme pronase and tryptophan was determined by spectrophotometical method (Spies et al. 1968).

g. Carcass Measurements.

Dressed weight was measured on the hot carcass while the following measurements were made on the chilled carcass. "A", "B" and "c" measurements were taken according to the procedure of Buck et al. (1962). The other carcass measurements were obtained according to Canadian Record of Performance for Swine (1967).

Maximum shoulder fat

Minimum middle fat

Maximum loin fat

Eye muscle

Area

"A" measurement of loin eye (width)

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* Phoenix Precision Instrument Co., 3083-05 North 5 Street, Philadelphia, Pa. 19140, U.S.A.

"B" measurement of loin eye (depth)

"C" measurement of fat at cut not including skin, i.e., 40 mm from mid line

Carcass length

h. Calculations.

Average daily gain was determined as the b value in the equation derived from weekly body weights y = a + bx, where "y" is body weight, "a" is initial weight, and "x" days on trial. The average daily feed consumption and feed conversion ratio (total feed eaten divided by total body weight gain, both values expressed in the same units) were calculated for each pen. Carcass measurements were determined for each pig.

i. Statistical Analysis of Data.

The data were subjected to an analysis of variance using, least squares technique as shown by Harvey (1960) which is suitable for comparing unequal sub-groups and hence relevant in this experiment with missing values. Means from comparisons showing a significant "F" value were tested according to the multiple range test of Duncan (1955). Daily weight gain was analyzed with starting weight as the covariable. Dressing percentage, carcass length, maximum shoulder fat, mid backfat, maximum loin fat, backfat 40 mm from midline, loin area and loin area index were analyzed with dressed weight as the covariable.

Table I. Composition of rations used in experiments I and II (Air dry basis)

	Treatment					
Ingredients	1 %	2 %	3 %	4 %	5 %	6 %
Barley	83.10	96.05	96.24	96.19	96.09	95.99
Soybean meal	13.76	---	---	---	---	---
L-lysine HCl*	---	0.69	0.50	0.50	0.50	0.50
L-threonine*	---	---	---	0.05	0.05	0.05
DL-methionine*	---	---	---	---	0.102	0.102
L-isoleucine*	---	---	---	---	---	0.10
Defluorinated rock phosphate	1.49	1.64	1.64	1.64	1.64	1.64
Limestone	0.57	0.54	0.54	0.54	0.54	0.54
Iodized salt	0.5	0.5	0.5	0.5	0.5	0.5
Copper sulfate	0.078	0.078	0.078	0.078	0.078	0.078
Trace mineral and vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5

The trace mineral and vitamin premix provided the following per kg of ration: manganese 44 mg as $MnSO_4.H_2O$, Zinc 110 mg as $ZnSO_4.7H_2O$, B.H.T. 500 mg, Vitamin A 3085 I.U., Vitamin D_3 440 I.U., Vitamin B_{12} 20 µg, riboflavin 2.9 mg, niacin 11 mg, calcium pantothenate 11 mg (45% equivalent).

* The L-lysine HCl was fed grade (Ajinomoto Co., Japan) and was 98% pure, containing 78% L-lysine. The L-threonine and L-isoleucine were pure. The DL-methionine was 98% pure.

Table II. Content of essential amino acids and proximate constituents of barley and soybean meal on a dry matter basis.

Component	Concentration	
	Barley	Soybean Meal
Arginine (g/100g d.m.)	0.529	1.526
Histidine (g/100g d.m.)	0.219	1.383
Isoleucine (g/100g d.m.)	0.345	2.135
Leucine (g/100g d.m.)	0.696	3.600
Lysine (g/100g d.m.)	0.434	3.578
Methionine (g/100g d.m.)	0.141	0.632
(Cystine) (g/100g d.m.)	0.178	0.614
Phenylalanine (g/100g d.m.)	0.617	3.025
(Tyrosine) (g/100g d.m.)	0.301	1.636
Threonine (g/100g d.m.)	0.293	2.010
Tryptophan (g/100g d.m.)	0.210	0.900
Valine (g/100g d.m.)	0.848	4.245
Crude Protein (%) (N x 6.25)	10.50	53.78
Ash (%)	3.19	6.30
Crude fibre (%)	5.87	6.50
Crude fat (%)	3.39	3.95

Table III. Content of essential amino acids of each ration (g amino acid/100g mixed feed) on an air-dry basis.

	Ration					
Amino Acid	1	2	3	4	5	6
Arginine	0.56	0.44	0.44	0.44	0.44	0.44
Histidine	0.33	0.18	0.18	0.18	0.18	0.18
Isoleucine	0.51	0.28	0.28	0.28	0.28	0.39
Leucine	0.90	0.57	0.57	0.57	0.57	0.57
Lysine	0.75	0.90	0.75	0.75	0.75	0.75
Methionine	0.18	0.12	0.12	0.12	0.17	0.17
(Cystine)	0.20	0.15	0.15	0.15	0.15	0.15
Phyenylalanine	0.81	0.51	0.51	0.51	0.51	0.51
(Tyrosine)	0.31	0.25	0.25	0.25	0.25	0.25
Threonine	0.46	0.24	0.24	0.29	0.29	0.29
Tryptophan	0.27	0.17	0.17	0.17	0.17	0.17
Valine	1.13	0.70	0.70	0.70	0.70	0.70
Dry matter content %	89.95	88.85	89.12	88.93	89.16	89.36
Crude protein % (N x 6.25)	15.81	10.15	10.18	10.12	10.25	10.31

B. RESULTS

The experimental data are summerized in Table IV. There was a significant difference between amino acid supplemented rations and the control ration in feed efficiency and daily weight gain but not in carcass quality. Adding 0.05% L-threonine to the lower level of lysine significantly* improved daily gain and feed conversion ratio compared to the lower level of lysine. Further addition of 0.10% DL-methionine either alone or in combination with 0.10% L-isoleucine did not improve performance but gave significantly higher daily gain and a better feed conversion ratio than the lower level of lysine alone.

Average daily gain and feed efficiency for pigs fed the lower level of lysine were significantly lower than those fed the higher level of lysine. The lower level of lysine gave significantly less mid backfat than the higher level of lysine but there was no significant difference between the two levels of lysine in other carcass measurements. However, the trend showed that the lower level of lysine produced better carcass quality than the higher level.

The addition of 0.1% DL-methionine to the lysine and threonine supplemented ration reduced feed intake resulting in a lower daily gain but did not significantly affect feed conversion ratio.

The barley-soybean control ration gave a significantly higher weight gain, better feed conversion ratio and measurements indicating leaner carcasses than the barley-amino acid rations. There was no signficant difference between treatments in dressing percentage and maximum shoulder fat. The barley-soybean control ration produced less backfat, and larger

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* Where significance is indicated, it refers to significance at the 5% level.

eye muscle areas and indices.

The lower level of lysine and supplementation gave a significantly greater dressing percentage than the ration to which both lysine and threonine were added. The minimum mid fat measurement of pigs receiving the barley-soybean control ration was significantly less than that of pigs receiving the higher level of lysine or the lower level of lysine with threonine and methionine. The barley-soybean control ration produced less maximum loin fat and less backfat at 40 mm from the mid line than the higher level of lysine. The barley-soybean control ration produced a significantly greater eye muscle area and eye muscle index than other treatments except for the lower level of lysine. Adding threonine, methionine and isoleucine to the lower level of lysine did not affect backfat or eye muscle measurements.

Barrows gave significantly higher daily weight gain than gilts while gilts had consistently less backfat, higher dressing percentage and larger eye muscle area. Treatment and sex interaction was obtained for daily weight gain and for carcass quality. Gilts fed the higher level of lysine gave a significantly higher daily weight gain than those fed the lower level of lysine. Supplementation with lysine, lysine and threonine, and lysine, threonine, methionine and isoleucine tended to show higher daily weight gain for barrows than gilts but supplementation with lysine, threonine and methionine produced an adverse effect.

Table IV. Summary of the effect of the addition of amino acids to barley on body weight gain, feed consumption (D.M.), feed conversion ration (D.M.) and carcass measurements in feeding trial.

Treatment	1 Soy Control	2 0.9% lys	3 0.75% lys	4 lys + threo	5 lys+threo + meth	6 lys + threo +meth+isol	Results of Significant Tests *
Starting Wt. (kg)	16.83	16.63	17.37	17.28	16.40	16.92	
Final Wt. (kg)	83.20	82.83	80.53	84.53	82.34	84.05	
Mean daily gain (kg)	.609	.461	.413	.525	.498	.501	1 4 6 5 2 3
D.M. Feed(kg) / Wt. gain (kg)	2.954	3.533	3.790	3.320	3.300	3.330	1 5 4 6 2 3
Mean daily feed intake(kg)	1.777	1.601	1.552	1.739	1.609	1.653	1 4 6 5 2 3
Dressing %	78.36	78.67	79.46	78.05	78.44	78.52	3 2 6 5 1 4
Length cm	79.15	77.10	74.25	78.07	78.49	77.41	1 5 4 6 2 3
Max shoulder fat (mm)	37.0	38.5	37.4	37.5	38.7	38.1	5 2 6 4 3 1
Min middle fat (mm)	21.1	25.4	21.9	23.7	23.1	24.3	2 6 4 5 3 1
Max loin fat (mm)	29.7	33.5	31.1	32.8	32.4	31.3	2 4 5 6 3 1
Backfat (40 mm)(mm)	18.8	22.6	20.5	21.2	20.5	20.8	2 4 6 3 5 1
Eye muscle index A x B (mm^2)	3714	3312	3382	3415	3376	3286	1 4 3 5 2 6
Eye Muscle area (mm^2)	2718	2476	2532	2454	2439	2477	1 3 6 2 4 5
Experimental period (days	101.4	146.2	146.8	128.1	135.3	136.2	

* Treatment means not underscored by the same line are significantly different at 5% level of probability (Duncan, 1955).

Table V. Comparison of the effects of supplementation with amino acids on daily weight gains and carcass measurements of gilts and barrows.

	Barrows (S_1)	Gilts (S_2)	Significant F_1 Test
Daily Wt. Gain	517	486	**
Carcass Measurement			
Dressing %	78.01	79.16	**
Carcass length (cm)	77.99	76.83	N.S.
Max. shoulder fat (mm)	39.44	36.19	**
Min. middle fat (mm)	24.69	21.80	**
Max. loin fat (mm)	32.92	30.85	*
Backfat 40 mm from mid (mm)	22.44	18.99	**
Eye muscle index (mm^2)	3243	3586	**
Eye muscle area (mm^2)	2374	2658	**

Treatment and sex interaction

Treat. x Sex		Daily Wt. Gain (g)	Treat. x Sex		Daily Wt. Gain (g)
1.	T_1S_1	601	7.	T_1S_2	618
2.	T_2S_1	476	8.	T_2S_2	446
3.	T_3S_1	467	9.	T_3S_2	359
4.	T_4S_1	546	10.	T_4S_2	505
5.	T_5S_1	483	11.	T_5S_2	513
6.	T_6S_1	530	12.	T_6S_2	473

Duncan's test of Treat. x Sex effect on weight gain at 5% level of probability.

7	1	4	6	11	10	5	2	12	3	8	9
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* P < 0.05
** P < 0.01
Treatment means not underscored by the same line are significantly different.

C. DISCUSSION

It might be concluded from the results obtained in this feeding trial, that the growing-finishing pig could perform reasonably well on a basal ration of Peace River barley supplemented with lysine and threonine, providing additional vitamins and minerals were given. However, the weight gain and feed efficiency were somewhat less with the lysine and threonine supplemented ration than with the soybean supplemented control ration. The addition of methionine to the ration alone or in combination with isoleucine did not improve performance beyond that obtained with the additional lysine and threonine.

Mean values indicated a signficantly better performance with 0.90% total lysine than with 0.75% total lysine. Obtained by the addition of lysine alone was inferior to that obtained when other amino acids were also added, indicating that lysine was not the only limiting amino acid in the barley.

L-lysine supplementation to a high barley diet (75% barley meal, 23% wheat offal) for growing pigs has been shown elsewhere to improve performance significantly (Braude _et al_. 1972). This ration contained 0.57% total lysine compared with 0.45% lysine in the unsupplemented high barley ration, thus approximating the lysine content of a standard British barley meal-wheat offal ration supplemented with 7% white fish meal. Many workers (Evans 1960; Jones _et al_. 1962; Ericson _et al_. 1962; Ostrowski 1969) have confirmed that lysine supplementation to poor-quality protein rations improved performance.

Sure (1954) demonstrated that wheat and rye were deficient in lysine and threonine and further that rye was deficient in valine for the growth of rat. Pond _et al_. (1958) indicated that lysine and threonine were probably the most limiting amino acids in milo for the growing rat. Rosenberg et al. (1959) established lysine and threonine as the first and the second limiting amino acids in rice protein for the growth of the weanling rat. Berry et al. (1966) using a corn-soybean combination reported a response to supplementation with lysine and methionine that was dependent on the addition of threonine, indicating that this amino acid was limiting in low-protein corn soybean meal rations for weanling pigs. Müller et al. (1967a) showed that lysine and threonine supplementation to a basal cereal diet had an extraordinarily favourable effect upon gains and feed efficiency. The supplementation of lysine alone enhanced gain in one case by 19% and in another case by 22%. The combination of lysine and threonine increased gain by 59% in the first trial and by 92% in the second, and the feed efficiency was improved by 31 - 34% over that obtained with the unsupplemented control ration. They considered from the viewpoint of nutrition that lysine was the first, threonine the second, tryptophan the third and methionine the fourth limiting amino acid in the cereal diet for the growing pigs. The cereal consisted of wheat, barley and oats. In the present experiment, the mean daily gain and feed efficiency values obtained with the lower level of lysine were markedly improved when 0.05% L-threonine was added to the diet. This indicated that threonine was the second limiting amino acid in the basal ration of barley which was in agreement with Sure (1954), Pond et al. (1958), Rosenberg et al. (1959), Berry et al. (1966) and Müller et al. (1967a).

The experiment (Table III) showed that the basal ration contained insufficient lysine, methionine, threonine and isoleucine for growing pigs according to the N.A.S.-N.R.C. standards (1968) but that there was adequate tryptophan.

Although the methionine + cystine requirements of growing pigs are widely accepted as being 0.5 - 0.6% of the ration (A.R.C. 1967), there are some experiments which indicate that 0.3 - 0.42% is adequate for optimal growth (Beames and Pepper 1969, Oestemer et al. 1970). It could be suggested that the lack of response to methionine supplementation under some circumstances was due to other factors in the ration being limiting. However, the results of Oestemer et al. (1970) would tend to refute this because they obtained good performance with rations in which opaque-2 corn provided all the sulphur-containing amino acids. The corn contained 0.23 - 0.28% methionine + cystine. Neither the rate of gain, gain/feed nor protein efficiency ratio were significantly improved by supplementing the basal corn rations (Crude protein 10.85%) with 0.07, 0.14, 0.21 or 0.28% DL-methionine. Jensen et al. (1965) indicated that the addition 0.20 or 0.25% lysine to the approximately 8.0% crude protein milo provided a balance of essential amino acids which supported gains equal to that of finishing pigs fed a corn-soybean 12.0% crude protein diet. They were unable to obtain a response to 0.10% DL-methionine in addition to the lysine supplementation even though the methionine plus cystine content of the milo ration was only 0.25%. Müller et al. (1967c) showed that supplementing with methionine and lysine was not as effective as adding lysine alone to cereal rations containing 0.31% methionine plus cystine, and that the enrichment of cereal diets with the combination of lysine plus threonine led to an increase in gain above the level attained by lysine supplementation alone. This agrees with the present study in which lysine and threonine supplementation of barley improved performance whereas the further addition of methionine had no effect.

It has been reported that feeding cereal mixtures supplemented with lysine, threonine, tryptophan, methionine and in some experiments also isoleucine resulted in performance equal to that obtained with diets containing protein concentrate (Robinson et al. 1963; Müller et al. 1967b). Müller and Malék (1967a, 1967b, 1967c) showed that 'monodiets', i.e., single cereal diets (wheat, barley or corn) with added amino acids could be used successfully to rear pigs from weaning (average liveweight about 20 kg).

Beames and Pepper (1969) indicated that the use of lysine, either with or without methionine, was not completely successful as a replacement of half the soybean meal in a grain plus 15 percent soybean ration for pigs of less than 45 kg body weight. From a body weight of 45 kg to the slaughter weight of approximately 90 kg, the above authors showed that half of 15% soybean meal could be replaced by amino acids without reducing the growth of the pigs, although results with this type of supplement in combination with sorghum over the body weight range (20 - 89 kg) were not as satisfactory as for wheat. In the present experiment, the replacement of protein concentrates such as soybean meal by lysine, lysine plus threonine, lysine plus threonine plus methionine plus isoleucine did not improve the performance to equal that of the barley-soybean control ration, but supplementation with these amino acids significantly improved performance above that obtained by adding lysine alone. Performance of animals on amino acid supplemented barley rations was inferior to the barley-soybean control ration. This is in agreement with Beames et al. (1968), Beames and Pepper (1969), and Barber et al. (1969), but in disagreement with Robinson et al. (1963) and Müller et al. (1967b). It is possible that the response to the addition of lysine and threonine to the barley ration was limited by either the level of total N in the ration, which was markedly lower than the level in the barley-soybean control ration, or a lower biological availability of lysine and threonine or other essential amino acids.

Bayley et al. (1968) indicated that the supplementation of a practical corn-soybean ration with either 0.1% lysine or 0.05% methionine had no beneficial effect on growth rate or feed efficiency, but with the levels used there was a positive effect on gain when both amino acids were added together. The effect was marked for the low-protein ration, but making up the difference in lysine and sulfur amino acids did not overcome the difference in gains between the two dietary protein levels.

Ericson et al. (1962) showed that supplementation with L-lysine HCl markedly increased daily gain (40 - 60%) and decreased the amount of feed per kg of gain (25 - 30%), when the basal ration consisted of 27% and 63% barley protein, and, that there was some response due to the L-lysine HCl supplementation in spite of the relatively high level of added fish meal and soybean meal. This experiment also indicated that supplementation with protein concentrates gave far better gain and feed efficiency than supplementation with amino acids.

Blair et al. (1969) showed that liveweight gain was not improved significantly by increasing the protein level above 16, 14 and 12% respectively, for the 23 - 45, 45 - 68 and 68 - 90 kg body weight categories. However, feed conversion efficiency was improved significantly during the 23 - 45 kg range by increasing the protein level to 18%. Lean meat production and the efficiency of conversion of feed to lean meat were improved by increasing the above protein levels slightly. Liveweight gain was not improved significantly by increasing the lysine level above 1.04, 0.74 and 0.70%, respectively, for the 23 - 45, 45 - 68 and 68 - 90 kg ranges. However, feed conversion efficiency was improved significantly during the 23 - 45 kg stage by increasing the lysine level to 1.22%. Raising the lysine level at each level of protein had no signficant effect on the rate and efficiency of lean meat gain.

Generally animals require more amino acids, especially lysine in early growth and gradually decrease their requirement with age. In the present experiment,higher levels of lysine (0.90%) gave a significantly higher mean daily weight gain and better feed efficiency than the lower level of lysine (0.75%). Pig performance showed that there was not much difference between the two levels of lysine in the early stages but the higher level of lysine gave improved mean daily weight gain and feed efficiency than the lower level in the later stages (Figure 2, Figure 3). It is possible that the response to the higher level of lysine was limited during early growth by the level of crude protein in the ration. In this experiment, threonine was the second limiting amino acid for pigs since supplements of lysine and threonine to the barley produced performance next to that of pigs fed the barley-soybean control ration. Braude et al. (1972) and Morrison et al. (1961) suggested thatllysine supplementation results in a progressive decrease in plasma threonine concentration. The higher level of lysine was perhaps more imbalanced in the early stage than in later stages. A level of 0.29% threonine in these rations was considerably lower than the published threonine requirement (0.45%) according to N.A.S.-N.R.C. (1968) but is adequate in the later stages since the requirement of threonine is 0.27% (crude protein 12%) by calculations which are based upon the assumption that the requirements for all amino acids expressed as a percentage of the protein, decrease linearly as the dietary protein increases (Becker et al. 1963). Therefore, in the later stage, pigs can utilize more lysine than in the early stage. Pierce and Bowland (1972) showed that the addition of 0.2% L-lysine HCl alone to the low protein diet

(C.P. 14%, lysine 0.57%) improved feed intake in the growing and finishing period and the overall period but not in the starting period compared with the low protein unsupplemented diet. Blair et al. (1969) showed that feed efficiency improved significantly during the 23 - 45 kg stage by increasing the lysine level to 1.22%. In the present experiment pigs fed the higher level of lysine gave better feed intake and growth rate than those receiving the lower level of lysine in the later stage but not in the early stage (Figure 2), but the higher level gave slightly better feed efficiency than the lower level of lysine in the early stage. These results were in agreement with Blair et al. (1969), and Pierce and Bowland (1972).

In particular, attention may be drawn to the carcass quality in this experiment. It is quite understandable that an energy excess will lead to fatter carcasses. In the present experiment daily feed intake was 1.55 - 1.78 kg over all period. This figure showed that the total energy intake would not be in excess since barley was used as a basal ration.

It is worthwhile in this respect to determine not only minimum but in particular optimum requirements, if quality meat is to be obtained economically from heavy pigs. Backfat measurements of pigs given the lower level of lysine were of lower magnitude than those of pigs receiving the higher level of lysine. Mid backfat was significantly less but maximum shoulder fat, maximum loin fat and backfat at 40 mm from the mid line were not significantly less. Dressing percentage, eye muscle area and eye muscle index were not significantly affected by levels of lysine supplementation but the trend showed that the lower level of lysine gave a higher dressing percentage, a larger eye muscle area and a larger eye muscle index. This trend might come from either amino acid imbalance at given protein levels or other limiting factors. The higher level of lysine (0.90%) at approximately 11% protein would be in excess of the growing pigs requirements compared to 0.58% lysine at 11.6% protein (Brinegar 1949), 0.71% lysine at 12.8% protein diet (McWard 1959) and 0.70% lysine when the ration contained 10 or 15% protein (Chance et al. 1958). This excess of lysine might cause an amino acid imbalance since performance showed that threonine was limited.

Low-protein rations based on barley and/or wheat or corn properly balanced by supplementation with lysine produced leaner carcasses than unsupplemented low-protein ration, as shown by Brooks et al. (1959), Bowland (1962), Cahilly et al. (1963), Nielsen et al. (1963), Braude et al. (1972). Adding threonine to the lower level of lysine had no significant effect on backfat, eye muscle area and dressing percentage. This would have been partly explained by a difference in feed intake as the feed intake on the lower level of lysine was 1.55 kg per day while further addition of threonine increased this to 1.74 kg per day for the whole period. The further addition of methionine, alone or in combination with isoleucine, did not affect backfat, eye muscle area, eye muscle index or dressing percentage. This indicated that there was no response from adding methionine and isoluecine on carcass quality as well as performance of pigs.

Clausen (1959-1964) collected a large quantity of data showing that amino acid supplementation of protein concentrates fed as supplements to barley diets have also effected a marked improvement in the carcass leanness of the Danish pigs.

In the present experiment, the barley-soybean control ration (about 17% C.P.) gave the lowest backfat thickness, larger eye muscle area and higher eye muscle index. Work by Bowland et al. (1959), Ashton et al. (1955), Becker et al. (1956) and results summarized by A.R.C. (1967), all support the view that lowering the crude protein level below about 16-17%, at least in the initial stages of the growing period, will produce adverse effects on carcass quality. These results are in agreement with the observations for this study that pigs fed a barley-soybean control ration produced better carcass quality than pigs fed lower protein (about 11%) amino acid supplemented barley but Clawson (1967), Meade et al. (1966) and Pierce and Bowland (1972) reported no influence of dietary protein on loin area and backfat thickness. Pierce and Bowland (1972) suggested that lack of treatment effects on total backfat thickness and on loin area was due to a wide tolerance level for dietary protein levels before gross carcass changes were noted (protein level was 14, 17 and 20%).

Barrows gave significantly higher daily weight gain than gilts which is in agreement with Tjong-A-Hung et al. (1972) but which is in disagreement with Newell and Bowland (1972) and Pierce and Bowland (1972). Gilts were superior to barrows in all carcass measurements except carcass length. These results are in agreement with Wong et al. (1968), Newell and Bowland (1972) and Pierce and Bowland (1972), and Tjong-A-Hung et al. (1972) who showed that gilts had lower backfat measurements and larger eye muscle area.

There was not much difference in weight gain between the two levels of lysine for barrows, but gilts had a significantly different response to lysine levels. This was in agreement with Robinson (1964) who showed that the response of gilts continued with increasing protein and lysine levels in the diets while that of barrows ceased at a much lower level. Adding lysine and threonine resulted in a similar improvement in the growth rate of both barrows and gilts when compared with the addition of lysine alone. Further adding methionine with or without isoleucine did not give consistant results. These results may be caused by a different amino acid requirement of sex. Therefore, further study on sex and supplementation with amino acids interaction would give more reliable results when a separated feeding system according to sex is adopted.

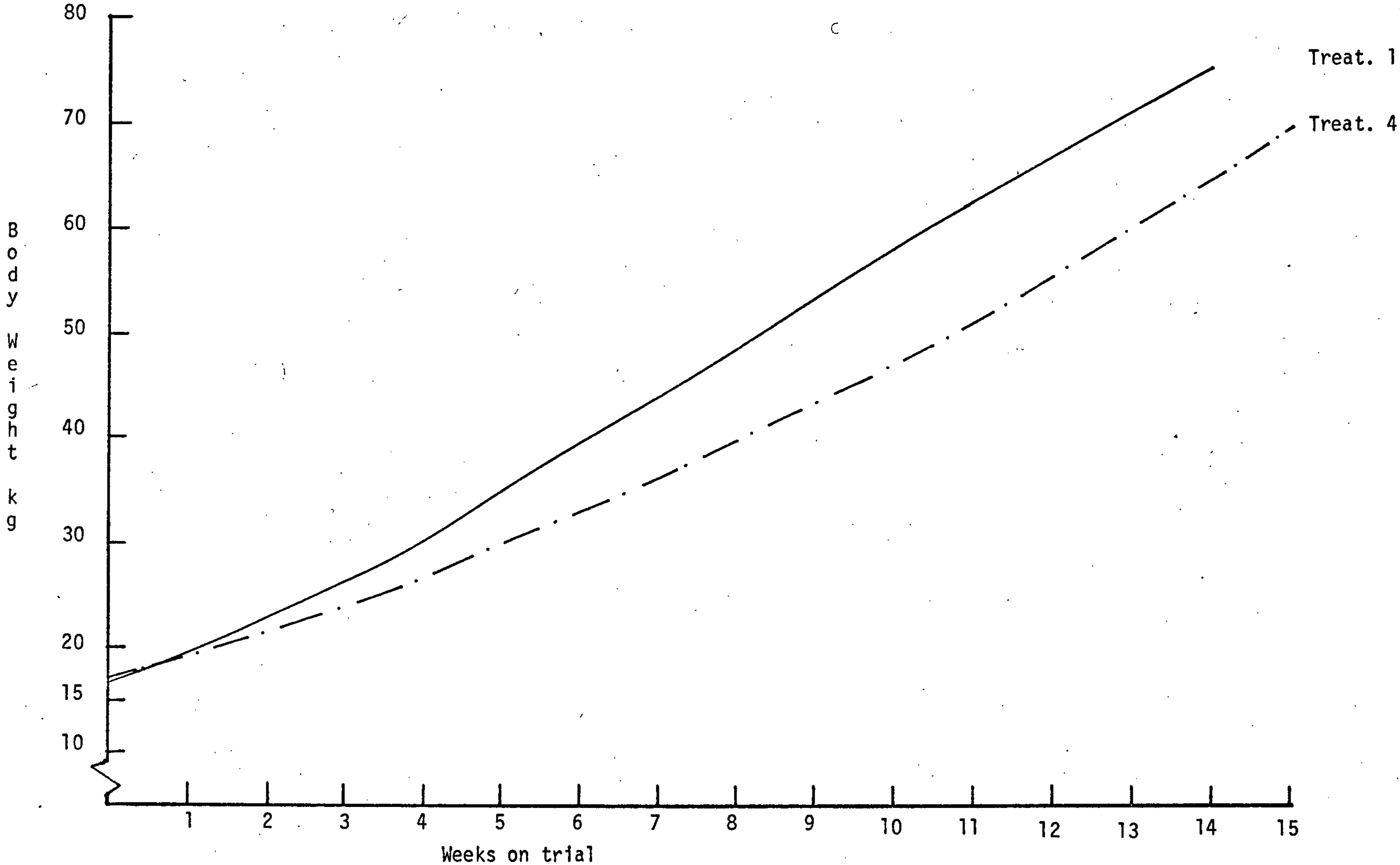

Figure 1. The effect of supplementation with lysine and threonine of basal ration of barley (Treat. 4) compared to the control ration (Treat. 1) on mean body weight from the start until first pigs sent for slaughter.

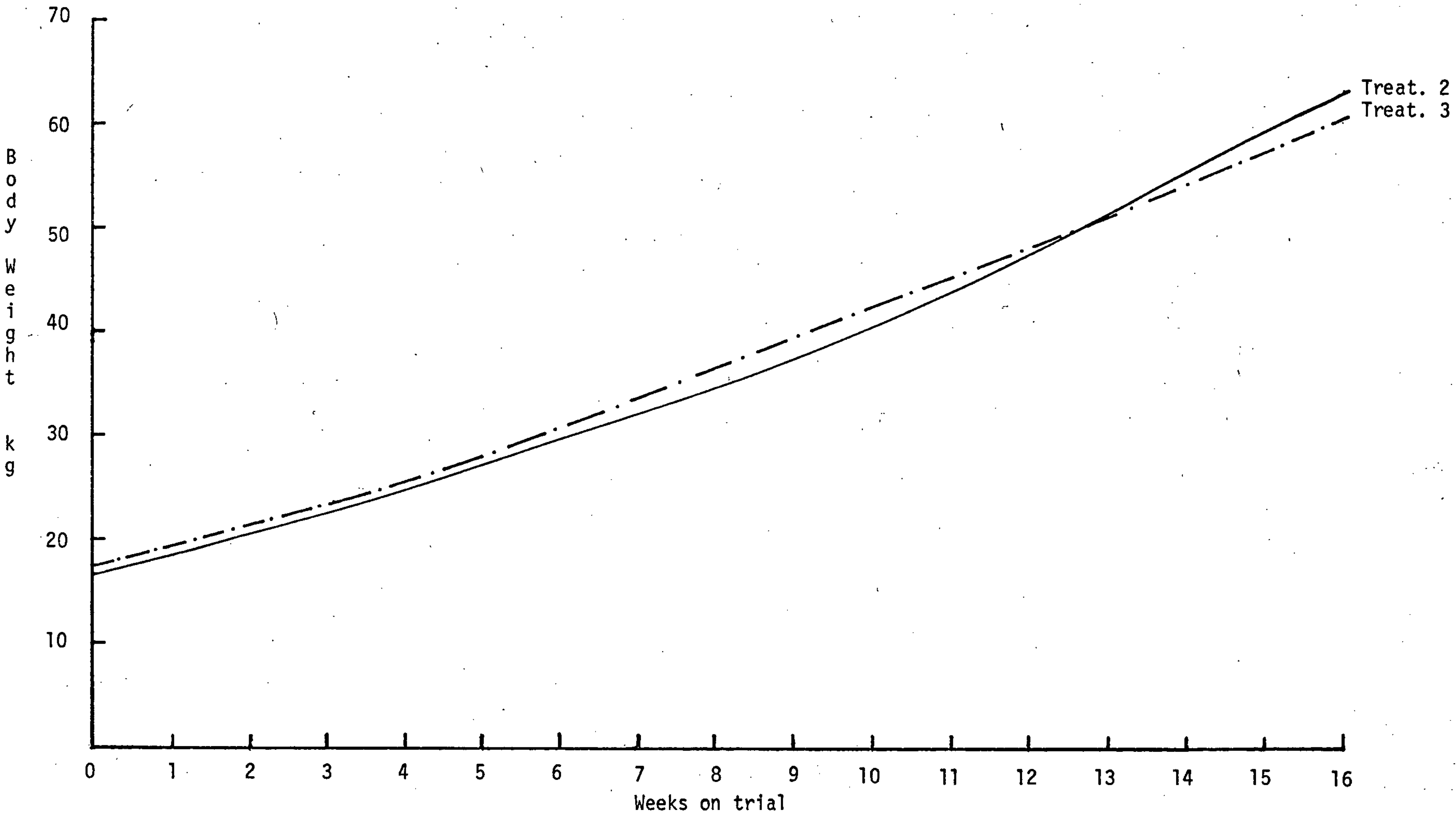

Figure 11. The effect of supplementation with higher level of lysine (Treat. 2) and lower level of lysine (Treat. 3) on mean body weight from the start until first pigs sent for slaughter.

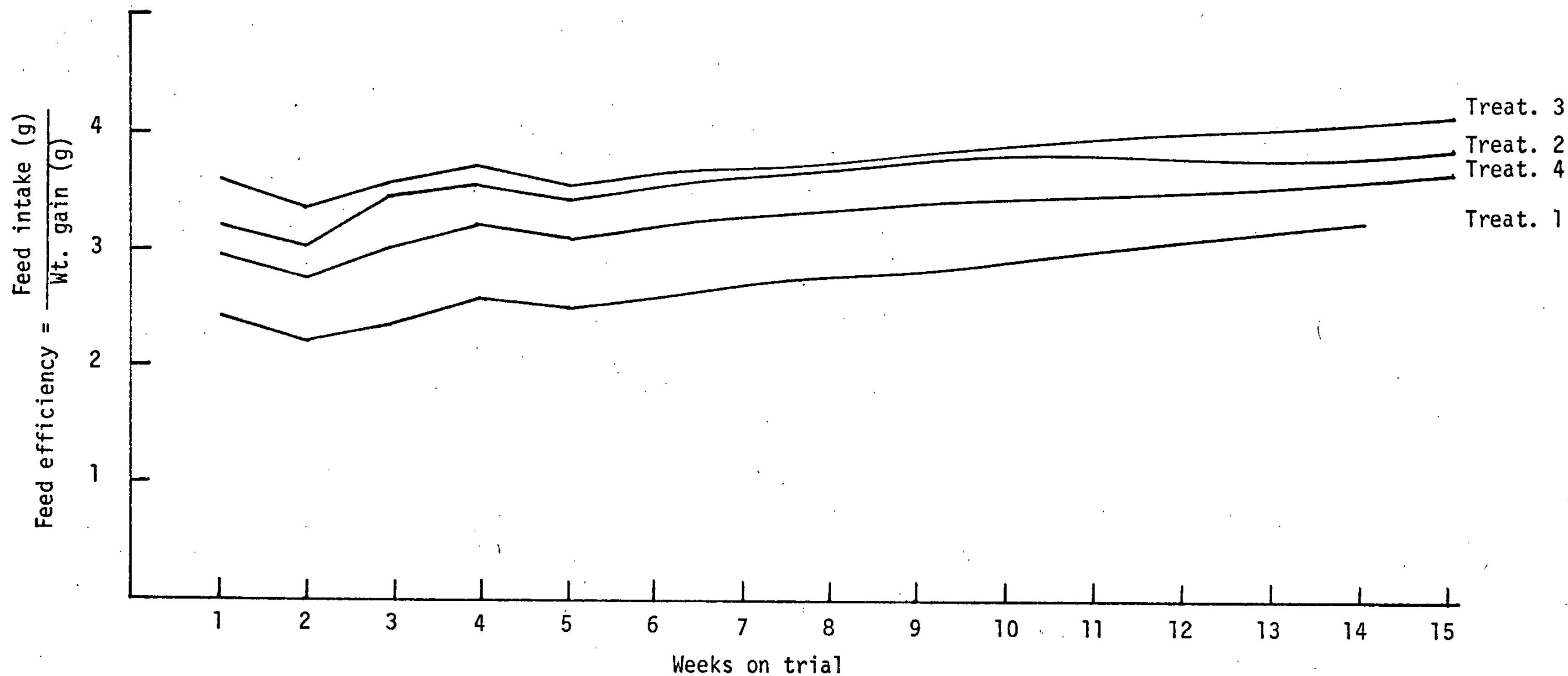

Figure 111. The effect of supplementation with lower level of lysine (Treat. 3), higher level of lysine (Treat. 2), lysine and threonine (Treat. 4) and control ration (Treat. 1) on Feed efficiency ratio from start until first pigs sent for slaughter.

D. CONCLUSION

Pigs fed the lower level of lysine had a significantly lower growth rate and poorer feed efficiency than those fed the higher level of lysine. The trend that pigs fed the lower level of lysine were leaner and with larger eye muscle areas than those fed the higher level of lysine. These results could be explained on the basis of a slower growth rate, everything else being equal, producing a leaner carcass.

Adding 0.05% L-threonine to the lower level of lysine improved feed intake, daily weight gain and feed efficiency but not carcass quality above the improvement resulting from the addition of lysine alone to the basal ration of barley. The further addition of 0.10% DL-methionine with or without 0.10% L-isoleucine did not improve feed intake, daily weight gain, feed efficiency or carcass quality. Nor did supplementation with these amino acids to the lower level of lysine improve carcass quality. In this experiment threonine was the second limiting amino acid in Peace River barley after lysine, which is well known as the first limiting amino acid in barley for growing pigs. By adding 0.05% L-threonine to the lower level of lysine mean daily gain improved by 27.1% and feed efficiency by 14%. However, supplementation with lysine and threonine resulted in a considerably inferior performance than that obtained with barley-soybean control ration, with daily weight gain, feed efficiency and carcass quality being significantly poorer. It was possible that the barley diet even with the addition of the four amino acids, was limiting either in the level of total N or as a result of an imbalance of amino acids was due to a low threonine level since addition of 0.05% L-threonine was deficient in the ration for growing pigs by N.A.S.-N.R.C. (1968). Either adding nitrogen or adding more threonine, or both may improve performance compared to that of the present experiment.

IV. EXPERIMENT II

A. EXPERIMENTAL PROCEDURE

a. General.

Although growth performance is an important parameter in practical animal production, weight gain and feed conversion ratio give only an inaccurate measure of nitrogen utilization. Nitrogen balance values are of much greater value in assessing protein quality. The following experiment was done to obtain nitrogen absorption and utilization data to compliment the results obtained in the main feeding experiment (experiment I) using the same rations.

The experiment utilized three groups each of six pigs, with each group maintained in digestibility crates for a three-week period. Within groups the six experimental rations were randomly allocated, with each pig receiving the same ration for the three-week period (Table VI). The first week was an acclimatization period. The second and third weeks were divided into the two one-week collection periods.

b. Animals.

A total of 18 male castrate Yorkshire pigs between 30 kg and 45 kg in three groups of six were used.

c. Rations.

Rations were the same as those used in experiment I.

d. Management.

(i) Housing

The metabolism room was insulated and supplied with space heaters which kept the temperature at approximately 21 ± 1° C. The metabolism crates were a modification of the Shinfield design (Frape *et al.* 1968). The urine tray which was inclined from front to reat at an angle of approximately 30° from the horizontal was made of fibre glass. The feces tray was made of plastic. In order to prevent some pigs from turning in the crates in the acclimatization period a sheet of plywood board was attached to both side of the crates. A fine wire screen was placed on the urine tray in order to retain the small amount of feces which fell anterior to the rear of the floor (Figure IV and V).

(ii) Feeding and watering

Pigs were fed twice daily *ad libitum* at 9:00 a.m. and 2:00 p.m. for 40 minutes on ration which was mixed with water (feed:water = 1:1) just before feeding. Water was given after the afternoon feeding *ad libitum* for 20 minutes. Feed residues were removed, put into plastic bags and stored at 3° C. At the end of each one-week collection period, the residues for each pig were pooled and dried at 60° C for 72 hours.

(iii) Mixing rations

Each ration was prepared by passing the barley through the fine screen (7 mm diameter) of a hammer mill. Feed samples were taken for determination of moisture and nitrogen at the time of feeding the ration.

e. Feces and Urine Collection

All feces from each pig were collected in plastic bags and stored at 3^{O}C until completion of the seven-day trial. The total fecal output of each pig was then pooled and weighed, and a 400 g aliquot dried at 60^{O}C for 72 hours (Saben and Bowland 1971). The dried feces were ground and stored for later measure of nitrogen and moisture. Urine was collected in a plastic jar to which was added 50 ml diluted sulphuric acid (50% V/V) each day. Each day urine volume was measured and a 10% aliquote retained at 3^{O}C for pooling at the end of the experiment. The pooled urine was used for subsequent nitrogen determination.

f. Statistical Analysis of Data.

The data for nitrogen balance, apparent nitrogen digestibility, nitrogen retained as a percentage of nitrogen absorbed, nitrogen retained as a percentage of nitrogen intake and dry matter digestibility were subjected to an analysis of variance (UBC-LSA8) and means from comparison showing significant F values were tested according to the multiple range test of Duncan (1955). Nitrogen balance data were analyzed by covariance (UBC-LSA8) with feed intake because of highly significant covariance.

Table VI. Allocation of pigs within replicates and periods for Experiment II

Period (Weeks)	Rations: 1 Soy Control	2 0.9% lys	3 0.75% lys	4 lys+threo	5 lys+threo +meth	6 lys+threo +meth+isoleu
	Pig No.					
1	1	2	3	4	5	6
2	1	2	3	4	5	6
3	1	2	3	4	5	6
4	7	8	9	10	11	12
5	7	8	9	10	11	12
6	7	8	9	10	11	12
7	13	14	15	16	17	18
8	13	14	15	16	17	18
9	13	14	15	16	17	18

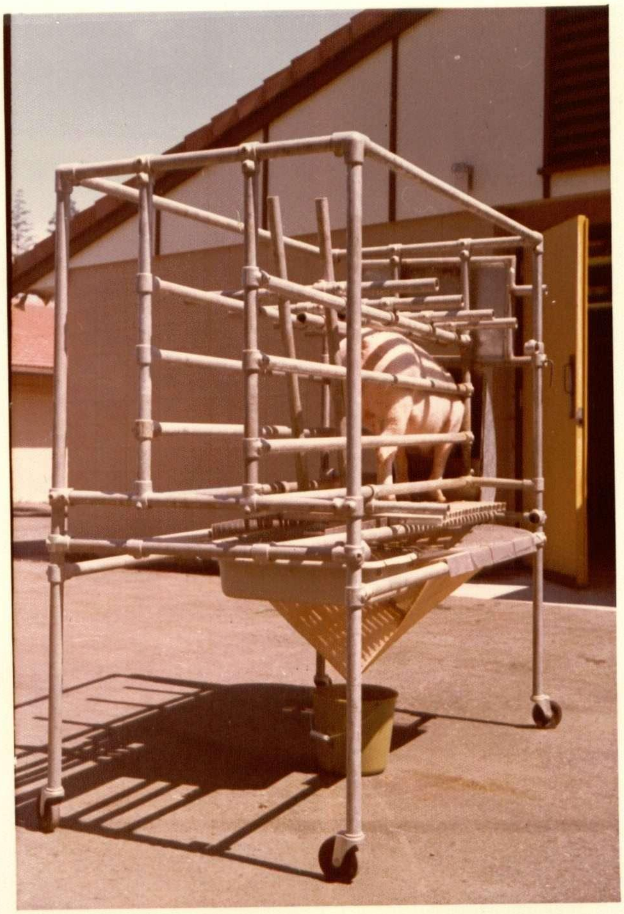

Figure IV. General view of the metabolism cage.

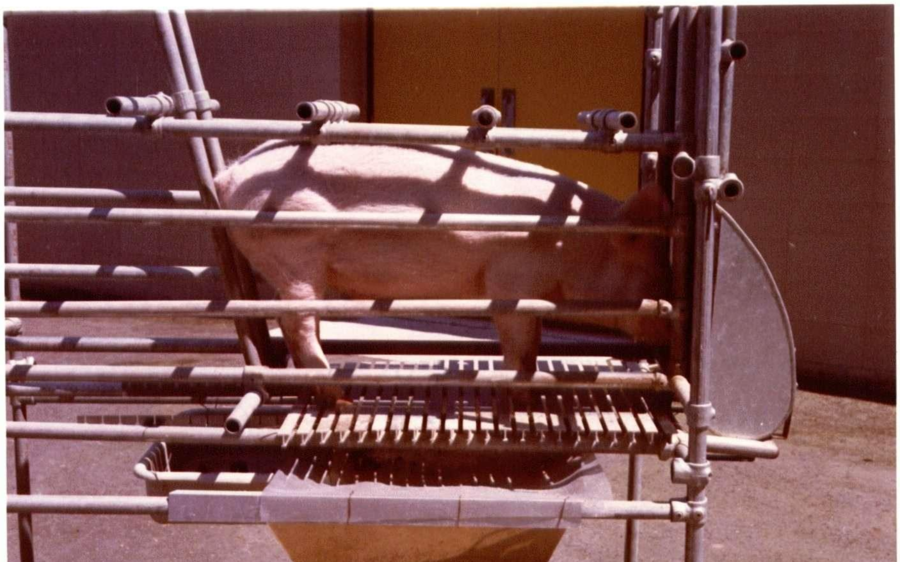

Figure V. Pig shown in the cage which could be adjusted for the size of pig.

B. RESULTS

The results of the metabolism trial are shown in Table VII. The addition of 0.50% L-lysine HCl (0.75% total lysine) to the basal ration of barley produced a significantly better nitrogen balance than the addition of 0.69% L-lysine HCl (0.90% total lysine). However, when nitrogen balance was adjusted for feed intake, there was no significant difference between two levels of L-lysine. The addition of 0.05% L-threonine to the lower level of lysine did not significantly improve nitrogen balance and also did not when nitrogen balance was adjusted for feed intake. No further improvement of nitrogen balance was achieved by the addition of 0.10% DL-methionine to the lower level of lysine. However, when nitrogen retention was adjusted for feed intake, adding 0.10% DL-methionine with or without 0.10% L-isoleucine further improved the quality of barley protein above that observed with lysine alone. Adding 0.10% L-isoleucine to the above ration improved nitrogen balance when nitrogen balance was adjusted by feed intake.

Nitrogen absorbed as a percentage of N intake tended to be improved more by the addition of 0.05% L-threonine to the lower level of lysine but this increase was not significant. Further improvement of N absorbed/N intake was obtained from the addition of methionine either alone or in combination with isoleucine. When nitrogen absorbed was expressed as a percentage of nitrogen consumed, the barley-soybean control ration was significantly higher than the barley-amino acids rations.

Nitrogen retained as a percentage of N absorbed was improved more by the addition of 0.05% L-threonine to the lower level of lysine than by adding the lysine alone to the barley ration. The lower level of lysine gave a better value for N retained as a percentage of N absorbed than the higher level of lysine. The barley-soybean control ration gave the second lowest N retained as a percentage of N absorbed. The lowest value was obtained with the barley plus the higher level of lysine. Further addition of methionine and isoleucine gave significantly higher biological value than the barley-soybean control ration and adding lysine alone to the barley.

Nitrogen retained, when expressed as a percentage of nitrogen consumed was not significantly different for the grain-soybean control ration and the rations containing threonine, with or without methionine and isoleucine, but all except the grain-threonine ration gave a higher retention than the grain-lysine combination.

The results showed that the major effect of improving protein quality was due to the threonine addition to the lower level of lysine. The addition of methionine and isoleucine gave non-significant improvement in N balance which was adjusted for feed intake, N digestibility, biological value and N retained as a percentage of N consumed, but a slight response.

Table VII. Summary of the effects of supplementation of barley with amino acids on apparent dry matter digestibility, nitrogen balance, apparent nitrogen digestibility and on nitrogen retained as a percentage of nitrogen absorbed (average 7-day figures).

Treatment	1 Soy Control	2 0.9% lys	3 0.75% lys	4 lys + threo	5 lys+threo + + meth	6 lys+threo +meth+isol	Results of Significant Tests *
Total feed intake (g)	9801	8660	10437	99841	8404	10199	
Total N intake (g)	267.03	150.70	179.221168888		144.33	171.17	
N balance (g)	120.37	55.16	68.56	70.84	63.61	70.50	1 4 6 3 5 2
N balance a adjusted by feed intake (g)	117.37	60.45	61.67	68.03	70.67	69.52	1 5 6 4 3 2
N absorbed / N intake %	76.05	64.58	61.95	64.43	66.01	66.18	1 6 5 2 4 3
N retained / N intake %	45.10	36.61	38.26	42.24	44.08	43.50	1 5 6 4 3 2
N retained / N absorbed %	59.39	56.62	61.76	65.43	66.80	64.60	5 4 6 3 1 2
D.M. digestibility	78.70	77.32	76.45	77.11	78.18	78.22	1 6 5 2 4 3

* Treatment means not underscored by the same line are significantly different at 5% level of probability (Duncan, 1955).

C. DISCUSSION

Although there were slight variations in the relative effects of treatments, the values for N balance, N absorbed/N intake, N retained/N absorbed and N retained/N intake were in reasonably close agreement in showing a progressive improvement in nitrogen retention by the addition of L-threonine, DL-methionine and isoleucine to the barley plus lysine. The results indicated that the threonine content of barley was deficient for growing pigs since the main improvement in protein quality was associated with the addition of threonine and lysine in contrast to the addition of lysine alone. Further addition of methionine with or without isoleucine did not significantly improve N balance, N digestibility, biological value and N retained/N absorbed above that obtained lysine and threonine only.

An improvement in nitrogen balance by the addition of L-lysine HCl to grain has been reported by many authors (Ericson et al. 1962; Bowland and Grimson 1969; Pick et al. 1971). Braude et al. (1972) indicated that N retention as a percentage of N intake tended to be higher with lysine supplementation to barley than with unsupplemented barley for growing-finishing pigs. Solberg (1971) showed that compared with a moderately lysine-deficient diet, lysine additions had no significant effect on either the digestibility or the metabolizable energy content of the diet but a lysine deficiency caused a decreased nitrogen retention, an increased uric acid biosynthesis and an increased feed intake in chicks.

Pick et al. (1971) showed that rats fed diets containing 0.73% lysine had significantly ($p \leq 0.01$) greater gain/feed ratio and retained more nitrogen than those fed 0.54% lysine diets. However, increasing the dietary lysine to 0.92% did not effect further improvements in any of the response criteria. Also these results indicated that the level of dietary isoleucine did not exert a significant effect on daily gain, gain/feed, N balance and N retained as a percentage of N absorbed. In the present experiment, the lower level of lysine gave better N balance and N retained/N absorbed than the higher level of lysine, and the lower level of lysine had a slightly better N retained/N intake value. Therefore, the lower level of lysine (0.75% total lysine) was more suitable than the higher level of lysine (0.90% total lysine) in the barley ration for growing pigs which was supported by Pick et al. (1971) who showed that 0.73% lysine was adequate in an opaque-2 corn ration for growing rats.

The lower level of lysine gave a significantly higher biological value and slightly better net protein utilization than the higher level of lysine although digestibility of nitrogen did not differ. Therefore, the different results of the two levels of lysine seemed to be derived from N retention but not N absorption. It is possible that the response to the higher level of lysine was due to either an excessive amount of lysine which would give rise to an imbalance of amino acids because the threonine was deficient in the ration according to N.A.S.-N.R.C. standard (1968) or to a lower amount of nitrogen intake than that of the lower level of lysine since nitrogen retention increases as nitrogen intake increases.

Bressani (1971) indicated that adding lysine and threonine to rolled oats improved the nitrogen balance in humans above that obtained with lysine. He obtained no further improvement in nitrogen balance by further addition of methionine. These results agree with the present experiment which showed a marked response to threonine when added to barley plus lysine. Müller and Rozman (1968) showed that lysine, threonine or tryptophan and methionine supplementation of barley for growing-finishing pigs improved N retained/N intake and N retained/N digested as well as daily gain and feed efficiency. This agrees with the present experiment in which adding lysine, threonine and methionine with or without isoleucine gave an improvement in N balance, N retained/N intake percentage in this order.

Bowland and Grimson (1969) indicated that a better N retained/N absorbed value was obtained with a lysine-methionine supplemented low protein diet (14% C.P.) than with a higher protein or an amino acid unsupplemented low protein diet for early weaned pigs. There was an increase in daily nitrogen retention when L-lysine was added to diets containing three percent urea but this did not occur where both L-lysine and DL-methionine was added. This observation suggested that for pigs fed the low protein diet plus urea, methionine was not the second limiting amino acid after lysine. This agreed with our experiment in which adding methionine produced only a slight response since threonine was the second limiting amino acid after lysine.

The barley-soybean control ration gave the highest N balance, the best opponent N digestibility and the highest value for N retained as a percentage of N intake but the second lowest value for biological value, after the higher level of lysine. It appears that the N of the barley-soybean ration was better digested but of a lower biological value than the N of the lysine plus threonine, lysine plus threonine plus methionine and lysine plus threonine plus methionine plus isoleucine rations. These results were in agreement with those of Bowland and Grimson (1969) who showed that apparent nitrogen digestibility was low on low protein diets but increased with urea or with protein supplementation of the low-protein diet and the highest apparent biological value was obtained with supplementation by lysine and methionine to low protein diet. It is possible that the response to the lower biological value of barley-soybean control ration was due to either a higher protein concentration since the barley-soybean ration was about 17% crude protein and the other rations were about 11% or a higher protein intake which was approximately one and half times more protein than those of the other rations. These results were in agreement with Metta and Mitchell (1956), and Rippon (1959) who found that the biological value of different proteins decreased linearly as the protein concentration or intake increased.

The results of the present study showed that barley protein could be improved by supplementation with four amino acids. However, barley-amino acids rations gave lower values for nitrogen retention than the barley-soybean control ration. Therefore, further studies might solve the problems of supplementation of cereal proteins with synthetic amino acids.

There was a possibility of nitrogen losses in feces and urine. Saben and Bowland (1971) suggested that either wet or dry pig fecal material may be used for nitrogen and energy determinations in pig digestion trials where feces is dried at 60°C for 72 hours. Martin (1966) indicated that losses of NH_3 from feces were negligible and the losses from urine depended on the temperature and pH at which it was collected. The average loss of nitrogen on collection of urine at a pH value below 2.0 was 1.33% when the ambient temperature was between 25 and 28°C and 0.97% when it was 15 and 18°C. In the present experiment, urine was collected in strong sulphuric acid so that the pH of urine was below 2 even though samples were stored for quite long periods in a freezer. Therefore losses of nitrogen from feces and urine would be negligible in this experiment.

D. CONCLUSION

The metabolism trial was conducted to determine the effect of amino acid supplementation of Peace River barley on nitrogen balance and apparent biological value.

The addition of L-lysine and L-threonine to the barley tended to improve nitrogen balance when adjusted for feed intake, apparent nitrogen digestibility, biological value and N retained/N intake above that obtained by the addition of lysine alone but the difference was not significant. Further addition of methionine with or without isoleucine markedly improved the above criteria although not all improvements were significant above the addition of lysine alone. The results showed that the major effect of improving protein quality appeared to be due to the threonine supplementation to the lower level of lysine since further supplementation with methionine and with or without isoleucine gave further slightly non-significant improvement in nitrogen metabolism measurements. Whereas, isoleucine did not affect the protein quality so much as methionine. Therefore, threonine was the second limiting amino acid after lysine in this experiment and methionine and isoleucine were probably limiting too.

The difference in apparent digestibility of protein between two levels of lysine was not significant but the biological value of the lower level of lysine ration was significantly higher than that of the higher level of lysine ration. This indicated that N retention varied with the level of lysine but N absorption was not influenced by the different lysine level. Therefore, 0.75% lysine seemed to be adequate for growing pigs in this experiment. Further studies in amino acid digestibility and blood amino acid patterns may explain these results. Also labelled amino acids would aid in determining amino acid absorption and retention.

Supplementation with lysine and threonine with and without methionine, and also isoleucine resulted in a significantly lower N balance, apparent N digestibility but a significantly higher biological value than the barley-soybean control ration.

It was concluded that both protein and amino acid supplementation to the basal ration of barley could affect nitrogen balance and biological value. Either adding nitrogen or adding more threonine, or both to the barley may improve nitrogen balance and biological value but not digestibility.

V. GENERAL CONCLUSIONS AND RECOMMENDATIONS

The supplementation with 0.50% L-lysine HCl (0.75% total lysine) to a basal ration of barley seemed to improve protein quality compared to the supplementation with 0.69% L-lysine HCl (0.90% total lysine). The biological value of the lower level of lysine ration (0.75% total lysine) was significantly higher than that of the higher level of lysine (0.90% total lysine), and the trend showed that pigs fed the lower lysine were leaner and had larger eye muscle areas than those fed the higher level of lysine even though the lower level of lysine gave a signficantly lower growth rate and poorer feed efficiency than the higher level of lysine.

Both the feeding experiment and nitrogen balance experiment indicated that threonine was the second limiting amino acid since supplementation with lysine and threonine to the barley improved growth performance significantly and tended to improve nitrogen retention and biological value to a greater extent than adding lysine alone. However, supplementation of barley with lysine and threonine resulted in significantly poorer daily gain, feed efficiency, carcass quality and nitrogen retention but significantly higher biological value than the barley-soybean control ration. Therefore, these treatments were unable to produce the results equivalent to that from normally supplemented soybean ration.

The result of the present study showed that barley protein could be markedly improved by supplementation with lysine and threonine and they demonstrated the importance of taking these into account in a ration. It is well known that one of the roles of protein concentrates is to counteract amino acid deficiencies and total nitrogen in the cereal components of a ration and this almost inevitably gives rise to an excess of the other amino acids which are not needed for protein synthesis. In order to reduce this excess as much as possible, it is necessary to supplement these limiting amino acids such as lysine and threonine and also to have a thorough knowledge of dietary amino acid content and availability at given components. The shortage and higher cost of protein supplements for use in feed is increasing. Therefore, further studies of the problem related to replacing protein concentrate with synthetic amino acids are required if they can be made available at suitable prices.

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APPENDIX

Table IA. Average daily body weight gain (kg) of the 107 pigs.

Treatment	Rep 1 Male	Rep 1 Female	Rep 2 Male	Rep 2 Female	Rep 3 Male	Rep 3 Female
1. Barley + Soybean (Control)	0.78	0.56	0.61	6.0	0.60	0.63
	0.70	0.62	0.65	0.65	0.45	0.64
	0.61	--	0.37	0.62	0.66	0.62
2. Barley + L-lysine HCl (0.9% total lysine)	0.50	0.50	0.45	0.41	0.47	0.51
	0.44	0.46	0.50	0.51	0.53	0.41
	0.52	0.39	0.53	0.41	0.35	0.37
3. Barley + L-lysine HCl (0.75% total lysine)	0.46	0.24	0.44	0.43	0.40	0.35
	0.47	0.42	0.54	0.32	0.56	0.33
	0.44	0.44	0.50	0.39	0.46	0.32
4. Treatment 3 + 0.05% L-threonine	0.58	0.44	0.56	0.51	0.55	0.49
	0.56	0.56	0.54	0.57	0.56	0.49
	0.57	0.46	0.50	0.41	0.56	0.59
5. Treatment 4 + 0.10% DL-methionine	0.39	0.59	0.52	0.50	0.57	0.48
	0.62	0.40	0.50	0.52	0.56	0.44
	0.45	0.46	0.57	0.44	0.54	0.35
6. Treatment 5 + 0.10% L-isoleucine	0.49	0.44	0.52	0.50	0.53	0.50
	0.58	0.48	0.55	0.50	0.56	0.39
	0.49	0.49	0.49	0.59	0.45	0.47

Table IB. Analysis of variance of the average daily body weight gain of 107 pigs. (Covariable starting weight).

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) p = 0.05	Variance Ratio F(nec.) p = 0.01
Total	106	0.859				
Treatment (a)	5	0.374	0.0748	19.939**	2.368	3.339
Block (b)	2	0.0094	0.0047	1.254	3.150	4.977
Sex (c)	1	0.0257	0.0257	6.864*	4.001	7.077
a x b	10	0.0231	0.0023	0.616	1.993	2.632
a x c	5	0.0561	0.0112	2.992*	2.368	3.339
b x c	2	0.0102	0.0051	1.363	3.150	4.977
a x b x c	10	0.0451	0.0045	1.202	1.993	2.632
Covariable	1	0.0333	0.0333	8.871**	4.001	7.077
Residual	70	0.263	0.0038			

* P < 0.01 ** P < 0.05

Table IIA. Average Feed intake (kg) per pig per day for each ration.

Treatment	Rep 1	Rep 2	Rep 3
1. Barley + Soybean (Control)	1.79	1.79	1.75
2. Barley + L-lysine HCl (0.9% total lysine)	1.64	1.61	1.55
3. Barley + L-lysine HCl (0.75% total lysine)	1.51	1.59	1.56
4. Treat. 3 + 0.05% L-threonine	1.74	1.67	1.81
5. Treat. 4 + 0.10% DL-methionine	1.61	1.62	1.59
6. Treat. 5 + 0.10% L-isoleucine	1.66	1.67	1.63

Table IIB. Analysis variance of average daily feed intake.

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	17	1.3012				
Treatment	5	1.1086	0.2217	23.9738**	3.2039	5.3160
Covariable starting weight	1	0.0253	0.0253	2.7382	4.8443	9.6460
Residual	11	0.1017	0.00925			

** P < 0.01
 * P < 0.05

Table IIIA. Average feed conversion efficiency ratio per pig. (D.M. feed intake (kg)/weight gain (kg).

Treatment	Rep 1	Rep 2	Rep 3
1. Barley + Soybean (Control)	2.83	3.07	2.94
2. Barley + 0.69% L-lysine HCl (0.9% total lysine)	3.55	3.46	3.59
3. Barley + 0.50% L-lysine HCl (0.75% total lysine)	3.72	3.70	3.95
4. Treat. 3 + 0.05% L-threonine	3.33	3.25	3.37
5. Treat. 4 + 0.10% DL-methionine	3.40	3.20	3.30
6. Treat. 5 + 0.10% L-isoleucine	3.38	3.21	3.41

Table IIIB. Analysis of variance of feed conversion efficiency ratio

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					p=0.05	p=0.01
Total	17	0.1328				
Treatment	5	0.1128	0.0226	12.72114**	3.2039	5.3160
Covariable starting weight	1	0.000157	0.000157	0.0886	4.8443	9.6460
Residual	11	0.0195	0.00177			

* P < 0.05
P < 0.01

Table IVA. Minimum middle fat (mm)

Treatment	Rep 1 Male	Rep 1 Female	Rep 2 Male	Rep 2 Female	Rep 3 Male	Rep 3 Female
1. Barley + Soybean	18	20	24	29	20	20
(Control)	--	25	23	24	18	20
	--	--	19	18	21	23
2. Barley + 0.69% L-lysine	32	32	32	21	21	24
(total 0.90% lysine)	30	28	33	24	28	18
	29	22	27	19	17	21
3. Barley + 0.50% L-lysine	25	10	29	28	16	14
(total 0.75% lysine)	24	23	24	17	25	15
	22	18	26	22	30	19
4. Treatment 3 + 0.05%	33	26	25	18	20	21
L-threonine	26	24	21	25	24	25
	26	22	27	15	33	21
5. Treatment 4 + 0.10%	26	22	23	18	26	18
DL-methionine	17	26	18	17	23	29
	29	--	28	22	16	29
6. Treatment 5 + 0.10%	32	24	27	25	24	19
L-isoleucine	25	25	28	18	25	18
	--	28	30	20	26	22

Table IVB. Analysis of variance of minimum middle fat.

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) p = 0.05	p = 0.01
Total	102	2344.427				
Treatment (a)	5	197.5564	39.5113	2.7410*	2.3683	3.3389
Rep (b)	2	74.9634	37.4817	2.6002	3.1504	4.9774
Sex (c)	1	206.0355	206.0355	14.29337**	4.0012	7.0771
a x b	10	270.2762	27.0276	1.8750	1.9926	2.6318
a x c	5	116.5186	23.3037	1.6167	2.3683	3.3389
b x c	2	44.5127	22.2564	1.5440	3.1804	4.9774
a x b x c	10	78.6352	7.8635	0.5455	1.9926	2.6318
Covariable	1	162.9588	162.9588	11.3050**	4.0012	7.0771

* $P < 0.05$ ** $P < 0.01$

Table VA. Eye muscle (width x depth) (mm^2) of the 103 pigs.

Treatment	Rep 1 Male	Rep 1 Female	Rep 2 Male	Rep 2 Female	Rep 3 Male	Rep 3 Female
1. Barley + Soybean	--	3496	3195	4080	3818	4131
(Control)	3666	3876	3744	3978	3456	3478
	--	--	3647	4131	4346	3773
2. Barley + L-lysine HCl	2451	3360	3216	3128	3818	3225
(0.9% total lysine)	2940	3408	2655	4080	3332	4480
	2280	3024	3168	3311	3848	3900
3. Barley + L-lysine HCl	3256	3724	3015	4272	3888	3654
(0.75% total lysine)	2747	3713	2418	3444	3240	3200
	2911	3621	3034	3698	3395	3096
4. Treatment 3 + 0.05%	3174	3330	2967	4437	3648	3036
L-threonine	3036	4374	3358	3420	3626	3266
	3034	3519	3036	3588	4000	3430
5. Treatment 4 + 0.10%	3600	3476	3266	4080	3182	3266
DL-methionine	2856	3360	2829	3080	3036	3901
	3696	--	3384	4042	3060	3219
6. Treatment 5 + 0.10%	3010	3042	3066	2970	2856	3358
L-isoleucine	3420	3124	3330	3680	3195	3975
	--	3285	3287	2775	3900	3640

Table VB. Analysis of variance of eye muscle (width x depth).

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) p=0.05	p=0.01
Total	102	21357320				
Treatment (a)	5	1628173	325634.6	2.457*	2.368	3.339
Block (b)	2	882920	441460.0	3.332*	3.150	4.977
Sex (c)	1	2889814	288981.4	21.808**	4.001	7.077
a x b	10	2019311	201931.1	1.524	1.993	2.632
a x c	5	968194	193638.7	1.461	2.368	3.339
b x c	2	1949199	974599.5	7.355**	3.150	4.977
a x b x c	10	1050038	105003.8	0.792	1.993	2.632
Covariable	1	997188	997188.4	7.525**	4.001	7.077
Residual	66	8745643	132509.7			

* $P < 0.05$ ** $P < 0.01$

Table VIA. Nitrogen retention (g/week).

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1. Barley + Soybean (Control)	123.8	126.5	115.1	116.0		
2. Barley + 0.69% L-lysine HCl (0.9% total lysine)	56.0	59.7	64.3	55.3	42.47	53.19
3. Barley + 0.50% L-lysine HCl	57.9	60.5	72.4	67.0	73.01	80.61
4. Treatment 3 + 0.05% L-threonine	74.2	70.2	88.2	73.0	53.43	65.98
5. Treatment 4 + 0.10% DL-methionine	56.2	63.0	64.7	76.4	56.42	64.80
6. Treatment 5 + 0.10% L-isoleucine	41.3	60.6	86.6	79.5	70.79	84.22

Table VIB. Analysis of variance for nitrogen retention.

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.) P=0.05	Variance ratio F(nec.) P=0.01
Total	33	14685.70				
Treatment	5	11565.06	2313.011	20.7535**	2.5336	3.6990
Residual	28	3120.642	111.4515			

** $P < 0.01$

Table VIC. Analysis of co-variance for nitrogen retention with feed intake as the covariable.

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.) p=0.05	p=0.01
Total	33	14697.28				
Treatment	5	9943.194	1988.639	63.4828**	2.5336	3.6990
Covariable starting weight	1	2275.892	2275.892	72.6526**	4.1709	7.5625
Residual	27	845.7928	31.3257			

Table VID. Analysis of co-variance for nitrogen retention with nitrogen intake as the covariable.

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.) p=0.05	p=0.01
Total	33	14685.70				
Treatment	5	945.2005	189.0401	5.2763**	2.5336	3.6990
Covariable starting weight	1	2153.281	2153.281	60.1002**		
Residual	27	967.3605	35.8282			

** P = <0.01

Table VIIA. Nitrogen retained as a percentage of nitrogen intake.

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1. Barley + Soybean (Control)	45.54	42.77	45.43	46.64	--	--
2. Barley + 0.69% L-lysine (0.90% total lysine)	38.07	35.94	38.57	30.64	37.09	39.37
3. Barley + 0.50% L-lysine (0.75% total lysine)	38.03	35.15	41.57	35.78	38.89	40.14
4. Treatment 3 + 0.05% L-threonine	49.95	45.35	45.73	37.41	36.12	38.90
5. Treatment 4 + 0.10% DL-methionine	45.27	43.50	45.81	46.15	42.00	41.74
6. Treatment 5 + 0.10% L-isoleucine	37.99	45.81	48.26	40.95	41.11	46.86

Table VIIB. Analysis of variance for nitrogen retained as a percentage of nitrogen intake.

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.)	
					p=0.05	p=0.01
Total	33	0.0668				
Treatment	5	0.0325	0.0065	5.3117**	2.5336	3.6990
Residual	28	0.0343	0.0012			

** P = < 0.01

Table VIIIA. Apparent nitrogen digestibility (%).

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1. Barley + Soybean (Control)	72.85	72.55	81.30	77.47	--	--
2. Barley + 0.69% L-lysine HCl (0.90% total lysine)	65.25	65.98	66.42	57.49	65.74	66.60
3. Barley + 0.50% L-lysine HCl (0.75% total lysine)	59.84	57.50	66.69	62.44	62.17	63.03
4. Treatment 3 + 0.05% L-threonine	69.88	67.31	63.99	59.14	63.40	62.84
5. Treatment 4 + 0.10% DL-methionine	66.35	68.82	68.72	65.37	63.52	63.26
6. Treatment 5 + 0.10% L-isoleucine	65.24	68.70	71.27	65.23	63.86	69.08

Table VIIIB. Analysis of variance for apparent nitrogen digestibility.

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.) p=0.05	Variance ratio F(nec.) p=0.01
Total	33	0.0840				
Treatment	5	0.0537	0.0107	9.957**	2.5336	3.6990
Residual	28	0.0302	0.0011			

** P < 0.01

Table IXA. Nitrogen retained as a percentage of nitrogen absorbed.

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1. Barley + Soybean (Control)	62.51	58.96	55.88	60.20	--	--
2. Barley + 0.69% L-lysine (0.90% total lysine)	58.35	54.50	58.07	53.30	56.42	59.11
3. Barley + 0.50% L-lysine (0.75% total lysine)	63.55	61.13	62.23	57.31	62.56	63.68
4. Treatment 3 + 0.05% L-threonine	71.47	67.37	71.46	63.26	56.86	61.90
5. Treatment 4 + 0.10% DL-methionine	68.23	63.21	66.66	70.59	66.13	65.97
6. Treatment 5 + 0.10% L-isoleucine	58.22	66.67	67.71	62.83	64.38	67.84

Table IXB. Analysis of variance for nitrogen retained as a percentage of nitrogen absorbed.

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.)	
					p=0.05	p=0.01
Total	33	0.0779				
Treatment	5	0.0437	0.0087	7.153**	2.5336	3.6990
Residual	28	0.0342	0.012			

** P <0.01