THREONINE AS THE SECOND LIMITING AMINO ACID IN BARLEY FOR GROWING-FINISHING PIGS AND GROWING RATS

by

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ABSTRACT

Supplementation with graded levels of threonine to an all barley-lysine diet (0.75% total lysine) improved the daily gain, feed efficiency and carcass quality of growing-finishing pigs. An addition of 0.10% threonine produced the optimum growth response in the experimental animals. No additional improvement was obtained with higher levels of threonine or threonine plus methionine supplementation of the diet. The 0.10% level of threonine supplementation gave performance criteria which were comparable to those obtained with the barley-soybean control diet, except the former diet resulted in significantly higher backfat measurements. Threonine added at levels of 0.15% resulted in higher nitrogen retention than the other barley-lysine-threonine diets. Nitrogen retention on this diet did not differ significantly from the control diet. Barley-amino acid diets resulted in better protein ultilization than barley-soybean control diets. Feeding trials and metabolism trials indicated that methionine was not limiting in barley and that threonine was the second limiting amino acid.

Growth trials with weanling rats confirmed the results obtained in the pig nutritional experiments. Rat experiments indicated that no additional beneficial effects were obtained when lysine levels were increased from 0.75% to 0.90% even when supplemented with additional threonine. Results indicated that supplementation with lysine to a total level of 0.75% and threonine at a level of 0.10% resulted in a highly balanced amino acid ratio for rats, and gave growth rates which approached

those obtained on the control diet. Supplementation of the barley-lysine diet with 0.20% threonine and all other essential amino acids resulted in growth rates and nitrogen retentions which resembled the results obtained with the control diet. The replacement of the essential amino acid mixture with glycine on an equal nitrogen basis did not result in adequate nitrogen retention or growth rates.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Contd.)

TABLE OF CONTENTS (Contd.)

TABLE OF CONTENTS (Contd.)

TABLE OF CONTENTS (Contd.)

LIST OF TABLES

LIST OF TABLES (Contd.)

LIST OF FIGURES

I. INTRODUCTION

Cereal protein is well known to be low in nutritive value, primarily due to a relatively low level of several essential amino acids such as lysine, threonine, methionine and tryptophan. Promising animal growth results have been reported when these limiting amino acids are used as supplements for cereal diets.

Soybean meal and fishmeal are the most common protein concentrates used as supplements for cereal diets, their beneficial effect arising from their overcoming both the amino acid deficiencies and the total nitrogen deficiencies in the grain. The competition between humans and livestock for natural protein sources has been a cause of concern for many years. Natural disasters such as drought can escalate prices and seriously interfere with the economics of swine production. It has long been hoped that complete dependence on conventional protein concentrates can be overcome. The production of amino acids either by fermentation or chemical synthesis offers a possible solution. Although some of these synthetic amino acids are still relatively expensive at present, an increase in demand and thus production level should result in a dramatic reduction in price, hopefully to the level where addition to commercial livestock diet would be economically attractive.

Barley is one of the major feed grains in western Canada. It is important that the limiting factors in barley protein be established so that the most economical form of supplementation either as conventional protein or pure amino acids can be practiced.

It is well established that lysine is the first limiting amino acid in barley as a feed for pigs. Although methionine has been suggested as the second limiting amino acid by some workers, a large number of experiments have shown either no response to methionine or in some cases, a growth depression. In contrast, threonine, when tested as the second limiting amino acid, has shown a response with barley, wheat, rice, milo and sorghum. Many recent reports have confirmed threonine as the second limiting amino acid in grain in the nutrition of several monogastric species including man.

The objective of this study was to further evaluate under practical conditions the requirements of threonine as the second limiting amino acid in Peace River barley for growing-finishing pigs. The design involved feeding graded levels of threonine in a barley diet in the presence of adequate lysine and measuring growth performance, carcass quality and nitrogen balance. Parallel studies were carried out with rats to further examine the nutritive value of barley with lysine, threonine and a mixture of other amino acids.

II. REVIEW OF LITERATURE

A. ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS

Amino acids found in protein were classified by Rose (1938) into two catagories, the essential and the non-essential amino acids, based on growth studies with rats. Essential are those amino acids which cannot be synthesized, at all, or at a rate fast enough to meet the metabolic requirements, and therefore must be supplied in the diet. Non-essential amino acids are those which can be synthesized by animals from normal food constituents via transamination reactions. The number of essential amino acids varies with species and the type of production desired. Those considered to be essential in all mammalian species are valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine and tryptophan.

Arginine and histidine were formerly included in this list. It is now clear that these amino acids are synthesized in adequate quantities to meet minimum metabolic needs. In some species, for example, the rat, better growth does occur when these amino acids are added to the diet. In marked constrast to the mammalian species, the chick cannot synthesize arginine at all and must obtain all its requirements for this amino acid from the diet. In addition, the chick apparently cannot synthesize glycine, serine, glutamic acid and proline at a sufficiently rapid rate for the demands of early growth (Almquist, 1972).

Cystine and tyrosine have been considered as semi-essential amino acids as they can be synthesized only from methionine and phenylalanine respectively. For example, when cystine in the diet is low, the amount of methionine must be sufficient to meet not only the specific methionine requirement, but also part of the cystine requirement.

The biological value of a protein depends on the level of each of the essential amino acids present and falls to a very low level if any one is completely absent.

All the non-essential amino acids are found in animal tissue protein, as they are required in the formation of body protein. They may be regarded as physiologically essential and must be either obtained from the diet or synthesized by animals from other amino acids. It has been reported that glutamic acid, a non-essential amino acid when eliminated from a diet with a low level of casein or synthetic amino acid mixture as the only source of nitrogen, causes a marked depression in growth rate and nitrogen retention (Harper, 1969; Rama Rao et al., 1960). Another role of non-essential amino acids is their 'sparing' action on the essential amino acids. The presence of non-essential amino acids in the diet reduces the necessity of synthesizing them from the essential amino acids (Rérat and Lougnon, 1968; Almquist, 1972). This sparing action is of much importance in practical nutrition.

B. DETERMINATION OF AMINO ACID REQUIREMENTS FOR GROWING ANIMALS USING GROWTH PERFORMANCE AND NITROGEN BALANCE

The amino acid requirements may be estimated in variety of ways. Growth rate and feed conversion efficiency are two commonly used criteria.

However, these criteria are not without disadvantages particularly with *ad libitum* feeding where it is difficult to specify whether the gain following the addition of a synthetic amino acid to a diet is due to an improved appetite or a better amino acid composition *per se*. (Rérat *et al*., 1962).

The determination of amino acid requirements by the use of growth performance as a criterion, can be improved upon by the additional information obtained from nitrogen balance measurements. The nitrogen balance technique has been widely used (Oslage *et al*., 1966; Thorbek, 1969 and Nielsen, 1971).

Another method for measuring nitrogen retention involves carcass analysis. The amount of nitrogen retained in a given interval can be estimated from the difference between the total amount of nitrogen found by analysis at slaughter of specifically treated animals and that contained in control animals slaughtered initially. This technique is widely used in laboratory animal experiments (Becker and Harnisch 1958; Rérat, 1961) and has also been employed in the piglet (Manners and McCrea, 1963; Wood and Groves, 1965) and the growing-finishing pig (Oslage, 1962).

Some discrepancies have been reported between the results obtained by these two methods of estimating nitrogen retention where nitrogen balance has given slightly higher retention figures than those obtained by carcass analysis (Nielsen, 1971; Oslage, 1962; Fuller and Boyne, 1971).

C. METHODS OF DETERMINATION OF AMINO ACIDS REQUIREMENTS FOR GROWING PIGS BY DIET, TISSUE AND BLOOD ANALYSIS

Estimates of specific amino acid requirements of animals vary widely depending partly on the method of assessment and partly on the levels of other dietary constituents e.g. the relationship between the protein content and the caloric content of the diet. Several methods, based on different principles, have been employed. Some of them depend upon the analysis of the diets (Becker *et al.*,1954a,b) which supply graded levels of a specific amino acid in conjunction with adequate levels of vitamins and minerals. Conversely, other methods rely upon tissue analysis, e.g. William *et al.* (1954).

a. Methods Based on Analysis of Diets

The methods used fall into two groups : (1) Analysis of adequate diets and (2) determination of the composition of an "ideal" protein.

(1) Analysis of adequate diets

The amino acid requirements are estimated from the analysis of diets on which satisfactory growth rate has been achieved. This is a rather imprecise method of estimation because one may overestimate all the requirements except for that of the limiting factor or factors of the proteins. However, this method has quite often been used for growing pigs (Evans, 1958; Rérat and Lougnon, 1968).

(2) Determination of the composition of an "ideal" protein

The principle on which this group is based consists of an attempt to build an ideally balanced protein in which the amino acids perfectly meet the requirements while including a minimum amount of additional nitrogen. Two methods are used : in the first case, the animals are fed a mixture of synthetic amino acids as the only nitrogen source of the diet (Mertz et al., 1949). This method is very expensive and consequently difficult to apply to large animals.

Most of the protein sources in the diet are nutritionally limiting in one or several amino acids. An attempt was made to balance the protein requirements by the addition of synthetic amino acids (Rérat and Henry, 1963, Rosenberg, 1959). The requirement can only be determined for the limiting amino acid and is equal to the amount of this amino acid allowing the best performance with that diet. In this method, the basal diet must be well balanced, and adequate in all amino acids except the first limiting, otherwise potentialities of growth of the pig cannot manifest themselves, and the requirements defined in this way are too low. This method can only be applied by means of successive approximation. Another limiting factor may appear when the response to supplementation of the first limiting nutrient causes this and subsequent deficits have to be made up until an "ideal" protein is obtained. The additional quantity of synthetic amino acids must naturally be calculated according to the other amino acids present in the diet and consequently according to nitrogen level in order to avoid an imbalance due to excess.

b. Methods Based on Tissue and Blood Analysis

William *et al*. (1954) were able to demonstrate the relationships between amino acid requirements and tissue amino acid composition of the animals. They showed that the estimation of amino acid requirements could be based on tissue analysis at different ages. The relationships between amino acids in the tissue were postulated as being those which should exist in the "optimum" diet. However, there has been criticism of this method. Ericson (1961) pointed out that the method could lead to the errors of underestimation by not taking into account the differences in the rate of release of different amino acids, the rate of synthesis of proteins; without knowing the partial synthesis of amino acids by the organism, nor their utilization for purposes other than tissue development. The possible sparing action of one amino acid on the requirements of others has not been fully evaluated.

Plasma amino acid (PAA) analysis has also been employed as a criterion to estimate the amino acid requirements of animals. The principle is based on the observations of Morrison *et al*. (1961) with the rat and especially of Zimmerman and Scott (1965) with chicks. These workers presented evidence that when an amino acid is added in graded levels to a diet which is deficient in this amino acid, the plasma concentration of this amino acid remains rather low and constant until the dietary requirement is reached. There is a rapid and approximately linear increase in the concentration of this limiting amino acid in the plasma when increasing levels above dietary requirement are fed.

Dietary amino acid requirements for animals have been estimated by this technique in chicks (Zimmerman and Scott, 1965), rats (Stockland et al., 1970) and pigs (Mitchell et al., 1968b; Bravo et al., 1970; Keith et al., 1972). Variable results have been obtained by employing this technique. However, one must take into account the significant variation in the circulating amino acid levels between animals (Devilat et al., 1970) and the effect of feeding regimes and environmental conditions preceeding the taking of samples. The interval between the time of samplings and the time of the last feed (Clark et al., 1963; Combs et al., 1967; Mitchell et al., 1968b; Ostrowski, 1969) is important. It is evident also that the duration of feeding is important (Pick and Meade, 1970; Mitchell et al., 1968b).

Most recently, an interesting new criterion was proposed by Brown and Cline (1974). They were able to demonstrated that the total urinary nitrogen excretion level could be used as an indicator of protein quality to assess the amino acid requirements of swine and other non-ruminant animals. The principle is based on the decrease of total urinary nitrogen output when an animal is fed with graded levels of an essential amino acid which is deficient in the diet.

D. VARIATION IN AVAILABILITY OF DIETARY PROTEIN

The task of precisely defining the amino acid requirements of the various animal species has long been complicated by adequately assessing the availability of the amino acids in the diet. It has been stressed that not all the amino acids in a protein may be available to synthesize the tissue of the animal. Complete utilization of the amino acid constituents of proteins and of those added occasionally in the free form is only possible under the two following conditions : they must be present at the same time at the site of synthesis (Cannon et al., 1947); the respective proportions of the supply must be balanced, all excesses with respect to needs resulting in a corresponding excretion. Consequently, when determining the requirements for amino acids from the composition of the diet, one must take into account all the factors which may modify the availability of amino acids. The availability of amino acids in the diet may be diminished when the diet contains substances modifying the digestibility of the dry matter or protein or when the protein structure is changed due to treatment such as the application of heat during processing.

Problems involved in determing availability of amino acids have been discussed by Grau and Caroll (1958) and Mauron (1961). Progress has also been made in the past decade in the development of procedures to assay amino acid availability. Despite progress having been made in some aspects, however, the problem is still far from resolved.

E. METHOD OF ASSESSING AMINO ACID AVAILABILITY

a. Chemical Methods

Chemical, microbiological and enzymic methods as well as bioassays have been developed to estimate amino acid availability. The most widely used chemical method is that developed by Carpenter to determine available lysine (Carpenter, 1960; Booth, 1971). The method depends upon the reaction of the dinitroflurobenzene with the ε-amino group of lysine in intact protein to produce a colored lysine derivative which can be estimated after acid hydrolysis.

In spite of there being a 14-28% overestimation of lysine availability when the method is applied to the pure N-α-formyl-N-ε-deoxy-fructosyl-derivatives of lysine, it certainly remains the most appropriate laboratory method for rapidly evaluating heat-damage to proteins (Finot, 1973). No chemical methods are available for the determination of the availability of the other individual amino acids, with the possible exception of methionine, which may be estimated as methionine sulphoxide. Methionine sulphoxide which represents a form of unavailable methionine can be employed to measure the methionine availability (Smith, 1972).

b. Microbiological methods

Microbiological method for determining available amino acids have been widely used, with the method of Ford (1960) who used Streptococcus zymogenes being one of the most popular. Ford (1962) was able to determine the availability of seven amino acids in this way : methionine, leucine, isoleucine, arginine, histidine, valine and tryptophan. The ultimate validity of the method rests upon its degree of correlation with biological assays *in vivo*. For methionine good correlations with *in vivo* results have been obtained (Carpenter *et al*., 1972).

c. Enzymic Methods

Mauron (1961) has been successful in evaluating heat-damage in some material, especially milk products using enzymic methods. The digestion is generally performed with pepsin, followed by pancreation (Mauron *et al*., 1955). A high correlation was obtained between the enzymic *in vitro* digestion of Mauron *et al*. (1955) and dinitroflurobenzene method of Carpenter (1960) with a series of milk samples (Bujard *et al*.,1967). However, the enzymic digestion method is not satisfactory in the routine determination of available amino acids because of incomplete digestion *in vitro* necessitating the use of the unheated material as a control, which in practice would not always be possible.

d. Bioassays

The ultimate standard for measuring amino acid availability is a bioassay with the animal itself. The most widely used procedures are based on the growth response of small animals such as rats (Calhoun *et al*., 1960) and chicks (Bragg *et al*., 1969; Oh *et al*., 1972). Procedures using the rat or the chick have been developed for several amino acids viz. lysine, methionine, isoleucine and tryptophan (Pellett, 1963). Oh *et al*. (1972) using a slope-ratio technique described by Firnney (1964) determined the biological availability of methionine for the chick in various protein supplements. 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. Using the chick to determine available lysine, Carpenter *et al*. (1972) concluded that materials cannot be ranked consistently, but that absolute estimates of methionine availability still vary between assay methods and further improvements are therefore necessary if bioassays are to be used for the calibration of *in vitro* procedures.

Several studies on availability of protein (Olsen *et al.*, 1968; Giovanetti *et al.*, 1970; Sauer, 1972) indicate that considerable variation exists among amino acids in their availability, although amino acid availability depends on the type of protein being studied.

Ideally, diets should be formulated according to amino acid availability rather than total amino acid content. However, this is not yet possible in commercial practice since no rapid method has been established to estimate availability. Moreover, the variation from batch to batch in dietary components further complicates the problem.

F. EFFECT OF PROTEIN DIGESTION AND ABSORPTION IN RELATION TO THE REQUIREMENT

Release of amino acids from proteins depends on the hydrolysis of protein by proteolytic enzyme in the gastro-intestinal tract. The rate at which amino acids are released from proteins may influence the amino acid requirement when determined by the most common techniques of providing graded levels of free amino acids to the basal diet. The delay of amino acid release may cause an underestimate of true amino acid availability provided by the protein source.

It is generally accepted that intestinal hydrolysis is the rate-limiting step in the absorption of the protein. However, the frequent lack of correlation between plasma amino acid levels and the quantities of amino acids ingested raises the question of whether rate of release of amino acids is the only factor affecting the availability of amino acids. Factors such as molecular weight, affinity for different transport system, and competition for absorption on sites have been reported to affect rate of absorption of individual amino acids (Kratzer, 1944; Delhumeau et al., 1962; Orten, 1963; Adibi et al., 1967). However, whether these factors apply to absorption of amino acids released from proteins has been challenged by several researchers (Bergen and Purser, 1968; Coulson and Hernandes, 1970; Nixon and Mawer, 1970a, b). Bergen and purser (1968) and Nixon and Mawer (1970a) concluded that there is no differential rate of absorption occurring among amino acids from food digesta in rat and man. Nixon and Mawer (1970b) reported that the rates of absorption of the amino acids from milk protein and gelatin protein were proportional to the concentration of amino acids in the proteins except for arginine, alanine, proline and glycine. Glutamic acid and methionine were absorbed at a similar rate although it has been reported in experiments with amino acid mixtures (Orten, 1963; Adibi et al., 1967) that glumatic acid was poorly absorbed relative to the rapid absorption of methionine. The evidence suggests the rate limiting factor in the digestion and absorption of a protein is the rate of amino acid release from the protein and not the rate of absorption of individual amino acids. Therefore, determinations on the requirements of amino acids should take this into account.

G. THE USE OF AMINO ACIDS IN PIG NUTRITION

Beeson et al. (1948, 1949) were probably the first workers to seriously attempt to determine the requirements for amino acids in pig nutrition. When they used a purified diet in combination with hydrolyzed fish protein for young pigs (the diet was practically free of tryptophan 0.01%) a remarkable response in growth was observed with the addition of 0.40% DL-tryptophan. During a four-week trial, pigs fed tryptophan gained 634 g per day whereas the control basal diet had zero gain. The result clearly demonstrated the significance of tryptophan in the diet. Beeson and his associates using a similar but more precise technique found later on that 0.2% DL-tryptophan was sufficient for normal growth of pigs (Shelton, 1951b). At the same time, extensive studies on other amino acids were also carried out at Purdue (Beeson et al., 1948; Mertz et al., 1949; Beeson et al., 1953; Mertz et al., 1955) and at Cornell (Bell et al., 1950; Brinegar et al., 1950a, b; Kroening et al., 1962).

Becker and his associates (Becker et al., 1955a, b; Becker, 1959; Becker et al., 1963) at Illinois are another group of workers that have done much to evaluate the quantitative needs of amino acids in the growing pig . A number of other scientist have also carried out such studies, e.g. Evans (1958, 1960, 1962, 1963) at Cambridge and Clausen et al. (1959-1962) in Copenhagen.

Most of the experiments which have been reported have been conducted on weanling pigs (eight weeks of age), although suckling pigs have been used to some extent. The method which is frequently used to assess requirements is to make up a basal diet 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 sources low in particular amino acids, such as tryptophan which is low in hydrolyzed feather meal, zein and gelatin; lysine which is low in zein and cereal protein; methionine which is low in expeller soybean meal and threonine which is low in rice protein. If the protein used is a poorer source of other amino acids than the one in question, a proper supplement of the others must be given.

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. (1950) for weanling pigs.

Clausen et al. (1959-1962) conducted extensive studies with the addition of one or more amino acids to practical diets and demonstrated the beneficial effect of amino acid supplementation.

It is well established that the protein quality in feed can be improved by appropriate supplementation with the first-limiting amino acid. However, the amount of supplementation that can be used effectively is governed mainly by the concentration of the second limiting amino acid present in the feed and the availability to the organism. Proper supplementation is achieved when the amount of the first-limiting is in balance with the amount of the second limiting amino acid and with the rest of the protein. All other nutrients must, of course, be presented in the diet to assure full utilization of the balanced protein.

H. AMINO ACID REQUIREMENT FOR GROWING-FINISHING PIGS

a. General

The protein requirement of animal can be expressed more precisely in term of amino acid requirement. Animals do not need protein *per se* for normal growth but require essential amino acids and an additional source of nitrogen for non-essential amino acid synthesis to support normal growth. In general, there is an increase in amino acid requirements which is directly proportional to the protein and caloric content of the diet (e.g. Bowland, 1962; Becker *et al*., 1963; Clawson, 1967).

b. The Requirement for Lysine

Mertz *et al*. (1949) were the first to demonstrate that lysine was an essential factor in the growing pig diet. Brinegar *et al*. (1949) concluded that 0.58% lysine was the requirement of weanling pigs fed a 10.6% crude protein diet. Later, it was found that the lysine requirement seemed to vary directly with the protein content of the diet. The feeding of a 22% crude protein diet to weanling pigs indicated a requirement based on growth data of 1.20% lysine (Brinegar *et al*., 1950a, b). Change *et al*. (1958) reported a lysine requirement of 0.7% in a diet containing 10 or 15% protein, and 0.9% in a diet of 20% protein content. Similarly, McWard *et al*. (1959) showed the requirement of lysine in a 12.8% protein diet to be 0.71% and in a 21% protein diet to be 0.95% .

Becker (1959) reporting experiments based on chicks and pigs concluded that the requirement for each amino acid is linear, but is not a fixed proportion of the protein content of the diet. He indicated that the requirement for each amino acid, expressed as a percentage of the total dietary protein decreases as the dietary protein level increases. Becker (1963) recommended 0.74% lysine for weanling pigs fed a 16% protein diet.

It has been suggested that dietary caloric density can influence amino acid requirements. Pigs eat more of a diet low in calorics than of a diet of high caloric density (McWard et al., 1959). A decreased feed intake results when lipid is added to the diet (A.R.C., 1967). It therefore seems logical that the amino acid requirements should increase with an increase in dietary energy concentration. Results to support this theory have been presented by many workers (Mitchell et al., 1965b; Lerner, 1968; Bell and Voldeng, 1968; Rérat et al., 1970). Mitchell et al., (1965b) showed that for an animal weighing 22 Kg, 0.65% lysine was sufficient in a diet of 2926 Kcal ME/Kg; but it must be 0.80% in diet of 3,718 Kcal ME/Kg. This was confirmed by Rérat et al., (1970) and Lerner (1968) by means of diets in which the energy content was varied by addition of vermiculite as an inert diluent. The lysine requirement was calculated by Mitchell (1965a) to be 2.4 gm/Kcal ME. Bell and Voldeng (1968) concluded that the lysine requirement for 23 to 57 Kg pigs fed a diet containing 3,330 Kcal DE/Kg was about 0.70%. Bell (1965) also demonstrated that increasing the dietary lysine level from 0.55 to 0.67%, was as effective as increasing the protein level from 13 to 16%, where the response criteria were growth rate and efficiency of feed ultization.

The lysine requirement varies according to sex (Henry et al., 1971). Henry showed that the lysine requirement for female and castrated male pigs ranging from 20 to 60 Kg was 1.02 and 0.82% respectively of a diet containing about 3,400 Kcal DE/Kg.

The British A.R.C. publication (1967) recommended 0.75-0.8 g dietary lysine/100 g air-dry feed for satisfactory performance in young pigs up to 50 Kg and thereafter about 0.6-0.65 g/100 g air-dry feed (0.9-0.95 and 0.7-0.75% of the dry matter in the diet respectively). This contrasts with the N.A.S.-N.R.C. (1968) recommendation of 0.7% in the air-dry feed for 20-35 Kg pigs and 0.5% for finishing pigs.

The lysine requirements for maximum weight gain and for maximum lean meat of finishing pigs have not yet been fully defined. Becker et al. (1966) and N.A.S.-N.R.C. (1968) suggested that a dietary level of 0.50% lysine was adequate for maximum rate of body weight gain in finishing pigs. More recently Brown et al. (1973a) reported a requirement of 0.48% lysine for maximum rate of gain and 0.62% for greatest feed efficiency, using a diet containing 3,501 Kcal ME/Kg and 13.3% crude protein. This result agrees with the results of many investigations on lysine requirements for maximum gain (Cahilly et al., 1963; Lee et al., 1967; Smith et al., 1967). However, Davidson et al. (1962) and Mitchell et al. (1965a) reported that the dietary lysine level required for maximum gain was approximately the same as that required for maximum feed efficiency. The predicted 0.48 ± 0.02% dietary lysine requirement reported by Brown (1973a), was considerably lower than 0.55% reported by Bell and Voldeng (1968).

Cahilly et al. (1963) reported that the lysine requirement for maximizing percentage of lean cuts and primal cuts was greater than for maximum gain. However, Hintz and Heitman (1967) found that lysine supplementation of a 10.5% crude protein diet did not affect carcass characteristics. More recently, Brown et al. (1973b) concluded that the lysine requirement for maximum carcass lean content was 0.51 ± 0.03% and for cross-sectioned area of the longissimus muscle was 0.60 ± 0.05%. The diets fed were corn-soybean containing 13.3% crude protein and 3,501 Kcal ME/Kg. From the results reviewed, it appears that the lysine requirements for maximum growth and feed efficiency are slightly lower than those for maximum carcass lean content.

c. The Requirement for Methionine

Bell et al. (1950) demonstrated the need for methionine in swine rations. Early works by Shelton et al.(1951a) indicated that the methionine requirement in the abscence of cystine was 0.6% for weanling pigs and that 50% of the total sulfur amino acid requirement can be provided by cystine in a diet of 21% crude protein contents. Curtin et al. (1952a, b) showed that when the cystine level was 0.38%, the requirement of methionine did not exceed 0.31% in a diet containing 22% crude protein. Evans (1959, 1960) has repeatedly achieved satisfactory growth and feed conversion efficiency up to a liveweight of 45 Kg with a low protein diet containing approximately 0.5% methionine plus cystine made up of low-protein vegetable foods plus 7% white fish meal. The dietary crude protein was 13 to 15%. Rérat et al. (1962) suggested that the optimal level of total sulfur amino acids for a high rate of growth was 0.6 to 0.7% of the diet for pigs from 20 to 60 Kg liveweight and 0.4 to 0.5% of the diet for pigs heavier over 60 Kg. Becker et al. (1966) indicated 0.5% methionine plus cystine to be adequate for growing pigs of initial weight 20 Kg fed a diet containing 16% protein. They found that cystine could satisfy forty percent of the total sulphur amino acid requirements. Rérat and Henry (1970) demonstrated that the requirement of sulphur amino acid varies with sex. Female and castrated male pigs between 20 and 60 Kg required about 0.47% and 0.52% total sulphur amino acid respectively in a diet containing 3,420 Kcal DE/Kg.

Oestemer et al. (1970) who conducted a series of experiments with growing swine to determine the capacity of opaque-2 corn to provide methionine suggested that the methionine plus cystine requirement of growing pigs from 21 to 40 Kg was somewhat less than 0.42 to 0.50% of the diet as reported by investigators previously cited. The corn diets (crude protein 10.85%) contained 0.275, 0.279 and 0.227% methionine plus cystine. There was no significant improvement in rate of gain, gain/feed or protein efficiency ratio (PER) as a result of supplementing the basal corn diet with 0.07, 0.14, 0.21 or 0.28% DL-methionine.

A recent report by Keith et al. (1972) indicated the methionine requirement of growing pigs (18 Kg) to be 0.46% of the diet. The serum free amino acids technique was employed in this experiment. More recently, Braude and Esnaola (1973) indicated that the optimum performance, nitrogen retention and carcass leaness was obtained with about 4 gm/Kg (0.4%) methionine plus cystine in the diet. The recommended levels of methionine plus cystine for growing pig are given as 6 gm/Kg (0.6%) and 5 gm/Kg (0.5%) of the diet by A.R.C. (1967) and N.A.S.-N.R.C. (1968) respectively.

In an attempt to summarize the work alone in the last two decades, it may be concluded that the methionine plus cystine requirement for the growing pig is within the ranges of 0.4 to 0.6% of the diet when dietary protein ranges from 12 to 18%. The only value outside of this range was reported by Oestemer _et al_. (1970). Braude and Enaola (1973) on the basis of their studies pointed out that the level and source of dietary protein seems to be important in determining requirements. Therefore, an accurate estimate of requirements is still virtually impossible.

Not much work has been done on the sulfur amino acid requirements of the finishing pig. Rérat _et al_., (1962) reported that total sulfur amino acid requirements of finishing pigs (> 60 Kg) was 0.4 to 0.5% of the diet. Studies from the Minnesota station (Meade _et al_., 1966a, b) indicated that the methionine plus cystine requirement of the finishing pig is equal to or less than the requirement of 0.30% of the diet reported by Becker _et al_. (1966). Welch _et al_. (1966) reported that methionine supplementation of a 12% crude protein corn-soybean meal diet containing 0.27% methionine plus cystine fortified with crystalline lysine and tryptophan did not increase nitrogen retention of the finishing pig. Most recently, Brown _et al_. (1974) indicated that total sulfur amino acid requirements for maximum nitrogen retention were 0.17% of a diet containing 14.1% protein and 3,700 Kcal ME/Kg. The values reported by Brown (1974) was strikingly lower than the values reported by the previous investigator cited. The authors points out that no estimated value of total sulfur amino acid lower than 0.17% of the diet has been studied. It is apparent, therefore that more work has to be done to clarify this estimation.

d. The Requirement for Threonine

A requirement for threonine was first demonstrated with diet containing an amino acid mixture in place of protein. Using a purified carbohydrate diet supplemented with amino acids, Shelton et al. (1950) demonstrated that threonine was essential as the pigs lost weight when threonine was omitted from the diet. Beeson et al. (1953) reported that the maximum weight gain and feed conversion efficiency of young pigs were observed when dietary threonine was provided up to a level of 0.4% of the diet (3% of the crude protein). The basal diet was based on maize and included nine essential amino acids. It provided 13.2% crude protein and 0.2% threonine. Mertz et al. (1952) also estimated the threonine requirement of pigs to be 0.4% of the diet. Sewell et al. (1952) investigated the threonine requirement of the 2-3 day old baby pig using semi-purified diets (25% crude protein) including casein and washed soybean protein. They concluded that the minimum L-threonine requirement of the piglet was 0.90 g/100 g DM diet.

Becker et al. (1954a) with a semi-purified diet based on dried skim milk powder providing 12% crude protein calculated that 0.61% L-threonine was a satisfactory dietary concentration for 5 to 9 week-old pigs. However, Evans (1958), using a similar method to estimate threonine requirements, calculated the threonine content of diets which had been proven to give satisfactory performance, namely diets containing 7% fish meal, 20% ground nut meal or 15% soybean meal, to be 0.52, 0.55 and 0.50% respectively. Further experiments conducted by the same author (1963) indicated 0.45% dietary threonine to be adequate for weanling pigs up to 36 Kg liveweight. Less dietary threonine was needed after 36 Kg liveweight. Robinson and Lewis(1963) fed a 95% barley diet supplemented with lysine, methionine, threonine, tryptophan and isoleucine for the growing pig and noted that the performance of growth was equal to the control diet. All these diets contained 16.4% crude protein and provided 0.46% of total dietary threonine.

Lougnon and Brette (1971) fed wheat-soybean meal diet containing 14% crude protein supplemented with lysine, methionine and different level of threonine to 17 Kg pigs concluded that the threonine content of the diet should not be below 0.50% under restricted feeding conditions with diets containing 3,125 Kcal ME/Kg.

Henry and Rérat (1970), using a semi-purified diet with 10% protein from a Norwegian herring meal, supplemental by a free amino acids mixture concluded that the threonine requirement of female pigs of 20 to 50 Kg liveweight was 0.48% of a diet of 3,350 Kcal DE/Kg.

Recently, Sowers and Meade (1972a) concluded the threonine requirement of 15 Kg pigs based upon ADG, G/F and plasma free threonine as criteria to be 0.39, 0.32 and 0.33 respectively. The basal diet containing 10.4% crude protein was based on corn, safflower meal, dried skim milk and crystalline essential amino acids except threonine. Glutamic acid was added to increase the crude protein content to 15%. Another experiment was conducted by the same author (1972b) based on Opaque-2 corn and crystalline amino acids. The basal diet was 10.4% crude protein. The threonine requirement was evaluated by increasing the crude protein level by adding glumatic acid and keeping the essential to non-essential amino acid ratio at 1 : 1 . It was found that the threonine requirement increased from 0.35 to 0.47% when dietary protein was increased from 10.4 to 15.0%. Sowers and Meade's reports indicated that adjustment for requirement is needed with different level of dietary protein.

The N.A.S.-N.R.C. (1968) and A.R.C. (1967) recommendations for weanling pigs are about 0.45 - 0.50% of the diet.

I. AMINO ACID REQUIREMENT OF THE GROWING RAT

Although the amino acid requirements for growing rats have been investigated very extensively and are relatively well established, differences still exist in the requirements listed in various publications. The most widely used and quoted figures for the amino acid requirements of growing rats are those reported by N.A.S.-N.R.C. (1972), and Rama Rao et al. (1959). The essential amino acid requirements of growing rats presented by N.A.S.-N.R.C. (1972), Rama Rao et al. (1959) and Pick and Meade (1971) are summarized in Table 1 for discussion. The N.A.S.-N.R.C. (1972) recommended values were concluded from many studies. The report by Rama Rao et al. (1959) was based on a series of their own experiments in which they gave the animals a basal diet containing 5% casein to which was added an amino acid mixtures varying in the content of each essential amino acid. The values presented were the

Table 1 . Essential amino acid requirements of young rat and growing pig

	Rama Rao *et al.* (1959) Rat	N.A.S.-N.R.C. (1972) Rat	Pick and Meade (1971) Rat	N.A.S.-N.R.C. (1968) Pig (20-35 Kg)
Arginine	0.28	0.60	-	0.20
Histidine	0.21	0.30	-	0.18
Isoleucine	0.55	0.55	0.36	0.50
Leucine	0.69	0.75	-	0.60
Lysine	0.90	0.90	0.70	0.70
Phenylalanine	0.42	-	-	0.50
Phenylalanine + tyrosine	0.72	0.80	0.59	-
Methionine	-	0.60	-	0.50
Methionine + cystine	0.50	-	0.40	-
Threonine	0.51	0.50	0.43	0.45
Tryptophan	0.11	0.15	-	0.13
Valine	0.56	0.60	0.54	0.50

minimum levels on which maximum growth was obtained. The figures of Pick and Meade (1971) were obtained by feeding a diet containing Opaque-2 maize supplemented with amino acids.

From the table, the values reported by Rama Rao et al. (1959) and N.A.S.-N.R.C. (1972) are generally in agreement with each other, except that the arginine requirement is considerably higher in the N.A.S.-N.R.C. recommendations. However, the values presented by Pick and Meade (1971) are generally lower except for the valine requirement which is in agreement with that of Rama Rao et al. (1959). Stockland et al.(1970, 1971) reported that the requirement of young rats with regard to lysine, phenylalanine and phenylalanine plus tyrosine were 0.60, 0.38 and 0.69% of the diet. Stockland et al. (1970, 1971) agreed with Rama Rao et al. (1959) on phenylalanine plus tryrosine levels but derived a lysine requirement only two-thirds that of Rama Rao et al. (1959) and N.A.S.-N.R.C. (1972). These are not inconsequential differences but it is very difficult to say which may be more accurate.

Since the amino acid requirements of animals are interrelated, it is still impossible, from existing data, to define the level of each essential amino acid for optimal growth. The problem is also further complicated by other factors such as the total nitrogen requirement, the essential and non-essential amino acid ratio and the amino acid and energy relationships.

It is generally recognized that the protein and amino acid requirements decline with age (Forbes and Rao, 1959; Hartsook and Mitchell, 1956). Because of a lack of any economic incentive to progressively change to cheaper diets with advancing age in most species the problem has not been studied extensively. Hartsook and Mitchell (1956), by use of a carcass analysis procedure, estimated that the requirements of protein and methionine plus cystine decline from about 28% and 1.3% respectively at 30 days of age to 10% and 0.42% respectively at 50 days of age.

The patterns of essential amino acids required by the pig (N.A.S.-N.R.C., 1968) and the rat (N.A.S.-N.R.C., 1972) as shown in Table 1 are similar except for arginine and lysine. However, the arginine requirements of Rama Rao et al. (1959) for the growing rat are similar to those of the pig (N.A.S.-N.R.C., 1968), while the lysine level for the rat given by Pick and Meade (1971) is similar to that for the pig given by N.A.S.-N.R.C. (1968).

The laboratory rat has been accepted as a satisfactory pilot animal in swine nutritional research although requirements differ in some respects and difficulty is encountered.

J. SUPPLEMENTATION WITH AMINO ACIDS OF GRAIN-PROTEIN CONCENTRATE DIETS AND GRAIN ONLY

Conventional pig diets are based mainly on cereal grains such as corn, barley, wheat, oats and sorghum. Cereals generally provide insufficient essential amino acids and sometimes insufficient nitrogen for the support of normal pig growth. As a result, protein supplements such as soybean meal and fishmeal are normally added in order to improve the protein content and to counteract any essential amino acid deficiency.

Since the protein concentrates are the most expensive components in a pig diet, the possible replacement of these ingredients with amino acid mixtures in commercial practice has reviewed considerable attention. A great deal of effort has been expended to investigate the nutrional and economical feasibility of using amino acid mixtures in high grain diets or diets containing only grain plus minerals and vitamins for pig feeding.

Lysine is the most limiting amino acid in cereal grains with the exception of corn, where tryptophan is generally equally limiting. In the past decade, a voluminous amount of literature has demonstrated that performance can be improved by supplementing grain-based diets with lysine (Evans, 1960; Jones et al., 1962; Rérat and Lougnon, 1965; Rozman et al., 1968; Ostrowski, 1969; Braude et al., 1972). Moreover, the addition of lysine to grain diets supplemented with white fishmeal has been reported to improve pig performance (Braude and Lerman, 1970). Besides lysine, some other amino acid such as threonine, methionine and tryptophan have been claimed as limiting in cereals for the growing pig when analysis are compared with N.A.S.-N.R.C. (1968) requirements. A series of studies were conducted by Müller and coworkers (1967a, b, c, 1968) to investigate the possibility of replacement of protein concentrate with various essential amino acids to single grain or grain mixtures for growing pigs. Müller et al. (1967b) were able to show that pigs fed a cereal mixture supplemented with lysine, threonine, tryptophan and methionine, and in some cases isoleucine, gave a performance equal to that obtained with diets containing conventional protein concentrates. Robinson and Lewis (1963) reported that the growth rate and feed efficiency improved markedly in growing pigs receiving barley diets where lysine and methionine are added and that the performance was equal to that obtained with a barley-soybean control when there was a further addition of DL-tryptophan, DL-isoleucine and DL-threonine.

Müller *et al.* (1967b) with weanling pigs of 14-18 Kg fed a cereal diet of barley, wheat and oats plus an amino acid mixture to 50 Kg liveweight found that the combination of lysine and threonine had an extraordinarily favorable effect upon gains and feed conversion. The supplement of lysine alone enhanced gains in one case by 19%, in another case by 22%. The combination of lysine and threonine increased gains by 59% in the first and by 92% in the second trial. The combination of lysine and 0.02% tryptophan showed a positive response, but caused a depression in the 0.04% tryptophan supplemented level. A supplement of lysine alone increased weight gain by 26-28% whereas the combination of lysine and tryptophan increased gain by 35-42%. Another trial compared lysine alone and lysine plus methionine and showed no significant difference in gain, indicating methionine not to be the second limiting amino acid in cereal mixtures for the growing pig.

Jensen *et al.* (1965) using the feeding standards of Becker *et al.* (1963) indicated that lysine and possibly methionine were required as a supplement to sorghum for pigs from 45 to 90 Kg liveweight, whereas lysine and tryptophan were the required amino acids in the supplementation of corn. The addition of 0.25% lysine to grain improved the growth rate but gave no response to the further addition of methionine. Beames *et al.* (1968) also indicated that up to 0.2% lysine addition improved growth rate and feed efficiency for the fattening pig but improvement in carcass quality was slight.

Bowland (1962) reported that the addition of 0.2% L-lysine to a barley, wheat and soybean meal diet containing 13.6% protein made the diet equivalent to one containing 16% protein as measured by gain and efficiency of feed ultization in pigs over the 14 Kg to 44 Kg body weight range.

Soldevila and Meade (1964) indicated that methionine is not seriously limiting in barley for they suggested that lysine is the first limiting amino acid and probably threonine is the second.

Recently, Rérat and Henry (1969) also reported that growth rate on barley only (10.1% crude peotein) was not improved by methionine supplementation. Chung (1973) also indicated that no growth improvement was obtained by methionine supplementation of a barley amino acid diet (10.3% crude protein).

Lysine and threonine have been repeatedly demonstrated to be the first and second limiting amino acids in rice protein (Rosenberg et al., 1959) and barley (Sure, 1955) for the rat. Sure (1954) showed also that wheat and rye were deficient in lysine and threonine and that rye was also deficient in valine. Lysine and threonine were reported to be limiting in milo also (Pond et al., 1958). Threonine was suggested to be the second limiting amino acid in milo for rats (Pond et al., 1958).

Recently, Veum et al. (1973) reported that normal and opague-2 corn diets supplemented with lysine, methionine and tryptophan apparently were deficient in isoleucine, valine, threonine and possibly phenylalanine when fed to growing rats. These results were supported by those of Pick and

Meade (1971) who reported that excellent daily gain, gain-feed ratio and PER could be obtained when a diet containing 89.5% opaque-2 corn was supplemented with 0.1% L-isoleucine, 0.18 to 0.35% lysine, 0.2% DL-methionine, 0.12% L-phenylalanine, 0.14% threonine and 0.08% L-valine. The final amino acid level of this diet was 0.70% lysine or less, 0.40% methionine + cystine, 0.43% threonine, 0.36% isoleucine, 0.54% valine and 0.59 to 0.65% phenylalanine + tyrosine.

K. FACTORS ASSOCIATED WITH AMINO ACID SUPPLEMENTATION OF GRAINS

a. Variation of Grain Amino Acids Profile and Availability

Maize, Barley, wheat and sorghum are the most commonly used cereal grains in pig diets. Although these grains are commonly limiting in the essential amino acids, lysine, threonine and methionine, the quantities of these and other amino acids vary from one to another. Accordingly, the optimal amino acid supplementation of grain will vary from grain to grain. For examples, lysine and tryptophan are equally and first limiting in maize since the expressed response for lysine becomes minimum without tryptophan addition (Gallo and Pond, 1968; Pond and Jones, 1964). However, in barley, lysine is the most limiting, followed by threonine with methionine possibly third limiting (Chung and Beames, 1972, 1974). Tryptophan is adequate in barley according to the N.A.S.-N.R.C. (1968) standards for growing pigs. Similar to barley, the first and second most limiting amino acids of wheat are lysine and threonine respectively (Shimada and Cline, 1974). Variation in availability of amino acids in different protein sources further complicate the amino acid supplementation problem. Olsen et al. (1968) reported considerable variation in amino acid absorption from wheat by-products. The absorption was highest for cystine, glutamic acid, histidine and arginine in all products tested whereas the lowest percentage absorption was found for threonine, lysine, alanine, glycine, aspartic acid, methionine and isoleucine. Some results (Carlson and Bayley, 1970; Sauer, 1972) have indicated that the true digestibilities of glutamic acid, proline, serine, lysine, phenylalanine, arginine and histidine in soybean meal for young pigs are higher than those of alanine, threonine, methionine, isoleucine and glycine. Giovanetti et al. (1970) and Sauer (1972) also reported lower availabilities for lysine, alanine, threonine and methionine in triticale, wheat and barley, in constrast to the high digestibilities of proline, glutamic acid, arginine, histidine and phenylalanine.

The evidence suggests that amino acid requirements should be expressed on an available basis and that the content of dietary components should be given on a similar basis.

b. Protein Level in Relation to Amino Acid Requirements

The total protein content of a diet may have an important effect on the amino acid requirement. According to Becker (1958) the requirement for essential amino acids increases with the protein content of the diet, but the increase is not in direct proportion. The argument of disproportional relationship is supported by an experiment in which the requirement of isoleucine, expressed as a percentage of the nitrogenous matter of the diet, decreased as the dietary protein increased (Becker et al., 1957).

This was further confirmed for the lysine requirement (McWard et al., 1959) and the tryptophan requirement of growing pigs (Boomgaardt and Baker, 1973).

Kroening et al. (1965) think that the necessary content of sulfur amino acids in the diet varies in the same direction as, but more slowly than the protein content,even when growth performances are almost identical.

Boomgaardt and Baker (1970, 1971) indicated that the lysine and tryptophan requirements of young chicks remain constant when expressed as a percentage of the protein. These results contradict the generally accepted concept that the requirement for an essential amino acid decreases linearly as a percentage of the protein and increase curvilinearly as a percentage of the diet.

Rosenberg (1959) indicated that the effect of a change in protein level is a problem of considerable practical importance because feeds for domestic animals range from 10% protein for the fattening pig to 30% protein for the turkey poult. Percentage protein in this corn-soybean diet was plotted against the percentage of amino acid in the protein for lysine, methionine and the combination of methionine and cystine in corn-soybean diets. As the level of protein was increased, the relative amount of lysine in the diet increased, while the relative amount of the sulfur-bearing amino acids decreased. Therefore, methionine deficiency is likely to occur as the protein level increases. Conversely, lysine deficiency is likely to occur as the protein level decreases (Rosenberg et al., 1959). Accordingly, the adjustment of most essential amino acid is needed when the protein level is altered.

c. Significance of Essential and Non-essential Amino Acid Ratio

Changes in nitrogen content of the diet will generally be associated with an alteration in the supply not only of essential but also of non-essential amino acids. A diet composed of minimum levels of only the essential amino acids supports a slow rate of growth only (Wretlind, 1949). Additional sources of nitrogen are required for normal growth and normal physiological function. The ratio of essential (EAA) to non-essential amino acids (NEAA) in a diet for optimum growth has recieved much attention.

Stucki and Harper (1962) reported that the NEAA/EAA ratio might vary in the rat from 4 to 1 without decreasing growth rate or nitrogen retention. They state that either wide or narrow ratios would give unfavorable results. Mitchell et al. (1968a) reported that the highest nitrogen retention of pigs was obtained when the diet contained equal amounts of essential and non-essential amino acid. Recently, Henry and Rérat (1970) indicated that the best growth performance and the greatest sparing of essential amino acids was obtained from a well balanced diet furnishing approximately 60% of the total amino acids in the non-essential form.

d. Relationships between Protein and Energy Content of a Diet

The ultization of protein can only be maximized when there is sufficient energy in the diet from non-protein sources to satisfy the requirement of the organism for calories. The energy content in the diet is of critical importance for the successful amino acid supplementation of animal feeds. Rosenberg et al. (1955) reported that no response was observed with the addition of methionine to a corn-soybean meal diet for chicks in spite of the fact that the first-limiting amino acid in the diet was considered to be methionine. However, the chicks responded to the supplemental dietary methionine with improved growth and feed efficiency when fat was added to the diet. This effect was also shown when fat was replaced by carbohydrate (Baldini and Rosenberg, 1957). These studies suggest a direct relation between caloric density and the amino acid requirements in the diet. The studies also indicated that three caloric levels required three different levels of methionine for optimum performance of the chick (Baldini and Rosenberg, 1955; Baldini and Rosenberg, 1957). Baldini et al. (1957), Rosenberg and Culik (1955), and Williams and Grau (1956) also showed that the caloric density is one of the factors governing the amino acid requirements of growing turkeys, rats and chicks.

The relationship between the protein content and the energy content of a diet has also been subjected to many studies using piglets (Bowland, 1964; Standish and Bowland, 1967) and the growing-finishing pig (Sewell et al., 1956; Abernathy et al., 1958; McWard et al., 1959; Henry and Rérat, 1964; Robinson and Lewis, 1964; Robinson et al., 1964 ; Robinson, 1965a; Clawson, 1967; Cooke et al., 1972a, b). Increases in the energy content of a diet reduce feed intake, but improve feed efficiency and in some cases also improve growth rate. However, the benefits of increased energy intake disappears when protein intake is insufficient. Conversely, an increased protein intake cannot be ultilized effectively for body protein synthesis when the diet contains insufficient energy.

The studies of the relationships between lipid content and amino acid content of a diet by a number of researchers (Anderson and Bowland, 1967; Mitchell et al., 1965b; Rérat et al., 1970) showed that the level of amino acids in the diet have to be increased with higher dietary lipid content because of the accompanying reduction in feed intake.

In terms of energy-lysine ratio, Robinson et al. (1964) concluded that the best growth performance and carcass lean content for pigs were recorded when the ratio of Kcal DE to Kg dietary lysine was approximately 3,500 at all level of energy concentration.

Lawrence (1971) indicated that the narrow caloric/protein/lysine (CPL) ratio diets[1] when compared with wide CPL ratio diets[2] gave better growth rates and energetic conversion efficiencies and higher cold carcass weights, smaller back fat deposits and greater percentage of lean and bone but smaller percentage of fat in the carcass.

The effect of the interaction between protein and energy contents of the diet on pigs performance and carcass quality has been investigated by many workers. In general, it has been observed that as the concentration of energy in the feed is raised, dietary protein level needed for best performance increases. Growth rate and efficiency of feed utlization are generally

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1. Digestible energy : crude protein ratio = 198 : 1
   Crude protein : Lysine ratio = 19.8 : 1

2. Digestible energy : crude protein ratio = 263 : 1 or 273 : 1
   Crude protein : Lysine ratio = 27.6 : 1 or 26.9 : 1

improved by raising the nutrient concentration whilst carcass quality benefits by an elevation of the protein intake or of the overall nutrient supply.

Although energy and protein intake influences growth performance and carcass characteristics of the growing pig, many experiments fail to demonstrate any significant interaction between energy and protein in terms of these criteria indicating that they are independent from each other (Cooke et al., 1972a, b; Lodge et al., 1972a). Cooke et al. (1972b) indicated that protein influenced growth and carcass lean content but that the effects were overridden by intake of energy so that there was little indication of an increased protein intake counteracting the adverse effects of an increased energy intake on carcass quality.

e. Amino Acids Imbalances and Interactions

It is generally recognized that amino acid imbalances can result in a decreased growth rate and a loss of appetite. An imbalance may be expressed as a depression in growth (or any other adverse effect). Nitrogen imbalances have been reported in pigs (Meade, 1956a, b; Miner et al., 1955), however, their effects have been studied mostly in the rat. Harper (1961) indicated two kinds of imbalances were possible. The first is induced by adding small amount of amino acid (but not the most limiting one) to a low protein diet and may be overcome by simply supplementing with the limiting amino acids. The second kind of imbalances is due to the protein source (or amino acid mixture) being totally devoid of a given amino acid. For example, addition of gelatin may decrease the growth of rats unless tryptophan is added (Harper, 1959). More examples of imbalance studies of the rat can be found in the report by Harper (1964).

A series of studies by D'Mello and Lewis (1970a, b, c) using the chick clearly demonstrated that the requirements for amino acids in animals are interdependent. The studies showed that graded levels of excess dietary lysine, leucine and theronine increased the quantitative requirements of arginine, isoleucine and tryptophan respectively. A subsequent experiment reported by D'Mello (1973) indicated that raising the isoleucine level in a diet increases the requirements for leucine and valine. These patterns of interactions are reflected in the plasma concentration of leucine, isoleucine and valine (D'Mello, 1974). The unique interaction between lysine and arginine in chick nutrition reported by D'Mello and Lewis (1970a,b,c) supports the observations of some other researchers (O'Dell et al., 1958; Jones, 1964; Smith and Lewis, 1966). Similarly, interrelationships between several amino acids have been observed in rats. These include interactions between leucine, isoleucine and valine (Harper et al., 1954, Benton et al., 1956; Rogers et al., 1967) and between threonine and trytophan (Salmon, 1954; Morrison and Harper, 1960; Florentino and Peason, 1962).

The specificity of interaction among leucine, isoleucine and valine is further exemplified by recent work with the pig (Oestemer et al., 1973).

f. Animal Variation : Age, Sex and Genetic Factors

Dietary protein and amino acids are utilized for body protein synthesis (anabolic function) and for maintenance. In the rapidly growing animal, the anabolic functions account for a large proportion of the amino acid requirements. Conversely, the replacement functions dictate the amino acid requirements in the mature animal. As a result, greater abundance of high quality amino acids are required by young growing animals than for mature animals. In addition, amino acid requirements progressively decrease as age increases.

Oslage et al. (1966) showed that the efficiency of nitrogen retention decreases with age. In their experiments, nitrogen retention efficiency was about 52% in young animals weighing 25 Kg and decreased to about 35% at 100 Kg and to 22% at 160 Kg liveweight. Similar results were obtained by some other workers (Rérat and Henry, 1964; Robinson, 1964). Evidence further indicates that amino acid allowances should be reduced with age.

Nitrogen utilization and thus nitrogen requirements are affected by sex. In general, females grow slower than castrated male and differ in body composition. However, at equal rates of growth and feed efficiency, females would have leaner carcasses indicating higher nitrogen retention (Bowland and Berg, 1959; Robinson et al., 1964; Lodge et al., 1972b). Consequently, the respective needs for protein and energy may vary according to sex (Bowland and Berg, 1959; Blair et al., 1969b). Similarly, the amino acid requirements of the female are different from those of the male. Germann et al.(1958) showed that females had optimum growth rate with less lysine than was necessary for males. Rérat and Henry (1970) indicated that the methionine plus cystine requirements of growing pigs (20 - 60 Kg) varied with sex. Female pigs needed 0.52% of methionine-cystine in the diet whereas castrated pigs required only 0.47% in the diet.

Bayley and Summers(1968) indicated that boars responded more than gilts to increased protein level in the diet. Bell (1965), Blair et al. (1969a) and Pierce and Bowland (1972) were able to demonstrate that gilts were more efficient converters of high protein diets than barrows with the differences reducing as the protein levels decreased.

It has been reported that there are important differences between breeds and within the same breed as regards growth performance and body composition (Hetzer et al., 1963). The high pressure of selection to improve the genetic potential for a leaner carcass may create a change of amino acid requirements. To date very little has been reported. The effect of the genetic origin of the animals on nitrogen retention has been demonstrated by Anderson and Bowland (1967) who reported that the Large White pig retains nitrogen better than the Large White × Lacombe pig. Bayley and Summers(1968) found a significant interaction between strain of pig and protein level. Lacombe and Yorkshire pigs increased liveweight gain with increased protein level from 13 to 16% whereas no response was found in the Hampshire × Landrace and Landrace.

On the basis of the above results recommendation for dietary nitrogen should take breed and strain into account.

g. Feeding Practices : *Ad Libitum* vs Restricted Feeding

Level of feeding influences the amino acid requirements of pigs. *Ad libitum* feeding is commonly employed in North America whereas restricted feeding is usually practiced in European countries. The purpose of restricting feed intake is to control the energy intake in order to improve the carcass quality of the animal. A review of energy and protein relationships, indicates that the level of essential amino acids in the diet must be adjusted according to the degree of feed restriction applied. According to Rérat *et al*. (1971), when energy intake is restricted to 80% of an *ad libitum* level, the increase in nitrogen and more particularly amino acids content must be about 10% to avoid modification of the protein synthesis.

It has been recommended (Rérat, 1972) that amino acid requirement should be expressed in the form of daily amounts. This recommendation could then be transformed into concentrations when formulating diets.

III. PIG EXPERIMENT I

A. EXPERIMENTAL PROCEDURE

a. General

Many experiments have shown that lysine is the first limiting amino acid in barley as a feed for pigs. Although methionine has been suggested as the second limiting amino acid by some workers a large number of experiments have shown either no response to methionine or in some cases, a growth depression. In contrast, threonine, when tested as the second limiting amino acid, has shown a response with barley (Müller et al., 1967c; Chung, 1973) and with sorghum (Pond et al., 1958). Several recent review papers (Scrimshaw and Altschul, 1971) have confirmed the wide-spread finding of threonine as the second limiting amino acid in small grains in the nutrition of several species, including man.

The present experiment is designed to evaluate the response to increasing levels of threonine in a diet of barley plus adequate lysine, and to investigate the effect of the addition of methionine to the diet containing the highest level of added threonine.

b. Design

The experimental layout was that of a completely randomized three-factor (diet — treatment, sex — male or female and period — rep) design with a factorial arrangement of treatments and three observations per each treatment combination. (e.g. appendix page 146)

c. Animals

A total of one hundred and eight Yorkshire and Yorkshire × Landrace cross pigs were randomly allocated to six dietary treatments with equal sex numbers (3 castrated males and 3 females) to a pen. These pigs were placed on trial at an average body weight of 20 - 21 Kg and thereafter were weighed weekly and were slaughtered when a body weight of 85 Kg was attained. The last two pigs in a pen were sent for slaughter when the body weight of the heavier one exceeded 85 Kg. Diets and pens were allocated on a random basis.

d. Diets

The six diets were fed in dry mash form. A discription of the diets is given below. Ingredients are listed in Table 2 .

1. Barley-soybean meal to supply 0.75% total lysine (control diet)
2. Barley + 0.444% L-lysine HCl to supply 0.75% total lysine
3. Barley + 0.444% L-lysine HCl to supply 0.75% total lysine + 0.05% L-threonine
4. Barley + 0.444% L-lysine HCl to supply 0.75% total lysine + 0.10% L-threonine
5. Barley + 0.444% L-lysine HCl to supply 0.75% total lysine + 0.15% L-threonine
6. Barley + 0.444% L-lysine HCl to supply 0.75% total lysine + 0.15% L-threonine + 0.10% DL-methionine

Table 2. Percentage Composition of Diets used in Pig Experiments I & II (Air dry basis)

Ingredients	Diet No					
	1	2	3	4	5	6
Barley	81.34	96.38	96.33	96.28	96.23	96.13
Soybean meal (45%)	15.60	-	-	-	-	-
L-lysine HCl*	-	4.44	4.44	4.44	4.44	4.44
L-threonine*	-	-	0.05	0.10	0.15	0.15
DL-methionine*	-	-	-	-	-	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	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral and vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50

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 feed grade and was 98% pure, containing 78% L-lysine. The L-threonine was pure, DL-methionine was 98% pure. All were produced by the Ajinomoto Co., Japan.

The amino acid content and chemical composition of the barley and the soybean meal are shown in Table 3 . The amino acid composition of each diet is listed in Table 4 . All diets were formulated to contain adequate amounts of all nutrients known to be required for growing-finishing pigs except for total protein and amino acid content.

e. Management

(i) Housing

This experiment was conducted at the Swine Research Unit, on the University of British Columbia Campus. The building is insulated, with air exhaust fans fitted with thermostatic controls set at 18.5° C. Pens are concrete and partially slatted and of total area 5.6 m^2.

(ii) Feeding method

Pigs were fed twice daily at 8:00 A.M. and 1:00 P.M.. Feeding was from troughs on an *ad libitum* basis with maximum allowance limited to 2.7 Kg per day. Feed consumption records were kept daily. Water was supplied *ad libitum* by drinking nipple.

(iii) Feed mixing and storage

All feed ingredients were purchased and stored prior to the commencement of the experiment to ensure a uniform diet composition throughout. The barley was stored in the whole form. Diets were prepared as required in 450 Kg batches by hammer milling (7 mm screen) the barley

Table 3. Content of essential amino acids and proximate constituents of barley and soybean meal on a dry matter basis.

Component	Concentration(g/100g d.m.) Barley	Soybean Meal
Arginine	0.539	3.172
Histidine	0.247	1.621
Isoleucine	0.471	1.800
Leucine	0.870	4.089
Lysine	0.491	3.041
Methionine	0.200	0.709
(Cystine)	0.241	0.701
Phenylalanine	0.631	2.521
(Tyrosine)	0.338	1.717
Threonine*	0.443	1.743
Tryptophan	0.749	0.126
Valine	0.808	2.052
Crude protein % (N × 6.25)	11.4	51.3
Ash %	2.5	6.3
Crude fibre %	6.72	5.7
Crude fat %	3.19	1.49

* Uncorrected value from destruction of acid hydrolysis (Blackburn, 1968)

Table 4. Content of essential amino acids and proximate constituents in diets (g amino acid/100 g) in Pig Experiments I and II .

Amino acid	Diets (g/100 g A.D.)					
	1	2	3	4	5	6
Arginine	0.82	0.52	0.52	0.52	0.52	0.52
Histidine	0.40	0.24	0.24	0.24	0.24	0.24
Isoleucine	0.58	0.45	0.45	0.45	0.45	0.45
Leucine	1.18	0.84	0.84	0.84	0.84	0.84
Lysine	0.75	0.75	0.75	0.75	0.75	0.75
Methionine	0.24	0.19	0.19	0.19	0.19	0.29
(Cystine)	0.27	0.23	0.23	0.23	0.23	0.23
Phyenlalanine	0.80	0.61	0.61	0.61	0.61	0.61
(Tyrosine)	0.48	0.33	0.33	0.33	0.33	0.33
Threonine	0.56	0.37	0.42	0.47	0.52	0.52
Tryptophan	0.19	0.11	0.11	0.11	0.11	0.11
Valine	0.98	0.78	0.78	0.78	0.78	0.78
Dry matter %	88.55	87.78	87.61	88.90	87.58	87.90
Crude protein %	17.58	11.01	11.36	11.36	11.32	11.41

and then mixing it with the other ingredients in a vertical mixer* for 5 to 10 minutes.

f. Records

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

g. Chemical Analysis

A.O.A.C. methods (1965) were employed in the analysis of all feed for moisture, ash and crude protein. Acid detergent fibre was determined by the method of Van Soest (1963). For amino acid analysis, the hydrolyzates of barley and soybean meal were prepared by acid hydrolysis (Kohler and Palter, 1967) except for cystine and tryptophan as these two amino acids are poorly recovered from acid hydrolyzates. Therefore, cystine was determined as cysteic acid by oxidation hydrolysis (Moore, 1963). Methionine was determined as methionine sulfone from this chromatogram. Tryptophan was determined by ion-exchange chramotography after alkaline hydrolysis by the method of Hugli and Moore (1972).

h. Carcass Measurements

Dressed weight was measured on the hot carcass (with head). The

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* Kelley Duplex Mill Machinery, size 220, serial 57364. The Duplex mill m f g. Co., Springfield, Ohio.

carcass was then chilled at 4° C for 96 hours and eye muscle area, eye muscle index (length "A" × width "B") and backfat thickness ("C" without skin) determined on the surface exposed by cutting the carcass at right angles to its length at the junction of the 7th and 8th vertebrae anterior to the dorso-sacral junction (Buck et al., 1962). Maximum shoulder fat, minimum middle fat and maximum loin fat were measured according to the Canada Department of Agriculture (1968) methods.

i. Calculations

Average daily gain was determined as the b value in the linear regression equation derived from weekly body weight $\hat{y} = a + bx$, where " $\hat{y}$ " is a estimated body weight, and "x" days on trial. The average daily feed consumption and feed conversion efficiency (total feed eaten divided by total body weight gain, both expressed in tha same units) were calculated for each pen. Carcass measurements were determined individually for each pig.

j. Statistical Analysis of Data

The data were subjected to analysis of variance using UBC-MFAV (Halm and Le, 1974) computer program. Since the program cannot compensate for missing values, carcass measurements of one missing value was estimated by missing data calculation (Snedecor and Cochran, 1967).
Two different analyses were made. For mean daily gain, dressing percentage, total back fat , "C" back fat, eye muscle index and area, and experimental days, the individual treatment combinations (diet, sex, period)

and their interactions were investigated (eg. page 147). For mean daily dry matter feed intake and feed conversion efficiency, the only factor was treatment (diet) with three replications (three groups of 6 animals each) each (eg. page 145). Mean daily gain, feed conversion efficiency, dressing percentage and days on trial were analyzed with starting weight as a covariable. Total back fat, "C" back fat, eye muscle area and eye muscle index were analyzed with final body weight, average daily gain and carcass length as covariables. Means from comparisons showing a significant "F" value were tested using Tukey's test (1953).

B. RESULTS

Nutrient contents of ingredients and diets are listed in Table 2. The experimental growth and carcass data analyses are summarized in Table 5. There was no difference in average daily intake among all treatments indicating that the effect of differences were not due to appetite. There was no significant effect of treatments on dressing percentage. The barley-soybean meal diet produced a significantly greater growth rate than all other diets, except for the 0.10% threonine supplemented diet. There were no significant differences in growth rates between 0.10% threonine, 0.15% threonine and 0.15% threonine plus 0.10% methionine diets, but all gave superior growth to that obtained on the diet containing no added threonine. The rate of growth was significantly greater on the soybean-control diet than on any other treatments over the body weight range 20 to 45 Kg whereas no significant differences were observed between diets containing 0.10% threonine, 0.15% threonine and 0.15% threonine plus 0.10% methionine additions and the barley-soybean control diet for liveweight gains from 45 Kg to finishing weight.

There were no significant differences in terms of feed conversion efficiency between the 0.10% threonine, 0.15% threonine, 0.15% threonine plus 0.10% methionine and soybean control diets. Feed efficiency was similar in diets containing 0.10% or greater added threonine, all of which produced a feed efficiency which was better than that obtained on the basal diet or the basal plus 0.05% threonine. The addition of 0.10%, 0.15% threonine and 0.15% threonine plus 0.10%

Table 5. Summary of the effect of the addition of amino acids to barley on body weight gain, feed consumption (D.M.), feed conversion (D.M.) and carcass measurement in Pig Experiment I .

Treatment	1 soy-control	2 0.75%lys (Basal)	3 B + 0.05%thr	4 B + 0.10%thr	5 B + 0.15%thr	6 B + 0.15%thr+ 0.10%met.	SE*	Result of significance test**
Starting wt.(Kg)	20.32	20.41	20.92	20.36	19.94	20.22	-	
Final wt.(Kg)	86.58	85.41	85.50	85.50	84.89	85.94	-	
Mean daily D.M. Feed intake (Kg)	1.943	1.821	1.839	1.911	1.823	1.856	±0.015	146352
Mean daily gain (Kg)								
Start to 45 Kg	0.648	0.466	0.522	0.556	0.536	0.565	±0.013	164532
45 Kg to finish	0.729	0.603	0.619	0.744	0.687	0.686	±0.018	145632
Start to finish	0.702	0.553	0.584	0.675	0.625	0.634	±0.013	146532
D.M. Feed(Kg) / Wt. gain (Kg)	2.831	3.407	3.286	2.951	3.017	3.020	±0.018	145632
Dressing %	78.59	79.37	80.15	78.18	79.04	79.26	±0.63	326514
Total back fat (mm)	82.45	96.74	94.93	91.51	88.92	90.95	±2.00	234651
Back fat 40mm from mid-line	14.53	20.18	18.53	17.14	16.66	18.07	±0.68	236451
Eye muscle index A×B (mm^2)	4274	3432	3694	4001	3902	3740	±78	145632
Eye muscle area (mm^2)	2991	2414	2645	2758	2658	2649	±17	145632
Experimental period(days)	96.9	121.9	116.8	100.8	106.6	106.5	±1.83	146532

* Standard error of treatment means.

** Treatment numbers not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

methionine to the basal diet improved feed conversion efficiency by 15.5%, 12.9 and 12.8% respectively.

There was no significant treatment effect on dressing percentage. The barley-soybean control pigs had the lowest total back fat thickness which was significantly different from the barley-lysine basal diet (Treatment 2), 0.05% threonine (Treatment 3) and 0.10% threonine (treatment 4) supplemented diets but not significantly different from the highest threonine (0.15%) (Treatment 5) and 0.15% threonine plus 0.10% methionine (Treatment 6) diet. However no significant differences were observed between the various barley-amino acid treatments for total back fat thickness. The carcass length had a significant negative relationship to total back fat thickness. Again the control diet produced the lowest back fat 40 mm from the mid-line, being significantly less than that of Treatments 2, 3 and 6 but not significantly differente from Treatments 4 and 5 . No significant differences were observed among the four diets containing added threonine. Back fat 40 mm from the mid-line in Treatment 2 did not differ significantly from measurements for Treatments 3,4 and 6 but was significantly higher than values for Treatments 5 and 1. The covariance analysis showed that carcass length was significantly negatively correlated with back fat 40 mm from the mid-line.

There was no difference in eye muscle index between the carcasses from pigs on the control diet and those on Treatment 4 but the former was significantly higher than that for all other treatments. There were no significant differences among the threonine supplemented diets. The basal diet showed the lowest eye muscle index though it was not significantly different from the means for Treatments 3 and 6. Similar results were observed for eye-muscle area except that the control diet produced significantly higher areas than all other treatments. Covariance analysis indicated that eye muscle index and eye muscle area were significant negatively correlated with average daily gain.

The mean daily gain was significantly higher in barrows than in gilts. Gilts had consistently significantly lower backfat measurements and larger eye muscle indices and eye muscle areas than barrows. Dressing percentage was not affected by sex. There was no diet × sex interaction in mean daily gain or carcass measurements (Table 6).

Table 6. Comparison of the effects of supplementation with amino acids on mean daily gains and carcass measurements[§] of gilts and barrows

	Gilt (S_1)	Barrow (S_2)	Significant F_1 Test
Mean Daily Gain (Kg)	0.612	0.646	**
Carcass Measurement			
Dressing %	79.69	77.08	N.S.
Total back fat (mm)	88.09	93.74	**
Back fat 40 mm from mid line (mm)	16.74	18.29	*
Eye muscle index (mm^2)	3986	3696	**
Eye muscle area (mm^2)	2782	2589	**

§ Carcass measurements were analysed with final body weight, mean daily gain and carcass length as covariables.

* $P < 0.05$

** $P < 0.01$

C. DISCUSSION

Based on the results obtained in this feeding trial, it might be concluded that the growing-finishing pig can perform well based on an all-barley low-protein diet with addition of an optimum level of lysine and threonine, providing additional vitamins and minerals are given. With the 0.75% lysine barley diet (basal diet), the addition of graded levels of L-threonine improved markedly the average daily gain and feed conversion efficiency of the growing pig. The 0.10% threonine addition to the basal diet produced results which were not significantly different from the results obtained on the conventional soybean-barley control diet indicating that this level of threonine (0.47%) in the diet, though less than in the barley-soybean meal diet, was adequate for the growth performance of growing-finishing pigs. Chung and Beames (1972) reported that an all barley diet is first limiting in lysine and that threonine is probably the second-limiting amino acid. With 0.75% total lysine in the lysine-barley diet, Chung (1973) observed a significant response to 0.05% threonine addition in growth and feed efficiency performance above the lysine-barley diet. The response of threonine in the present experiment is in good agreement with the results of Chung and Beames (1974). However, 0.05% threonine addition to a 0.75% lysine diet in the present experiment improved the pig performance but not significantly. The reason is probably because the threonine level of the barley used by Chung and Beames (1974) was lower than the level in the present experiment. The basal diet with 0.75% lysine contained 0.24% in the experiment of Chung and Beames (1974) whereas in the present experiment the threonine level was 0.37% .

Supplementation with lysine of a high cereal-diet has been shown by several workers to improve growth and feed efficiency (Evans, 1960; Jones et al., 1962; Ericson et al., 1962; Ostrowski, 1969; Braude et al., 1972). Bloch et al. (1972) from an experiment with pigs receiving an all-barley diet showed that barley protein is poor-quality without amino acid supplementation.

Although Chung and Beames (1974) reported that 0.90% lysine content in a barley-amino acid diet was significantly different from 0.75% lysine-barley diet as measured by growth rate and feed conversion efficiency of growing-finishing pig, the significant diet × sex interaction indicated that the higher level of dietary lysine content was beneficial only for the gilts. The addition of 0.05% threonine to 0.75% lysine-barley diet significantly improved the above performance criteria compared to the barley diets with only 0.75 and 0.90% lysine. This suggested that 0.75% lysine might be the optimal level for growth and feed efficiency, but that threonine is limiting in such a diet. The level of dietary lysine for the growing and the finishing pig was recommended by N.R.C.-N.A.S. (1968) to be 0.70 and 0.50% respectively, and 0.75 - 0.80% and 0.6 - 0.65% by A.R.C. (1967). This appears to support that 0.75% dietary lysine is adequate for the growing-finishing pig.

Müller et al. (1967a) demonstrated that lysine and threonine supplementation of a basal cereal diet had a large favorable effect upon gains and feed efficiency. With lysine supplementation alone, the improvement in gains was found to be 19% in one case and 22% in another case. However, with lysine and threonine supplementation, the gains were improved by 59% in one trial and 92% in the other. Respective improvements in feed efficiency were 31 to 34% . Müller *et al*. (1967a) considered that in growing-pig diets based on cereals (wheat, barley and oats) lysine is the first limiting amino acid, followed by threonine, tryptophan and methionine. Threonine was the second limiting amino acid in barley in the present experiment. This supports the findings of previous investigations with barley and other cereals (Sure, 1955; Pond *et al*., 1958; Rosenberg *et al*., 1959).

In the present experiment, tryptophan was sufficient in the basal barley diet according to N.A.S.-N.R.C. (1968) Feeding Standards for growing-finishing pigs. Consequently, it was not investigated.

Although total sulfur amino acid (methionine + cystine) requirements of growing pig are widely accepted as being 0.5 - 0.6% of the diet (N.A.S.-N.R.C., 1968; A.R.C., 1967), many researchers over recent years have indicated that the requirements of sulfur amino acid of pigs may be overestimated. Beames and Pepper (1969) indicated that 0.3 - 0.42% is adequate for optimal growth. Chung and Beames (1974) obtained no response with supplementation of 0.10% methionine to an all barley-amino acid diet containing 0.27% methionine + cystine. Similar evidence was reported by many other researchers such as Oestemer *et al*. (1970), Jensen *et al*. (1965) and Brown *et al*. (1974) strongly supporting the contention that the total sulfur amino acid requirements of growing and finishing pigs should be lower than N.A.S.-N.R.C. (1968) and A.R.C. (1967) recommended levels. The failure in response when adding 0.10% methionine to an all barley-amino acid diet containing 0.44% methionine + cystine in the present experiment further confirmed that methionine is not limiting in barley for growing pigs. This is in agreement with results of Soldevila and Meade (1964), Rérat and Henry (1969) and Chung and Beames (1974).

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 grain diets containing protein concentrate (Robinson and Lewis, 1963 ; Müller et. al., 1967b).

The simplified diet of amino acids added to single cereal diets (wheat, barley or corn) used by Müller and Malek (1967a, b, c) indicated that this kind of diet could be employed to raise pigs from weaning (approximately 20 Kg liveweight). Many reports have indicated that the performance of animals fed a diet with a single cereal plus amino acids diet fail to produce performance as good as that achieved with natural protein concentrate supplemented diet (Chung and Beames, 1974).

Beames and Pepper (1969) also reported that the use of lysine, either with or without methionine, was not completely successful as a replacement of half soybean concentrate to sorghum diet for pigs less than 45 Kg body weight. However, no difference in performance was observed in pigs growing from 45 Kg to 90 Kg between the amino acid replaced diet or the original soybean-sorghum diet.

Results from the present experiment indicated that diets composed of a single cereal supplemented with the required amino acids could induce high pig performance as good as conventional protein concentrate supplemented diets. Pigs fed with 0.444% lysine-HCl and 0.10% threonine added to barley in a diet containing adequate minerals and vitamins were not significantly inferior to those on the barley-soybean meal diet. However, when the crude protein, lysine and threonine levels in these two diets is compared, the barley-amino acid diet is seen to contain less crude protein (11.4% vs 17.6%), a similar lysine level (0.75% vs 0.75%) and a lower threonine level (0.46% vs 0.57%). The threonine levels recommended by N.A.S.-N.R.C. (1968) and A.R.C. (1967) are 0.45% and 0.45 - 0.50% of the diet for 20 to 45 Kg pigs. The dietary threonine level of 0.47% threonine is the best diet of the present experiment which agrees with these recommendations. Henry and Rérat (1970) reported that on a 10% protein diet, 0.48% dietary threonine was the optimal level for growth of 20-50 Kg female pigs. The further increase of threonine level with or without additional methionine slightly depressed growth below that obtained with 0.1% threonine addition. This may indicate that the addition of 0.05% extra L-threonine created a slight stress or an amino acid 'imbalance' in the diet. Methionine addition to 0.15% threonine diet tended to improve performance slightly, possibly as a result of reducing the imbalance.

Although the diet containing 0.10% added threonine was not statistically inferior to the barley soybean meal diet by using Tukey's test, it appeared that the control soybean-barley diet still gave slightly better performance. It is possible that response to the addition of lysine and threonine to the barley was limited by other factors such as the level of total nitrogen in the diet, a lower biological availability of lysine and threonine or other essential amino acids, or that the amino acid pattern in the all barley-amino acid diet was not as well balanced as in the soybean-barley diet although the requirements of all essential amino acids appeared to have been met.

The soybean-barley diet was significantly better than the diet containing higher threonine (0.15%) additions with or without methionine in the present experiment. This is possibly as a result of a greater imbalance. It is possible that better growth may have been obtained with an increased lysine level in the higher threonine diet. The results of Harper (1964) obtained with rats indicated that an excess of a particular amino acid or of an imbalanced protein required further addition of the primary-limiting amino acid.

Bayley and Summers (1968) reported that the supplementation of practical corn-soybean diet (14% crude protein) with 0.1% lysine or 0.05% methionine did not give a beneficial effect to growth and feed efficiency. However, there was a positive effect if the same level of both amino acids were supplemented together particularly on the lower protein diet (12% crude protein).

Although the pig does not require crude protein _per se_. the crude protein level appears to affect performance to some extent. Rérat and Henry (1963) indicated that the performance of pigs receiving a low lysine diet could be improved by raising the nitrogen level or by adding lysine to the diet. The high nitrogen level of the soybean-barley may have contributed to the good performance on this diet. Blair _et al_. (1969a) reported that liveweight gain was not improved significantly by increasing the protein level above 16, 14 and 12% for the 23-45, 45-68 and 68-90 Kg body weight categories, respectively. However, feed conversion efficiency was improved significantly over the 23-45 Kg weight range by increasing the level to 18%. Liveweight gain was not improved significantly by increasing the lysine level above 1.04, 0.74 and 0.70 for the 23-45, 45-68 and 68-90 Kg ranges, respectively. Feed efficiency was, however, improved significantly during the 23-45 Kg stage by increasing lysine levels to 1.22%. It appears that the protein level (about 11%) and lysine level in the barley-amino acid diets of the present experiment may have been the factors limiting the growth and feed efficiency performances of pigs during the early stage of growth. This was revealed by the growth rate differences occuring only from 20 to 45 Kg body weight between 0.10% threonine or over diets and the control diet. It is possible that the growth rate can be improved by raising the nitrogen level of the diets through amino acid addition during that period. The lysine level apparently was sufficient but not the nitrogen level when compared to the reports of Blair _et al_. (1969a). In the present experiment, the growth rate difference occured only from 20 to 45 Kg body weight between the control diet and the 0.10% threonine supplemented diet.

Chung and Beames (1974) showed significant growth improvement with gilts but not with barrows by increasing the lysine level from 0.75% to 0.9% of the all barley diet containing about 10% crude protein. However, it is difficult to explain the significant increase in daily weight gain obtained by adding 0.05% threonine to the barley diet containing 0.75%

lysine when fed to gilts; a significant increase could also be obtained by further increasing the lysine level to 0.90%. The response to other limiting amino acids obtained when the first limiting amino acid is not at an optimal level is contrary to the general theory of a stepwise response, as illustrated by the contour maps of Rosenberg et al. (1959); although where several amino acids are equally limiting, a response is obtained only when all are supplied together (Fisher, 1965). It is therefore apparent that 0.75% lysine in the diet is adequate for the growing and finishing of the pig. The increased growth response and feed efficiency obtained by Chung and Beames (1974) indicated that threonine was a limiting amino acid in these all barley diets. The limiting factor of threonine may have been one of the main factors which resulted in a failure to show the response of increasing lysine from 0.75% to 0.90% of the diet. The evidence observed by Morrison et al. (1961) and Braude et al. (1972) indicated that lysine supplementation results in a progressive decrease in plasma threonine concentration. The high level of lysine could be more imbalanced in the early stage than in later stages of growth. It is therefore suggested that perhap levels higher than 0.75% lysine with progressively increasing threonine levels commencing at 0.47% in all barley-amino acid diets should be further investigated to see if additional improvements in pig performance could be achieved.

The evidence based on pig growth and feed efficiency in the present experiment suggested that threonine is adequate for optimal growth at 0.47% of the low-protein barley-amino acid diet containing 0.75% lysine for the growing-finishing pig.

Dressing percentage did not differ significantly between treatments and sex. Jurger (1967), Meade et al. (1969) and Pierce and Bowland (1972) obtained no effect on dressing percentage from varying dietary protein levels, thus supporting the results of the present experiment showing the high protein control diet (about 17.6% crude protein) to produce a dressing percentage similar to that obtained with the low protein treatment (about 11% crude protein).

Many reports in the literature have shown lysine supplementation of low protein cereal to markedly improve carcass lean content (Brooks et al., 1959; Bowland, 1962; Cahilly et al., 1963; Nielsen et al., 1963; Braude et al., 1972). However, very little information has been presented on the effect of threonine addition to a low-protein cereal with adequate lysine supplementation on carcass lean content. The data in the present study indicated a gradual decrease (not significant at $P < 0.05$) in backfat thickness corresponding to increased level of threonine supplement. However, the thickness of backfat 40 mm from mid-line was significantly ($P < 0.05$) reduced only at 0.15% level of threonine supplementation. This suggests that to achieve a significant improvement in carcass quality, higher level of threonine supplementation may be required. Chung and Beames (1974) were also unable to show any difference from the addition of 0.05% threonine to a barley-lysine diet (0.75% lysine).

Higher threonine additions in Treatments 5 and 6 gave backfat figures not significantly different from values obtained on the barley-soybean control diet. Similarly, Treatments 4 and 5 were statistically as good as the control diet in respect to backfat 40 mm from the mid-line. In general, the results indicate that the addition of threonine to the barley-amino acid (0.75% lysine) diet tends to decrease the backfat and improve carcass quality. Variation in feed intake can also influence carcass fatness. However, as there were not significant differences between treatments in mean daily feed intake this would not have been a contributory factor.

Eye muscle index and eye muscle area were not affected significantly over the 0.05% to 0.15% range of threonine addition although the level of threonine supplementation was the only level on which the eye muscle index did not differ significantly from that obtained on the barley-soybean meal control diet.

The overall results of the present experiment showed that the lowest back fat thickness, largest eye muscle area and eye muscle index was obtained from the barley-soybean control diet, although some of the threonine supplemented diets gave values which were not statistically lower than the control .

Many reports have indicated that lowering the crude protein level below about 16 - 17%, at least in the early stages of the growing period, will produce adverse effects on carcass quality (Ashton et al., 1955; Bowland et al., 1959 and A.R.C., 1967; Tjong-A-Hung et al., 1972). The inferiority of carcass quality on the present barley-amino acid diets (about 11% crude protein) compared with the carcasses from pigs on the barley-soybean control (17.5 crude protein) diet may be due to inadequate nitrogen contents in the former diets for optimal body protein synthesis.

Conflicting reports by Clawson (1967), Meade (1966b) and Pierce and Bowland (1972) indicate no influence of dietary protein on loin area and back fat thickness. However, it appeared that the protein level in diets used by above authors were over 14% crude protein and therefore not designed to critically test the effect of protein levels.

Higher mean daily gains were observed in the barrow than the gilt which was in agreement with the results of Tjong-A-Hung et al. (1972) and Chung and Beames (1974). However it is in disagreement with the findings of Newell and Bowland (1972) and Pierce and Bowland (1972).

Gilts were superior to barrows in all carcass measurements. This result is supported by many previous investigators (e.g. Wong et al., 1968; Young et al., 1968; Newell and Bowland, 1972 ; Tjong-A-Hung et al., 1972; Lodge et al., 1972b; Chung and Beames, 1974).

There was no interaction between diet and sex in the present experiment. Chung and Beames (1974) in a similar experiment observed interaction between diet and sex. It appears that diet and sex interaction has been inconsistently found in several previous investigations.

D. CONCLUSION

It may be concluded that threonine was the second limiting amino acid in barley as used in this experiment for the finishing pig and that barley can be used as the sole protein source for finishing pigs providing that the proper levels of lysine and threonine are added.

With 0.75% lysine in the all barley diet, a 0.10% L-threonine addition to the diet (0.47% total threonine) produced the best mean daily gain and feed conversion efficiency. Further addition of 0.05% L-threonine and 0.05% threonine plus 0.10% DL-methionine did not give any additional improvement in pig performance. The barley-lysine diet containing 0.75% total lysine gave the poorest performance in both rate of growth and feed conversion efficiency. The 0.10%, 0.15% threonine and 0.15% threonine plus 0.10% methionine improved the mean daily gain by 22.1%, 13.0% and 14.7% respectively and feed conversion efficiency by 15.5%, 12.9% and 12.8% respectively when they were added to a barley-lysine diet containing 0.75% total lysine. Differences in the rate of growth on the barley-soybean diet (17.6% crude protein) and barley amino acid diets containing 0.10% or more added threonine (about 11% crude protein) occured only at the 20 to 45 Kg body weight period. The essential amino acid content of the higher barley-lysine diets containing 0.10% or more threonine are sufficient to meet the requirements of the growing pig according to the N.A.S.-N,R,C, (1968) feeding standards. However the total nitrogen content of the amino acid supplemented diets may have been insufficient during the growing (prior to 45 Kg body weight) period of growth. It is possible that growth rate could have been improved by increasing the nitrogen level of diet during this period.

The barley-soybean meal diet gave the best carcass quality. The addition of graded levels of L-threonine to the barley-lysine diet improved carcass quality.

Gilts grew more slowly and produced leaner carcasses than barrows.

IV. PIG EXPERIMENT II

A. EXPERIMENTAL PROCEDURE

a. General

In feeding experiments, growth performance as assessed by growth rate and feed efficiency gives no indications of the variation in nitrogen retention in the tissues, particularly when the animals are fed *ad libitum*. Although carcass measurements are reasonably well correlated with lean content and total carcass nitrogen these give no indication of the causes of variations between treatments in nitrogen storage. It is generally accepted that nitrogen balance techniques are much more valuable in assessing protein quality. Therefore, the nitrogen metabolism experiment was designed to determine the causes of variations in nitrogen retention in tissues of pigs receiving the diets used in Experiment I.

b. Animals

A total of 18 male castrate Yorkshire-Landrace crossbred pigs weighing between 39 and 51 Kg were assigned to three groups. Each group consisted of six animals which were placed in six metabolism crates for a three-week period. Within groups six experimental diets were assigned randomly. The first week was an acclimatization period. The second and third weeks were divided into two one-week collection periods.

c. Diets

Diets were the same as those used in Pig Experiment I. Each diet was prepared by passing the barley through the fine screen (7 mm diameter) of a hammer mill. Feed samples were taken for moisture and nitrogen determination at the start of the trial.

d. Management

(i) Housing

The metabolism room was maintained at a temperature of 21° C with thermostatically-controlled space heaters.

The metabolism crates were a modification of the Shinfield design (Frape et al., 1968). The fibre glass urine tray was inclined from front to rear at an angle of approximately 30° from the horizontal. A fine wire screen was placed on the urine tray in order to retain the small amount of feces falling beyond the feces tray which was located at the rear of the floor as shown in Figure 1 .

Each pig had attached a canvas belt to which was attached a piece of rubber tubing. The canvas belt was stuck with cement around the abdomen of the pig with the rubber tube hanging just in front of the penis. The purpose of this design was to lead the urine down to the urine tray and to minimize possible urine loss from spraying during urination (Figure 2).

(ii) Feeding method

Pigs were fed twice daily ad libitum at 9:00 a.m. and 1:00 p.m.

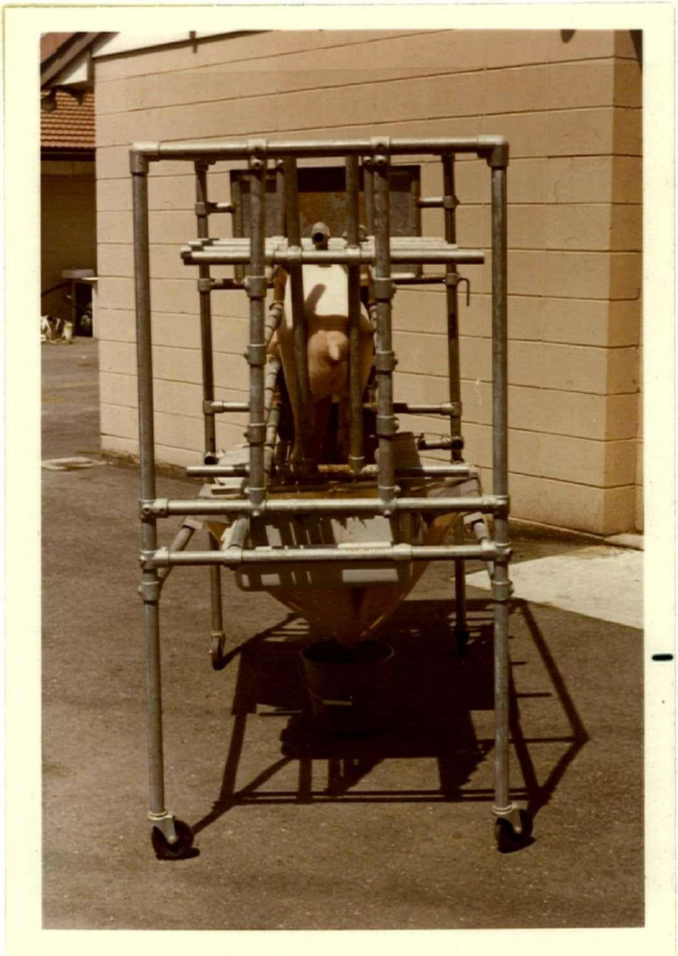

Figure 1. General view of metabolism cage

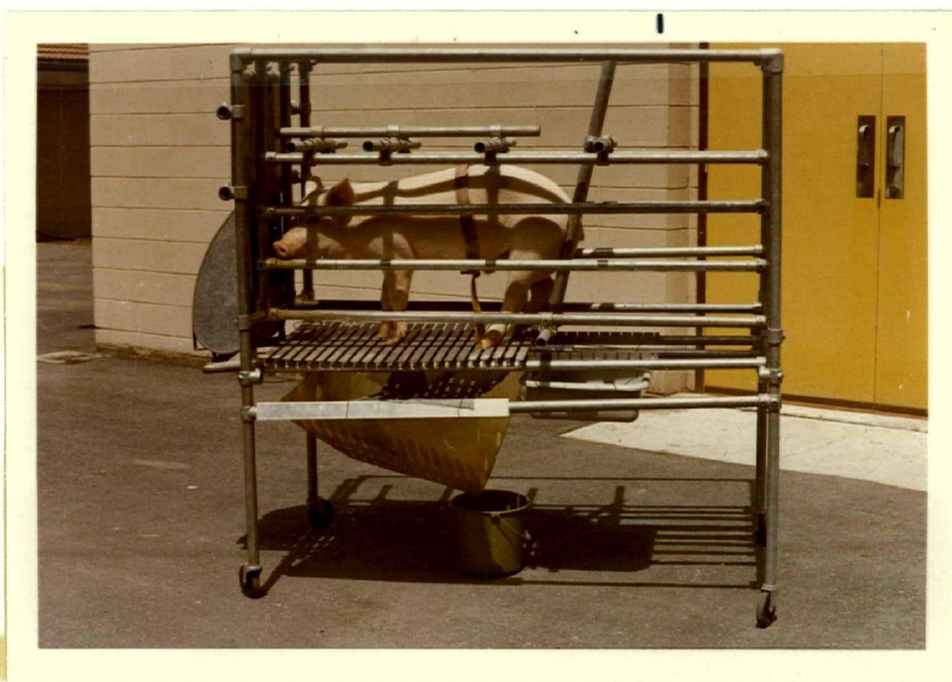

Figure 2. Pig in the modified adjustable cage, held with canvas belt and rubber tubing to minimize urine loss

for 40 minutes per feed. The diet was mixed with equal amounts of water by weight just before feeding. Water was provided ad libitum for a 20 minute period after each feeding. Prior to the provision of water, feed residues were removed, placed in 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 to constant weight (approximately 72 hours).

e. Feces and Urine Collection

All feces were collected daily in plastic bags and stored at 3° C until completion of the seven-day trial. The total fecal output of each pig was then pooled and weighed and an accurately weighed aliquot of approximately 500 g was dried at 60° C for 72 hours (Saben and Bowland, 1971). Urine was collected in a plastic jar to which was added 50 ml of diluted sulphuric acid (50% V/V) each day to avoid loss of nitrogen. Urine volume was measured each day and a 10% aliquot collected into a plastic jar and stored at 3° C for pooling at the end of each one-week collection period. The sample of pooled urine was used subsequently for nitrogen determination.

f. Statistical Analysis of Data

The analyses were done as for a one-way classification analysis of variance (diet as treatment) with or without a covariable. The data for nitrogen balance, apparent nitrogen digestibility, nitrogen absorbed as a percentage of nitrogen intake, nitrogen retained as a percentage of nitrogen absorbed and dry matter digestibility were subjected to an analysis of variance (UBC-MFAV, Halm and Le, 1974) and the means from comparison showing significant F values were tested according to Tukey's Test (1953). Nitrogen balance data were also analyzed by covariance analysis UBC-MFAV (Halm and Le, 1974) with feed intake and nitrogen intake as covariables.

B. RESULTS

The summarized results of the pig metabolism studies are shown in Table 7 . With equal levels of lysine (0.75%) in the diet, the supplementation of L-threonine to the barley-amino acid diet improved the nitrogen balance significantly. The nitrogen balance improved progressively but not significantly from 0.05 to 0.15% threonine addition. No further response was obtained from methionine supplementation of the highest threonine diet. The nitrogen balance figures of the barley-amino acid diets were significantly inferior to those of the barley-soybean control diet except for the 0.15% threonine supplemented diet which did not differ significantly from the control. When nitrogen balance was adjusted for feed intake, comparative results were similar to those without feed intake adjusted except that the non-threonine supplemented diet became insignificantly different from Treatments 3, 4 and 6. However, when nitrogen balance was adjusted for nitrogen intake the 0.15% threonine added diet became the best although it was insignificantly different from all other threonine supplemented and control diet. However, it was significantly better than the non-threonine supplemented diet. Moreover, the diet without threonine added did not differ significantly from Treatments 1, 3, 4 and 6.

Nitrogen absorbed as a percentage of nitrogen intake showed no significant differences between amino acid supplemented diets but the lysine only and lysine plus 0.05% threonine supplemented diets gave significantly lower values than that of control. The control diet gave a significantly lower nitrogen retention as a percentage of nitrogen intake than all barley-amino acid diets. The diets with added threonine did not differ significantly from each other but tended to improve from the low to the higher levels of threonine addition. The picture for nitrogen retained as a percentage of nitrogen absorbed was similar to that obtained when nitrogen retention was expressed as a percentage of nitrogen intake.

Dry matter digestibility showed no significant differences between diets. However, nitrogen intake and nitrogen digestibility were significantly higher in the barley-soybean control treatment but there were no significant differences among the barley-amino acid supplemented diets.

In summary, the results showed that the barley diet with 0.444% lysine-HCl addition only was still limiting in threonine values for nitrogen balance. Nitrogen absorbed as percentage of nitrogen intake, nitrogen retained as percentage of nitrogen intake and nitrogen retained as percentage of nitrogen absorbed were generally improved by threonine supplementation.

Table 7 . Summary of the effects of supplementation of barley with amino acids on apparent dry matter digestibility, nitrogen balance, apparent nitrogen digestibility and on various nitrogen retention indices

Treatment	1 Soy-barley (control)	2 0.75%lys (Basal)	3 Basal + 0.05%thr	4 Basal + 0.10%thr	5 Basal + 0.15%thr	6 Basal + 0.15%thr+ 0.10%met.	SE*	Result of significant test**
Total feed intake(g)	13424	11833	12701	12538	12567	12244	±595	135462
Total N intake(g)	337.60	208.36	230.72	227.88	227.66	223.49	±11.78	134562
D.M. Digestibility(%)	78.43	77.76	76.26	77.95	77.61	77.14	±1.11	142563
N digested	289.80	143.66	152.40	159.37	158.09	154.73	±7.39	145632
N balance(g)	133.27	87.78	103.65	107.63	114.48	102.12	±6.23	154362
N balance† adjusted by feed intake(g)	128.53	91.67	102.84	107.70	114.40	103.78	±5.42	154632
N balance§ adjusted by N intake(g)	104.14	97.07	107.83	112.50	119.40	107.98	±5.72	546312
N absorbed / N intake (%)	76.78	69.11	66.15	69.91	69.54	69.77	±2.41	146523
N retained / N intake (%)	35.44	42.07	44.84	47.32	50.52	45.99	±2.12	546321
N retained / N absorbed (%)	46.24	61.18	67.91	67.70	72.26	66.16	±3.37	534621

* Standard error of treatment means.

** Treatment numbers not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

† Covariable feed intake is significant ($P < 0.05$).

§ Covariable N intake is highly significant ($P < 0.01$).

C. DISCUSSION

The results of the experiment showed that the addition of threonine to the barley-lysine diet progressively improved the dietary protein quality. There were slight variations for nitrogen balance, nitrogen absorbed/nitrogen intake, nitrogen retained/nitrogen intake and nitrogen retained/nitrogen absorbed for all the threonine supplemented diets.

The 0.15% threonine supplemented diet gave the best results but not significantly so except for nitrogen absorbed/nitrogen intake. The 0.15% threonine supplemented diet also appeared to be as good as ($p < 0.05$) the barley-soybean control diet in terms of nitrogen balance.

An improvement in nitrogen balance of animals by the addition of L-lysine-HCl to grain has been reported by many investigators (e.g. Ericson et al., 1962; Soldevila and Meade, 1964; Bowland and Grimson, 1969; Pick and Meade,1971; Braude et al.,1972). Braude et al. (1972) reported that nitrogen retained as percentage of intake was significantly higher in the lysine supplemented high barley diet (0.57% lysine) than non-lysine supplemented diet (0.45% lysine) for the growing pig. Chung and Beames (1974) showed no response to adding further lysine to a barley plus lysine diet containing 0.75% lysine, indicating some other amino acid to be limiting at this level.

Pick and Meade (1971) reported that rats fed diets containing 0.73% lysine has significantly ($p < 0.01$) 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.

Improvement of nitrogen balance by synthetic threonine supplementation to a low protein diet has been studied by some researchers (Evans, 1963; Müller and Rozman, 1968; Bressani, 1971; Chung and Beames, 1974).

Evans (1963) reported that maximum nitrogen retention was obtained when the diet was supplied with crystalline amino acids with the threonine content up to 0.43% of the diet. Further addition of threonine up to 0.58% of the diet did not improve nitrogen retention, rate of growth or feed conversion efficiency under the metabolism cage conditions. Both diets contained approximately 0.70% lysine and 0.64% methionine. The present experiment showed that with 0.75% total lysine in the basal diet, nitrogen retention improved progressively up to 0.10% threonine addition (0.47% of the diet). The higher level addition of 0.15% threonine (0.52% of the diet) did not show a significant improvement with respect to nitrogen retention. This closely supported the results of Evan (1963).

Bressani (1971) reported that lysine and threonine supplements to rolled oats improved nitrogen balance in children above that obtained with lysine alone. No further nitrogen balance improvement was obtained by methionine addition. Chung and Beames (1974) obtained the similar results with barley diets for growing pigs. They demonstrated that nitrogen retention was improved by threonine supplementation of a barley-lysine diet. The marked response for improvement in nitrogen retention by threonine addition confirmed Chung and Beames's result.

Müller and Rozman (1968) showed that either lysine, threonine or tryptophan, and methionine supplementation of barley for growing-finishing pigs improved the nitrogen retained/nitrogen digested ratio as well as daily gain and feed efficiency. Chung and Beames (1974) also indicated that lysine, threonine and methionine supplementation of barley gave an improvement in nitrogen balance and nitrogen retained/nitrogen intake. The present experiment gave results which were in agreement with those of Müller and Rozman (1968) and Chung and Beames (1974). However, the addition of methionine to the barley-lysine-threonine diet did not improve the nitrogen retention, nitrogen retained/nitrogen intake and nitrogen retained/nitrogen absorbed but rather slightly depressed the maximum values for the above criteria obtained for lysine and threonine additions only. This was in agreement with the results reported by some other investigators (Soldevila and Meade, 1964; Bowland and Grimson, 1969). The reason for methionine addition depressing nitrogen balance is probably that the methionine content in diet may have been adequate for the growing-pig and that the addition of methionine may have created a slight imbalance.

Bowland and Grimson (1969) indicated that a better nitrogen retained/nitrogen absorbed value was obtained with a lysine-methionine supplemented low protein diet (14% crude protein) than with a higher protein or an amino acid unsupplemented low protein diet for early weaned pigs. Daily nitrogen retention was increased when only L-lysine , instead of both L-lysine and DL-methionine, was added to diets containing three percent urea. The observation suggested that the methionine was not the second limiting amino acid after lysine. This was also in agreement with the present experiment which showed no improvement in pig performance with methionine addition to the barley amino acid diet. On the other hand, the great response to threonine in the all barley diet strongly suggested that threonine is the second limiting amino acid after lysine for the growing-finishing pig. Moreover, Chung and Beames (1974) demonstrated that isoleucine added to a barley + lysine + threonine + methionine diet did not give further improvement in nitrogen retention indicating that isoleucine was not limiting at this levels of inclusion of the other amino acids in these diets for growing pigs.

The dry matter digestibility of barley-soybean and barley-amino acid diets were not significantly different . The barley-soybean control diet gave the lowest figure for nitrogen retained/nitrogen intake and nitrogen retained/nitrogen absorbed, which would be understandable as this diet contained a high level of non-essential amino acids and thus a higher total nitrogen level than that required for maximum performance. The results of Chung and Beames (1974) with pigs and Bowland and Grimson (1969) with rats support this finding.

According to Metta and Mitchell (1956) and Rippon (1959), the biological value of different proteins decreased linearly as the protein concentration or intake is increased. (e.g. Becker et al., 1963, A.R.C., 1967). It is suggested that the lower apparent biological value of the soybean-barley control diet was due to higher protein (17.6% of the diet) intake. This is above the need of the growing pig. The protein ratio of barley-soybean and barley-amino acid diet was about 1.5 to 1 in the present experiment.

It was shown in this experiment that 0.15% threonine added to the barley-lysine diet gave a nitrogen retention not significantly less than that obtained with the barley-soybean control but significantly higher in nitrogen retained/nitrogen intake and nitrogen retained/nitrogen absorbed. The data suggested that a 0.15% threonine supplemented barley-lysine diet may be able to replace protein concentration in order to support the equal nitrogen retention.

In nitrogen metabolism experiments, there is the possibility of nitrogen loss in feces and urine if one does not take adequate precautions. Martin (1966) indicated that NH_3 loss from feces was negligible and 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% at the lower temperature of 15 to 18° C. The losses of nitrogen from feces and urine should have been negligible in this experiment as the urine was collected in strong sulphuric acid with a pH below 2 and stored at 3° C until the end of the one-week collection period. The samples were analysed immediately after the end of each one-week collection period.

D. CONCLUSION

It may be concluded, from the metabolism experiment that threonine is the second limiting amino acid in barley for growing pigs, thereby supporting the results of the growth experiment.

The addition of graded levels of L-threonine of the barley-lysine diet progressively improved the nitrogen balance, nitrogen balance adjusted for feed intake and nitrogen intake, nitrogen retained/nitrogen intake and nitrogen retained/ nitrogen absorbed. Although not all results from the graded L-threonine additions differed significantly from each other, the highest threonine (0.15%) supplemented level was significantly better than the basal barley-lysine diet. At this level of threonine addition (0.52% of the diet), the nitrogen balance and nitrogen balance adjusted for feed intake were statistically non-significantly different from the barley-soybean control diet. All threonine supplemented diets were significantly better than barley-soybean control diet in terms of nitrogen retained/ nitrogen intake and nitrogen retained/nitrogen absorbed. This phenomenon occurred because the barley-soybean diet contained 17.5% crude protein, and in excess of the requirements of growing pigs. The excess nitrogen intake was excreted in the urine. Therefore its biological value was reduced.

The methionine supplement did not further improve nitrogen retention indicating that methionine was mot limiting in the diet. There was no significant difference in dry matter digestibility between diets.

V. RAT EXPERIMENT I

A. EXPERIMENTAL PROCEDURE

a. General

The results obtained from Pig Experiment I indicated that the performance of the pigs receiving diets with graded levels of threonine added to the barley-lysine basal diet, was still inferior to that obtained with the barley-soybean control diet, although performance on one of the graded threonine supplemented diet was not statistically inferior to the performance on the control diet on the basis of Tukey's test (1953). Consequently it was considered desirable to investigate the effect of modifying the levels of amino acid supplementation on the complete removal of this gap.

Owing to the time and expense involved with carrying out this investigation with any degree of thoroughness with pigs, it was decided to use rats in this investigation.

It is generally known that the laboratory rat has been accepted as a satisfactory pilot animal in swine nutrition research as the pattern of amino acid requirements for both species are quite similar (N.A.S.-N.R.C. 1968, 1972). This experiment was designed as a preliminary investigation to compare the response in rats given diets similar to those received by the pigs in Pig Experiment I. If similar growth patterns were obtained, it was intended to do further experiments as first experiments for possible subsequent work with pigs in an attempt to formulate the "ideal" barley-amino acid diet.

This experiment consisted of six treatments with 6 rats individually housed per treatment. Three extra rats were needed as the control for initial body composition analysis. The experiment was conducted for 26 days.

b. Animals

A total of 39 albino male rats (Woodlyn/Wistar strain) aged 30±1 days with average body weight approximately 80 g were employed. Thirty-six of these were randomly allocated to individual wire cages (25.4 × 17.8 cm^2). Diets were assigned randomly to the rats. Three rats were killed by diethylether over-anesthetization and used as controls for body composition analysis.

c. Diets

The six test diets were of a similar formulation to those used in Pig Experiments I and II as shown in Table 2 . However, all the ingredients for rat diets were finely ground through 30 mesh screen by C & N laboratory mill* before mixing in a Hobart planetary feed mixer.

d. Management

(i) Housing

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* Size 8 inches laboratory mill, Christy & Norris Ltd., Chelmsford, England.

The experiment was conducted in the rat laboratory of the Department of Animal Science, U.B.C. The temperature of the room was maintained between 24 to 25° C throughout the experiment.

(ii) Feeding methods

Rats were fed ad libitum. Feed was added daily. Fresh water was provided daily and was available continuously from automatic drinkers:

e. Records

All rats were weighted initially and thereafter at 24 hours intervals throughout the experiment. Daily feed consumption was recorded. Spillage of feed, when it occured, was collected daily and subtracted from gross intake.

f. Chemical analysis

(i) Feed

All diets were sampled and analysed similar to Pig Experiment I.

(ii) Carcass

Control rats were killed by diethylether over-anaesthetization at the beginning of the experiment. The gut of each rat was cleaned by flushing with tap water before storing at -10° C for later analysis. Similarly all the experimented rats were killed at the end of the feeding period by the same method, cleaned and stored at -10° C .

For analysis, all rats were dried at 95° C for 72 hours and weighed. Each dried rat carcass was extracted in a soxhlet apparatus using size 43 × 123 mm thimbles. The carcass was then dried and weighed and ground in a C & N Laboratory mill (30 mesh screen) and then ball milled for 24 hours. The powder from the carcass sample was then used to determine protein and ash content. Analyses were done according to A.O.A.C. (1965).

g. Calculations

Average daily gain, feed conversion efficiency and average daily feed intake of each rat was calculated for each week* and the over all period. Protein ultilization ratio for each rat was also calculated. The overall average daily gain was used as a covariable for analysis of all the carcass data. Means of factors with significant F values were tested by Tukey's test (1953). Standard errors of treatment means were calculated from analysis of variance tables. Carcass analyses were used to calculate total carcass protein, protein retention during the whole experiment period, protein retained/protein intake (%), protein % in dry carcass, protein % in fat free carcass, total carcass fat, fat % in dry carcass, total carcass ash, ash % in dry carcass and ash % in fat free carcass.

* The fourth "week" was only of four days duration.

h. Statistical Analysis

All the data were analysed by analysis of covariance using computer program, UBC-MFAV (Halm and Le, 1974) for a one way classification. Average daily gain and feed conversion efficiency for each week and for the overall period were analysed with weekly and overall average dry matter feed intake respectively and weight at the beginning of the experiment as covariables. Carcass measurement data were analysed with average daily gain as a covariable.

B. RESULTS AND DISCUSSION

The results obtained with rats in average daily gain (ADG) on a weekly and overall basis, feed conversion efficiency (FCE) and overall protein efficiency ratio are summarized in Table 8 .

The addition of 0.444% L-lysine-HCl alone to the all barley diet (basal diet) gave the lowest ADG and poorest FCE on both weekly and overall figures while the addition of graded levels of L-threonine progressively improved ADG and FCE. The rats appeared to respond better to 0.15% L-threonine addition than to 0.10% addition, but the improvement was not significant. The addition of 0.10% methionine to 0.15% added L-threonine improved the response slightly but not significantly. The 0.15% threonine and 0.15% threonine + 0.10% methionine added diets were significant better then the basal diet. The overall ADG and FCE of rats receiving the barley-lysine diets supplemented with 0, 0.05, 0.10, 0.15% threonine and 0.15% threonine plus 0.10% DL-methionine were 3.98, 3.71; 4.18, 3.51; 4.38, 3.34; 4.55, 3.21; 4.73, 3.09, respectively. The trend obtained was similar to that obtained with the pigs which received the same diets in Pig Experiment I . The rat growth results indicated that threonine is also limiting in barley for the growing rats. This is in agreement with the results reported by Sure (1955). No further significant response to the addition 0.10% methionine suggests that methionine is adequate in barley for the growing rat . This in turn indicated that threonine, but not methionine, is the second limiting

Table 8 . Effect of amino acid supplementation of low protein barley on average daily gain, feed conversion efficiency and protein efficiency ratio of rats

	Week	1 Barley-soy control	2 lys (Basal)	3 B + 0.05%Thr	4 B + 0.10%Thr	5 B + 0.15%Thr	6 B + 0.15%Thr+ 0.10%Met.	SE*	Result of significance test**	Significant covariables
Average Daily Gain(g)	1	5.35	3.36	3.78	4.02	4.27	4.72	±0.15	1 6 5 4 3 2	Initial weight(-) Average feed intake
	2	5.58	3.68	4.07	4.45	4.47	4.58	±0.20	1 6 5 4 3 2	Initial weight(-) Average feed intake
	3	5.25	4.22	4.26	4.50	4.71	4.83	±0.21	N.S.	Average feed intake
	4[a]	5.47	4.24	4.42	4.60	4.81	5.11	±0.26	N.S.	Initial weight(-) Average feed intake
	overall period	5.28	3.98	4.18	4.38	4.55	4.73	±0.10	1 6 5 4 3 2	Initial weight(-) Average feed intake
Feed Conversion Efficiency	1	2.30	3.77	3.30	3.08	3.02	2.67	±0.13	2 3 4 5 6 1	Initial weight Average feed intake (-)
	2	2.49	3.96	3.49	3.23	3.20	3.11	±0.16	2 3 4 5 6 1	Initial weight
g feed / g gain	3	3.15	3.74	3.95	3.65	3.43	3.31	±0.23	N.S.	N.S.
	4[a]	3.07	3.90	3.90	3.61	3.41	3.27	±0.22	N.S.	N.S.
	overall period	2.79	3.71	3.51	3.34	3.21	3.09	±0.07	2 3 4 5 6 1	Initial weight
Initial weight		84	78	78	86	77	79	±2.81	N.S.	
Protein Efficiency Ratio		2.03	2.36	2.48	2.63	2.69	2.80	±0.06	6 5 4 3 2 1	N.S.

[a] Computed from 5 days data.

* Standard error of treatment means.

** Treatment means not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

amino acid in barley. The overall ADG on the barley-soybean control diet was significantly better than the gain on any amino acid-supplemented barley diet which tends to show that at these levels of amino acid addition to barley, protein requirements still were not adequately met. However, the 0.15% threonine plus 0.10% methionine addition to the basal diet produced a FCE which did not differ significantly from that of the control diet. In this case, methionine did show an effect by improving FCE of the growing rats.

The weekly ADG showed that the protein and amino acid requirements decreased as the age of rat increased. The rats had responded to the higher levels of threonine supplementation during the first two weeks on trial. There was no significant respond to the higher levels in the latter two weeks. This reduction in proteins and amino acid requirements with age confirms the work of Forbes and Rao (1959) and Hartsook and Mitchell (1956).

The weekly FCE results indicated that more units of feed were required per unit of gain as age increased. This generally appeared in all the treatments. The 0.15% threonine plus 0.10% methionine supplemented diet and the control diet showed the best FCE, and did not differ significantly from each other. The former diet also did not differ from the 0.10% and 0.15% threonine addition diets. The basal diet required more feed per unit gain than all other treatments. The fact that threonine and methionine added to the basal diet produced a FCE similar to the control indicated that a low protein diet (about 11% crude protein) with a proper amino acid balance can perform as well as a higher protein diet (about 17.5% crude protein) containing a conventional protein supplement.

The protein efficiency ratio (PER) was improved progressively with an increase in the level of threonine supplementation. Diets with 0.10% or over threonine addition gave significantly better PER values than the basal diet indicating threonine to be limiting in the basal diet. This agrees with the ADG and FCE results and indicates that threonine is the second limiting amino acid in barley for growing rats.

The barley-soybean control diet produced the poorest PER although the ADG and FCE were the best based on this diet. This is probably because the control diet contained a higher crude protein level than required per se. The results agree with those of Metta and Mitchell (1956) who indicated that the biological value of different protein decreased linearly as the protein concentration in the diet increased.

Covariance analysis showed that there was a significant negative relationship between ADG and initial weight and between FCE and feed intake.

The rat carcass analysis data are summarized in Table 9 . No significant differences in total protein retention were obtained among the control, 0.10% threonine and 0.15% threonine plus 0.10% methionine added diets. Levels of 0.10% threonine or higher produced significantly better protein retention than the basal diet. No significant differences

Table 9 . Effect of amino acid supplementation of low protein barley on carcass characteristics of rats.

	1 Barley-soy control	2 lys (Basal)	3 B + 0.05%thr	4 B + 0.10%thr	5 B + 0.15%thr	6 B + 0.15%thr+ 0.10%met	SE*	Result of significance test**	Significant covariable
Total carcass protein(g)	38.31	29.96	31.82	35.07	33.26	34.25	± 1.17	1 4 5 6 3 2	N.S.
Protein retention during 26 days on trial (g)	28.07	19.72	21.58	24.82	23.03	24.01	± 1.17	1 4 6 5 3 2	N.S.
Protein retained / Protein intake %	45.29	44.51	47.73	54.47	52.07	55.93	± 2.21	6 4 5 3 1 2	N.S.
Protein % in dry carcass	63.02	48.62	53.18	53.60	53.21	60.42	± 1.51	1 6 4 5 3 2	Average daily gain(-)
Protein % in fat free carcass	81.46	80.26	80.21	81.66	80.54	81.00	± 0.53	N.S.	N.S.
Total carcass fat (g)	13.21	23.07	20.63	22.79	21.55	14.29	± 1.47	2 4 5 3 6 1	Average daily gain
Fat % in dry carcass	22.37	38.37	33.85	34.30	33.81	25.32	± 1.60	2 4 3 5 6 1	Average daily gain
Total carcass ash (g)	6.48	5.42	5.72	6.06	6.03	5.99	± 0.17	N.S.	N.S.
Ash % in dry carcass	10.78	8.94	9.57	9.30	9.68	10.61	± 0.26	1 6 5 3 4 2	Average daily gain(-)
Ash % in fat free carcass	13.88	14.51	14.47	14.13	14.62	14.21	± 0.16	N.S.	N.S.
Protein+fat+ash / Dry carcass wt. %	96.39	96.75	96.46	97.12	96.77	96.43	± 0.39	N.S.	N.S.

* Standard error of treatment means.

** Treatment means not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953) were obtained among the threonine supplemented diets. The results indicate that the barley-lysine diet was limiting in threonine. The protein retained as percentage of protein intake was progressively improved by threonine addition, similar in manner to improvements in PER. The methionine supplemented diet appeared to produce a higher protein retained/protein intake value but the difference was not significantly different as measured by Tukey's (1953) test. The barley-soybean control diet resulted the poorest value indicating an excess protein intake.

Carcass fat was lowest in both the control group and the methionine supplemented group. The addition of threonine had no influence on the high carcass fat content obtained on the barley-lysine diet. Total ash showed no variation with treatment.

The threonine plus methionine supplemented diet resulted in significantly lower carcass fat. This indicated that methionine addition effectively reduced total carcass fat in growing rats. However, total backfat thickness was not reduced in the previous pig nutritional trial with the same level of methionine supplementation. This evidence suggests that the growing rat can probably ultilize this level of methionine more effectively then the growing-finishing pig by enhancing nitrogen retention resulting in less fat deposition. Thus, the growing rats do not correlate well with the growing-finishing pigs in this respect.

C. CONCLUSION

The results of the rat experiments indicate a rat response similar to that given by pigs in Pig Experiment I. The addition to the barley-lysine diet of graded threonine levels improved the ADG, FCE and PER of the rat. The further addition of methionine did not improve the above criteria significantly. The carcass protein retention, expressed either in absolute terms or as a percentage of protein intake also gave results in favor of threonine supplementation. The data measured gave strong evidence that threonine is the second limiting amino acid in barley for growing rats. The result is in agreement with the pig experiments.

Carcass fat was lowest in the control group and the methionine supplemented group. However, the results obtained from the methionine supplemented group of rats, of low carcass fat and high protein retention, did not occur in the pigs.

Fat content in the rat was not reduced by threonine supplementation of the barley-lysine diet. Total ash did not vary with treatment.

VI. RAT EXPERIMENT II

A. EXPERIMENTAL PROCEDURE

a. General

Results of Rat Experiment I indicated that the response in rats to amino acid supplementation of barley was somewhat similar to that in pigs. However there were quantitative differences in that the rats appeared to be responding to the higher level of threonine and, at least in the first week, growth and carcass composition were responding to the addition of methionine. However, it was obvious that even 0.15% threonine plus 0.10% methionine addition did not give optimal performance.

This experiment was designed to investigate the effect of (1) an improved control diet (higher protein) (2) Additional lysine (3) Additional threonine and (4) The provision of an amino acids mixture to the barley diet on rat growth and carcass composition.

The experimental design consisted of 48 albino male rats which were fed for 4 weeks. Four extra rats were slaughtered at the beginning of the experiment as an initial control group for the assessment of body nutrient storage.

b. Animals

A total of 52 albino male rats (Woodlyn/Wistar strain) average weight 86 g and age 30 ± 1 days were used. Method of allocation of rats was similar to that used in Rat Experiment I.

c. Diets

The diet formulation is shown in Table 10. The amino acid mixture was formulated to provide a slight margin of each of the essential amino acids over requirement levels listed in "Nutrient Requirements of Laboratory Animals." N.A.S.-N.R.C (1972). The listed requirements for non-essential amino acids were not adhered to as these had been merely calculated from levels included in "successful" diets, and thus were not shown to be essential. Total level of added amino acid mixture (air dry basis) was 1.75%. The composition of the added amino acid mixture and treatments were given below. The amino acid content of each diet is shown in Table 11.

Amino acid mixture

L-arginine 0.20%

L-tryptophan 0.05%

L-histidine 0.20%

L-leucine 0.20%

L-isoleucine 0.20%

L-phenylalanine 0.30%

DL-methionine 0.20%

L-valine 0.10%

L-Glutamic acid 0.30%

Treatments

1. Barley only to supply 0.41% total lysine.
2. Barley + soybean meal to supply 0.75% total lysine.
3. Barley + soybean meal to supply 0.90% total lysine.
4. Barley + 0.637% L-lysine-HCl to supply 0.90% total lysine.
5. Barley + 0.444% L-lysine-HCl to supply 0.75% total lysine + 0.10% L-threonine.
6. Diet 5 + 0.10% L-threonine (i.e. 0.20% added L-threonine)
7. Diet 4 + 0.10% L-threonine.
8. Diet 4 + 0.20% L-threonine.
9. Diet 5 + amino acid mixture.
10. Diet 6 + amino acid mixture.
11. Diet 7 + amino acid mixture.
12. Diet 8 + amino acid mixture.

d. Management

Same as Rat Experiment I.

e. Records

Same as Rat Experiment I.

f. Chemical Analysis

Same as Rat Experiment I.

g. Calculations

Same as Rat Experiment I.

h. Statistical Analysis

Same as Rat Experiment I.

Table 10. Percentage composition of diets used in Rat Experiment II (Air dry basis)

Ingredients	Diet No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Barley	96.82	81.34	75.16	96.19	96.23	96.18	96.04	95.99	94.63	94.43	94.34	94.24
Soybean meal	-	15.60	21.80	-	-	-	-	-	-	-	-	-
L-lysine HCl*	-	-	-	0.637	0.444	0.444	0.637	0.637	0.444	0.444	0.637	0.637
L-threonine*	-	-	-	-	0.10	0.20	0.10	0.20	0.10	0.20	0.10	0.20
L-Arginine*	-	-	-	-	-	-	-	-	0.20	0.20	0.20	0.20
L-tryptophan*	-	-	-	-	-	-	-	-	0.05	0.05	0.05	0.05
L-histidine*	-	-	-	-	-	-	-	-	0.20	0.20	0.20	0.20
L-leucine*	-	-	-	-	-	-	-	-	0.20	0.20	0.20	0.20
L-isoleucine*	-	-	-	-	-	-	-	-	0.20	0.20	0.20	0.20
L-phenylalanine*	-	-	-	-	-	-	-	-	0.30	0.30	0.30	0.30
DL-methionine*	-	-	-	-	-	-	-	-	0.20	0.20	0.20	0.20
L-valine*	-	-	-	-	-	-	-	-	0.10	0.10	0.10	0.10
L-glutamic acid*	-	-	-	-	-	-	-	-	0.30	0.30	0.30	0.30
Trace mineral and vitamin promix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Defluorinated rock phosphate	1.64	1.49	1.42	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64
Limestone	0.54	0.57	0.62	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

The composition of trace mineral and vitamin premix is same as that used in Pig Experiments I, II and Rat Experiment I.

* The L-lysine HCl was feed grade and was 98% pure, containing 78% L-lysine. The threonine was pure and DL-methionine was 98% pure. All were produced by the Ajinomoto Co., Japan.

Table 11. Content of essential amino acids of each diet for Rat Experiment II (g amino acid/100g mixed feed) on air dry basis.

Amino Acid	Diet No 1	2	3	4	5	6	7	8	9	10	11	12
Arginine	0.45	0.82	0.96	0.45	0.45	0.45	0.45	0.45	0.64	0.64	0.64	0.64
Histidine	0.21	0.40	0.48	0.21	0.21	0.21	0.21	0.21	0.40	0.40	0.40	0.40
Isoleucine	0.40	0.58	0.66	0.40	0.40	0.40	0.40	0.40	0.59	0.59	0.59	0.59
Leucine	0.73	1.18	1.36	0.73	0.73	0.73	0.73	0.73	0.91	0.91	0.91	0.91
Lysine	0.41	0.75	0.90	0.90	0.75	0.75	0.90	0.90	0.75	0.75	0.90	0.90
Methionine	0.17	0.24	0.27	0.17	0.17	0.17	0.17	0.17	0.36	0.36	0.36	0.36
(Cystine)*	0.20	0.27	0.29	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phenylalanine	0.53	0.80	0.90	0.53	0.53	0.53	0.53	0.53	0.82	0.82	0.82	0.82
(Tyrosine)*	0.28	0.48	0.55	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Threonine	0.37	0.56	0.63	0.37	0.47	0.57	0.47	0.57	0.47	0.57	0.47	0.57
Tryptophan	0.11	0.19	0.23	0.11	0.11	0.11	0.11	0.11	0.16	0.16	0.16	0.16
Valine	0.68	0.86	0.93	0.68	0.68	0.68	0.68	0.68	0.76	0.76	0.76	0.76
Glutamic acid**	2.36	2.97	3.21	2.36	2.36	2.36	2.36	2.36	2.60	2.60	2.60	2.60
Dry matter content %	89.93	90.26	90.34	89.76	89.75	90.22	90.22	89.74	90.47	89.91	90.21	90.13
Crude protent% (N × 6.25)	10.93	17.91	19.35	11.54	11.81	11.38	11.39	11.66	12.58	12.58	12.99	12.93

* Semi-essential amino acid

** Non-essential amino acid

B. RESULTS AND DISCUSSION

The results of the experiment for ADG, FCE and PER are summarized in Table 12 .

Rats fed a sole barley diet gave the poorest ADG, FCE and PER. The above criteria were improved by addition of lysine alone. Further improvements were obtained by various levels of graded lysine, threonine and other amino acid mixtures.

With the 0.444% lysine-HCl added diet (0.75% of the diet), 0.10% and 0.20% threonine (diets 5 and 6 respectively) supplementation gave similar ADG and FCE, i.e. no beneficial effect was obtained by increasing the threonine level from 0.10% to 0.20%. In addition, no improvement was obtained based on these two levels of threonine by increasing lysine levels of the diets from 0.75% to 0.90%. The results suggests that 0.75% lysine was adequate for growing rats, although in the first week daily growth rate was greater on the 0.20% added threonine diet when the lysine level was 0.90% than when it was 0.75%. These results are in agreement with the findings of Pick and Meade (1970) who reported that the lysine requirement of growing rats was 0.70% of the diet although Rama Rao et al. (1959) and N.A.S.-N.R.C. (1972) indicate a higher requirement (0.90% of the diet).

The supplementation of amino acid mixtures to diets 5 and 6 (i.e. diets 9 and 11 respectively) did not improve ADG and FCE

Table 12. Effect of amino acid supplementation of low protein barley on average daily gain, feed conversion efficiency and protein efficiency ratio of rats.

	Week	1 Barley only (Basal)	2 Barley-soy (0.75%lys)	3 Barley-soy (0.90%lys)	4 B + lys(0.90%)	5 B + lys(0.75%)+ 0.10% thr.	6 B + lys(0.75%)+ 0.20% thr.	7 B + lys(0.90%)+ 0.10% thr.	8 B + lys(0.90%) 0.20% thr.	9 B + lys(0.75%)+ 0.10% thr + amino acid mixture	10 B + lys(0.75%)+ 0.20% thr + amino acid mixture	11 B + lys(0.90%)+ 0.10% thr + amino acid mixture	12 B + lys(0.90%)+ 0.20% thr + amino acid mixture	SE*	Result of significance test**	Significant covariables
Average Daily Gain (g)	1	2.83	5.76	5.90	3.21	4.06	3.59	3.74	4.47	4.17	5.01	4.29	5.38	±0.15	3 2 12 10 8 11 9 5 7 6 4 1	Initial weight(-) Average feed intake
	2	3.40	5.85	5.57	3.60	4.90	4.71	4.38	4.84	4.42	5.65	4.77	6.12	±0.22	12 2 10 3 5 8 11 6 7 9 4 1	Average feed intake
	3	2.70	6.03	5.62	3.48	4.00	4.62	4.34	4.70	4.88	5.69	4.78	5.51	±0.22	2 10 3 12 9 11 8 6 7 5 4 1	Initial weight(-) Average feed intake
	4	2.67	5.38	5.52	3.63	4.05	3.92	3.49	3.76	4.87	5.17	4.42	4.94	±0.25	3 2 10 12 9 11 5 6 8 4 7 1	Initial weight(-) Average feed intake
	Overall period	3.32	5.41	5.35	3.94	4.33	4.32	4.16	4.40	4.62	5.18	4.52	5.15	±0.09	2 3 10 12 9 11 8 5 6 7 4 1	Initial weight(-) Average feed intake
Feed Conversion Efficiency g feed / g gain	1	4.36	2.25	2.17	4.61	2.95	3.50	3.25	2.87	2.92	2.62	2.88	2.40	±0.25	4 1 6 7 5 9 11 8 10 12 2 3	Average feed intake(-)
	2	4.40	2.55	2.71	4.36	3.14	3.24	3.33	3.15	3.49	2.67	3.20	2.46	±0.17	1 4 9 7 6 11 8 5 3 10 2 12	N.S.
	3	5.39	2.43	2.69	4.50	4.01	3.48	2.71	3.39	3.40	2.69	3.43	2.81	±0.19	1 4 5 7 6 11 9 8 12 10 3 2	Initial weight Average feed intake(-)
	4	5.79	2.81	2.66	4.91	4.17	4.37	4.85	4.97	3.39	3.02	3.70	3.14	±0.31	1 8 4 7 6 5 11 9 12 10 2 3	Initial weight Average feed intake(-)
	Overall period	4.51	2.73	2.80	3.84	3.45	3.49	3.62	3.41	3.24	2.92	3.32	2.92	±0.06	1 4 7 6 5 8 11 9 12 10 3 2	Initial weight Average feed intake(-)
	Initial weight	86	87	86	86	85	90	88	85	87	85	82	87	±2.20		
	Protein Efficiency Ratio	2.03	2.02	1.85	2.23	2.45	2.52	2.44	2.54	2.46	2.76	2.31	2.66	±0.05	10 12 8 6 9 5 7 11 4 1 2 3	Initial weight Average feed intake(-)

* Standard error of treatment means.

** Treatment means not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

significantly. However, the addition of amino acid mixtures to diets 6 and 8 (i.e. diets 10 and 12 respectively) gave results which were significantly better than those obtained with all other barley-amino acid diets. Moreover, the results obtained with these two diets were equivalent to those resulting from the barley-soybean diets (diets 2 and 3).

When comparing all the diets containing 0.75% lysine (i.e. diets 5, 6, 9 and 10), diets 5 and 6 containing 0.10% and 0.20% threonine respectively has similar ADG and FCE. However, the marked improvement of diet 6 after addition of the amino acid mixture and relatively no improvement in diet 5 with similar amino acid mixture addition tend to suggest that threonine became limiting when the amino acid mixture was added to the diet containing 0.10% added threonine. Increasing the supplemented threonine level to 0.20% brought the diet into proper balance, thereby improving the ADG and FCE dramatically. Higher lysine addition with amino acid mixture did not further improve the ADG and FCE as in diets 11 and 12. Diet 12 contained 0.90% lysine and 0.20% threonine plus amino acid mixtures gave the result similar to diet 10 with 0.75% lysine. The evidence further comfirmed that 0.75% lysine was sufficient in this barley diet for the growing rats. Again 0.10% threonine added to the barley diet containing 0.90% lysine plus amino acid mixture (diet 11) gave inferior result to those with 0.20% added threonine plus the amino acid mixture. The observation suggested that with the amino acid mixture, addition of 0.20% threonine must be required in this barley diet (or 0.57% total threonine) in order to attain the proper balance. However, this balance could not be attained without the amino acid mixture supplementation.

The results obtained with diets 10 and 12 were similar to the results obtained with the barley-soybean diet, indicating that optimum ADG and FCE can be achieved by amino acids supplementation of the barley diet. The adequacy of the 0.52% threonine in the diet for optimal growth was in agreement with the reports by N.A.S.-N.R.C. (1972) and Rama Rao et al. (1959) but lower than levels suggested by Pick and Meade (1971).

Rosenberg et al. (1959) studied the effect of supplementation of rice diet with lysine and threonine and indicated that the ratio of total lysine to total threonine in the diet for optimum response was 1.4 to 1 by weight. This ratio was coincidentally in agreement with diets 2, 3, 6 and 10 in the present experiment. However, this differed from the ratios of 1.66 : 1 or 2.01 : 1 suggested by Rose (1937) and Rose et al. (1949) for the growing rats.

The barley-soybean diet (2) containing 17.9% crude protein produced the maximum growth. Further increasing the protein level (diet 3) to 19.4% crude protein did not improve the ADG and FCE. The lysine and threonine of diet 2 were 0.75%, 0.56% respectively and of diet 3 were 0.90%, 0.63% respectively.

The overall results for ADG and FCE of diets 2, 3, 10 and 12 were significantly better than those of any other barley-amino acid diets.

Diets 10 and 12 gave the best PER but values were not significantly better than those of diets 6 and 8. The latter diets were not significantly better than the other threonine and lysine supplemented diets but were significantly better than values obtained with the 0.90% lysine supplemented diet and the sole barley diet. The barley-soybean diets produced the poorest PER's as in Rat Experiment I due to the high intakes of non-essential nitrogen. The results indicated that the protein of diets 10 and 12 was best utilized followed by diets 8 and 6.

The rats carcass analysis results are presented in Table 13 . Barley-soybean meal diets gave the highest carcass protein retention but gave the lowest figures (except for the sole barley diet) in terms of protein retained as a percentage of protein intake. As with the PER figures, the unnecessarily high intake of protein produced smaller values for protein retained as percentage of protein intake. Diets 10 and 12 had the highest protein retention and protein retained as percentage of protein intake when compared with all other barley-amino acid diets. The sole barley diet gave lowest protein retention and protein retained/protein intake. Similar to the picture produced by the PER figures, results obtained with the barley amino acid diets tended to indicate that addition of the first limiting amino acid lysine to barley improved the above criteria. Addition of graded levels of threonine further improved the performance. Increasing dietary lysine level from 0.75% to 0.90% did not further improve performance. This again suggested that 0.75% lysine is sufficient for the growing rat.

Carcass fat content was negatively correlated with protein

Table 13. Effect of amino acid supplementation of low protein barley on carcass characteristics of rats.

	1 Barley only (Basal)	2 Barley-soy (0.75%lys)	3 Barley-soy (0.90%lys)	4 B + lys(0.90%)	5 B + lys(0.75%) +0.10%thr	6 B + lys(0.75%) +0.20%thr	7 B + lys(0.90%) +0.10%thr	8 B + lys(0.90%) +0.20%thr	9 B + lys(0.75%) +0.10%thr+ amino acid mixture	10 B + lys(0.75%) +0.20%thr+ amino acid mixture	11 B + lys(0.90%) +0.10%thr+ amino acid mixture	12 B + lys(0.90%) +0.20%thr+ amino acid mixture	SE*	Result of significance test**	Significant covariable
Total carcass protein(g)	32.25	41.31	40.44	34.29	35.66	37.63	36.93	37.35	38.81	40.69	37.31	39.92	± 0.89	2 10 3 12 9 6 8 11 7 5 4 1	Average daily gain
Protein retention during 28 days on trial (g)	20.46	29.51	28.64	22.49	23.86	25.83	25.14	25.55	27.02	28.89	25.51	28.15	± 0.89	2 10 3 12 9 11 8 5 6 7 4 1	Average daily gain
Protein retained / Protein intake %	33.98	43.21	38.81	41.49	46.76	51.59	48.96	51.14	51.63	58.83	47.69	55.90	± 1.34	10 12 9 6 8 7 11 5 2 4 3 1	Average daily gain
Protein % in dry carcass	41.81	68.33	65.79	48.57	51.57	57.14	54.82	59.80	62.46	64.51	57.83	61.78	± 1.72	2 3 10 9 12 8 11 6 7 5 4 1	Average daily gain(-)
Protein % in fat free carcass	77.90	83.19	83.09	79.93	80.37	80.73	80.37	80.82	80.18	82.98	81.82	81.61	± 0.66	N.S.	N.S.
Total carcass fat(g)	31.75	9.65	12.04	26.18	24.87	19.52	21.26	16.41	15.05	13.56	19.22	15.53	± 1.65	1 4 5 7 6 11 8 12 9 10 3 2	Average daily gain
Fat % in dry carcass	46.09	17.81	20.81	39.12	38.63	29.21	31.75	26.06	24.03	22.26	29.34	24.33	± 1.78	1 4 5 7 11 6 8 12 9 10 3 2	Average daily gain
Total carcass ash(g)	6.22	6.55	6.72	6.08	6.20	6.69	6.50	6.69	6.44	6.49	6.13	6.47	± 0.13	3 6 8 2 7 10 12 9 1 5 11 4	Average daily gain
Ash % in dry carcass	8.36	10.98	11.02	8.68	8.99	10.19	9.67	10.71	10.36	10.41	9.50	10.10	± 0.30	3 2 8 10 9 6 12 7 11 5 4 1	Average daily gain(-)
Ash % in fat free carcass	15.52	13.98	13.93	14.30	14.00	14.37	14.18	14.48	13.65	13.38	13.44	13.32	± 0.14	1 8 6 4 7 5 3 9 11 10 2 12	Average daily gain(-)
Protein+fat+ash / Dry carcass wt. %	96.29	97.10	97.61	96.39	96.36	96.54	96.25	96.57	96.85	97.16	96.67	96.19	± 0.45	N.S.	N.S.

* Standard error of treatment means.

** Treatment means not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

retention. It appeared that the better balanced diet such as diets 2, 3, 10 and 12 produce minimum fat and maximum protein retention. The sole barley diet which was limiting in both lysine and threonine produced maximum fat and minimum protein retention in the carcass.

Diet had a rather small significant effect on carcass total ash content. However, these differences could not be detected by Tukey's Test (1953).

The response of rats to lysine and threonine addition appeared to be similar to that obtained with the pig. The increase of added threonine over 0.10% to the 0.75% lysine barley diet did not further improve the growth of rats. Rats were observed to improve growth by addition of a complete amino acid mixture. However, it is difficult to predict whether the addition of some amino acids to the pig diet would further improve the growth as shown by the rats,as the barley diet has actually satisfied the other essential amino acid for the growing-finishing pigs except for lysine and threonine as indicated by comparing analysis figures with the N.A.S.-N.R.C. (1972) feeding standards.

C. CONCLUSION

It may be concluded that diets based on barley alone with adequate mineral and vitamin supplementation do not support optimal growth and feed conversion efficiency in rats. The PER, protein retention and protein retained as percentage of protein intake indicated that such a diet is of poor protein quality. Using the above criteria, performance was improved by lysine supplementation with a further improvement produced when graded levels of threonine were added to the diet. Threonine was probably the second limiting amino acid in barley for the growing rat.

A 0.75% dietary lysine was observed to be adequate for normal growth in conjunction with 0.10% threonine addition (0.47% total threonine in the diet), as supplementation of 0.90% dietary lysine did not further improve performance of the rats. The results also indicated that 0.20% threonine supplementation was required in this barley-lysine diet (either 0.75 or 0.90% dietary lysine) to maximize nitrogen balance when an amino acids mixture was added to the diet. The evidence obviously showed that a low quality barley diet with proper supplementation of limiting amino acids can result in performance equal to that obtained with a barley-soybean diet. The barley-soybean diets produced poor values for PER and protein retained as percentage of protein intake because of excess protein intake.

VII. RAT EXPERIMENT III

A. EXPERIMENTAL PROCEDURE

a. General

The evidence of Rat Experiment II indicated that a 0.75% lysine barley diet with 0.20% threonine (0.57% total threonine in the diet) and an amino acid mixture provided optimal growth equivalent to that obtained with a barley-soybean control diet. A requirement for attaining optimal growth appeared to be the addition of the essential amino acid mixture. However, it is still possible that the improvement may have been attained by addition of other nitrogen sources instead of essential amino acids. Such an addition of non-specific nitrogen would be economically more desirable than the addition of amino acids either in pure form or as proteins in practical pig feeding. Therefore the following experiment was designed to investigate the effect of supplementation of glycine as a nitrogen source on growth performance, feed conversion efficiency, protein utilization and retention of rats and to compare glycine addition with addition of an amino acid mixture.

The experiment consisted of 10 treatments with 4 rats per treatment. Duration was 4 weeks.

b. Animals

A total of 44 albino male rats (Woodlyn/Wistar strain) with average weight of 88 g and 30 ± 1 days of age were employed. Four of these were killed initially to obtain a control measure of body composition. All other procedures were exactly the same as in the previous experiment.

c. Diets

The composition of the diet is shown in Table 14. The preparation of these diet were the same as for Rat Experiment I. The experiment consisted of 10 treatments as follows :

1. Barley only to supply 0.41% total lysine.
2. Barley + 0.444% L-lysine-HCl to supply 0.75% total lysine.
3. Barley + 0.637% L-lysine-HCl to supply 0.90% total lysine.
4. Diet 2 + 0.10% threonine.
5. Diet 4 + amino acid mixture.
6. Diet 4 + glycine.
7. Diet 2 + 0.20% threonine.
8. Diet 7 + amino acid mixture.
9. Diet 7 + glycine.
10. Barley-soybean to supply 0.75% lysine.

The composition of the amino acid mixture was the same as that in Rat Experiment II.

d. Management

Same as Rat Experiment I .

e. Records

Same as Rat Experiment I .

f. Chemical Analysis

Same as Rat Experiment I .

g. Calculations

Same as Rat Experiment I .

h. Statistical Analysis

Same as Rat Experiment I .

Table 14. Percentage composition of diets used in Rat Experiment III (air dry basis)

Ingredients	Diet No.									
	1	2	3	4	5	6	7	8	9	10
Barley	96.82	96.38	96.19	96.28	94.53	94.93	96.18	94.43	94.83	81.34
Soybean meal	-	-	-	-	-	-	-	-	-	15.60
L-lysine HCl*	-	0.444	0.637	0.444	0.444	0.444	0.444	0.444	0.444	-
L-threonine*	-	-	-	0.10	0.10	0.10	0.20	0.20	0.20	-
L-Arginine*	-	-	-	-	0.20	-	-	0.20	-	-
L-tryptophan*	-	-	-	-	0.05	-	-	0.05	-	-
L-histidine*	-	-	-	-	0.20	-	-	0.20	-	-
L-leucine*	-	-	-	-	0.20	-	-	0.20	-	-
L-isoleucine*	-	-	-	-	0.20	-	-	0.20	-	-
L-phenylalanine*	-	-	-	-	0.30	-	-	0.30	-	-
DL-methionine*	-	-	-	-	0.20	-	-	0.20	-	-
L-valine*	-	-	-	-	0.10	-	-	0.10	-	-
L-Glutamic acid*	-	-	-	-	0.30	-	-	0.30	-	-
L-Glycine*	-	-	-	-	-	1.355	-	-	1.355	-
Trace mineral and vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Defluorinated rock phosphate	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.49
Limestone	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.57
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

The composition of trace mineral and vitamin premix was the same as that of used in Pig Experiments I and II .

* The L-lysine HCl was feed grade and was 98% pure, containing 78% L-lysine. The L-threonine was pure and DL-methionine was 98% pure. All were produced by the Ajinomoto Co., Japan.

Table 15. Content of essential amino acids of each diet for Rat Experiment III (g amino acid/100g mixed feed) on air dry basis .

Amino Acid	Diet No. 1	2	3	4	5	6	7	8	9	10
Arginine	0.45	0.45	0.45	0.45	0.64	0.45	0.45	0.64	0.45	0.82
Histidine	0.21	0.21	0.21	0.21	0.40	0.21	0.21	0.40	0.21	0.40
Isoleucine	0.40	0.40	0.40	0.40	0.59	0.40	0.40	0.59	0.40	0.58
Leucine	0.73	0.73	0.73	0.73	0.91	0.73	0.73	0.91	0.73	1.18
Lysine	0.41	0.75	0.90	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Methionine	0.17	0.17	0.17	0.17	0.36	0.17	0.17	0.36	0.17	0.24
(Cystine)*	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.27
Phenylalanine	0.53	0.53	0.53	0.53	0.82	0.53	0.53	0.82	0.53	0.80
(Tyrosine)*	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.48
Threonine	0.37	0.37	0.37	0.47	0.47	0.47	0.57	0.57	0.57	0.57
Tryptophan	0.11	0.11	0.11	0.11	0.16	0.11	0.11	0.16	0.11	0.19
Valine	0.68	0.68	0.68	0.68	0.76	0.68	0.68	0.76	0.68	0.86
Glutamic acid**	2.36	2.36	2.36	2.36	2.60	2.36	2.36	2.60	2.36	2.97
Glycine**	0.42	0.42	0.42	0.42	0.41	1.77	0.42	0.41	1.77	0.56
Dry matter content %	90.14	89.13	89.69	90.12	90.00	89.93	89.94	89.57	89.85	90.45
Crude protein % (N × 6.25)	10.58	11.17	11.39	11.15	12.74	12.86	11.24	12.89	12.91	17.40

* Semi-essential amino acid

** Non-essential amino acid

B. RESULTS AND DISCUSSION

The results of ADG, FCE and PER are presented in Table 16 while the carcass analysis data are summarized in Table 17 .

The rats fed with diets the same as those in Rat Experiment II obtained basically similar results in terms of ADG, FCE, PER and carcass protein analysis values. This further confirmed the importance of threonine addition to barley-lysine to improve the protein quality. The evidence also confirmed threonine as the second limiting amino acid in barley. The rats' performance on the 0.75% and 0.90% lysine diets did not differ significantly, further confirming the previous experimental results that 0.75% lysine is adequate for the growing rat . With the same level of lysine in the diets, addition of 0.10% threonine was slightly but non-significantly better than 0.20 % threonine addition. However, the results were reversed and significantly in favorof the 0.20% threonine added diet when further amino acid mixtures were added. This observation was in agreement with Rat Experiment II.

Replacement of the amino acid mixture with glycine on an isonitrogenous basis failed to show the same performance in all measured criteria indicating that the improved performance in diet 8 was not due to nitrogen level per se but rather due to the proper balance of essential amino acids in the diet. As a matter of fact, glycine addition depressed the rat performance. This suggests that the addition of glycine at these levels creats an imbalance. Rama Rao et al. (1960) reported that glycine might depress

Table 16. Effect of amino acid supplementation of low protein barley on average daily gain, feed conversion efficiency and protein efficiency ratio of rats.

	Week	1 Barley only (Basal)	2 B + lys(0.75%)	3 B + lys(0.90%)	4 B + lys(0.75%) +0.10%thr	5 B + lys(0.75%) +0.10%thr+ amino acid mixture	6 B + lys(0.75%) +0.10%thr+ glycine	7 B + lys(0.75%) +0.20%thr	8 B + lys(0.75%) +0.20%thr+ amino acid mixture	9 B + lys(0.75%)+ 0.20%thr + glycine	10 Barley-soy (control)	SE*	Result of significance test**	Significant covariables
Average Daily Gain(g)	1	2.77	3.21	3.54	4.35	4.82	4.66	4.37	5.01	4.52	5.93	± 0.17	10 8 5 6 9 7 4 3 2 1	Initial weight(-) Average feed intake
	2	3.28	3.93	4.04	4.34	5.22	4.11	4.31	5.50	4.50	5.73	± 0.23	10 8 5 9 4 7 6 3 2 1	Average feed intake
	3	3.04	3.68	3.90	4.69	4.70	4.61	4.37	5.81	5.27	5.68	± 0.21	8 10 9 5 4 6 7 3 2 1	Initial weight(-) Average feed intake
	4	2.59	3.49	3.47	4.30	4.93	4.23	3.69	5.63	4.39	4.71	± 0.30	8 5 10 9 4 6 7 2 3 1	Initial weight(-) Average feed intake
	Overall period	3.28	3.78	3.93	4.42	4.81	4.41	4.28	5.18	4.56	5.19	± 0.11	10 8 5 9 4 6 7 3 2 1	Initial weight(-) Average feed intake
Feed Conversion Efficiency (g feed / g gain)	1	4.47	4.06	3.47	2.90	2.69	2.48	2.87	2.47	2.67	2.25	± 0.16	1 2 3 4 7 5 9 6 8 10	Initial weight Average feed intake(-)
	2	4.62	3.89	3.78	3.53	3.00	3.82	3.51	2.75	3.35	2.78	± 0.18	1 2 6 3 4 7 9 5 10 8	N.S.
	3	5.01	4.40	4.05	3.48	3.45	3.52	3.68	2.68	2.97	2.79	± 0.17	1 2 3 7 6 4 5 9 10 8	Initial weight Average feed intake(-)
	4	5.74	4.61	4.63	3.75	3.18	3.77	4.41	2.63	3.56	3.60	± 0.36	N.S.	Initial weight Average feed intake(-)
	Overall period	4.58	3.97	3.78	3.37	3.12	3.35	3.47	2.88	3.22	2.94	± 0.08	1 2 3 7 4 6 9 5 10 8	Initial weight Average feed intake(-)
	Initial weight	86	88	89	89	89	86	85	87	88	92	± 2.33		
	Protein Efficiency Ratio	2.04	2.22	2.32	2.69	2.56	2.30	2.60	2.71	2.37	2.02	± 0.06	8 4 7 5 9 3 6 2 1 10	N.S.

* Standard error of treatment means.

** Treatment means not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

Table 17. Effect of amino and supplementation of low protein barley on carcass characteristics of rats .

	1 Barley only (Basal)	2 B + lys(0.75%)	3 B + lys(0.90%)	4 B + lys(0.75%) +0.10%thr	5 B + lys(0.75%) +0.10%thr+ amino acid mixture	6 B + lys(0.75%) +0.10%thr+ glycine	7 B + lys(0.75%) +0.20%thr	8 B + lys(0.75%) +0.20%thr+ amino acid mixture	9 B + lys(0.75%) +0.20%thr+ glycine	10 Barley-soy (control)	SE*	Result of significance test	Significant covariable
Total carcass protein (g)	31.19	33.91	34.33	37.38	37.76	35.90	36.80	39.35	36.39	42.81	± 0.68	10 8 5 4 7 9 6 3 2 1	Average daily gain
Protein retention during 28 days on trial (g)	18.39	21.12	21.55	24.60	25.00	23.12	24.03	26.58	23.61	30.06	± 0.68	10 8 5 4 7 9 6 3 2 1	Average daily gain
Protein retained / Protein intake %	33.84	41.65	42.29	52.90	49.01	44.23	50.76	55.45	45.97	42.71	± 1.23	8 4 7 5 9 6 3 10 2 1	Average daily gain
Protein % in dry carcass	40.39	47.58	49.65	60.34	59.04	53.27	58.53	63.06	59.47	71.88	± 1.67	10 8 4 9 5 7 6 3 2 1	Average daily gain(-)
Protein % in fat free carcass	78.15	79.86	79.48	80.09	80.63	80.88	81.45	81.78	81.08	82.39	± 0.78	N.S.	N.S.
Total carcass fat (g)	34.35	28.83	26.42	15.28	16.68	20.41	17.62	13.46	16.58	3.40	± 1.58	1 2 3 6 7 5 9 4 8 10	Average daily gain
Fat % in dry carcass	49.86	41.63	38.23	24.43	25.76	31.26	27.94	21.93	26.81	10.77	± 1.69	1 2 3 6 7 9 5 4 8 10	Average daily gain
Total carcass ash (g)	5.84	6.02	6.23	6.81	6.45	6.39	6.37	6.63	6.34	7.18	± 0.15	10 4 8 5 6 7 9 3 2 1	Average daily gain
Ash % in dry carcass	7.49	8.30	8.95	11.03	10.31	9.99	10.18	10.87	10.33	12.51	± 0.28	10 4 8 9 5 7 6 3 2 1	Average daily gain
Ash % in fat free carcass	14.95	14.28	14.49	14.60	13.84	14.51	14.12	13.89	14.12	14.07	± 0.23	N.S.	N.S.
Protein+fat+ash / Dry carcass wt. %	96.18	96.42	96.19	96.01	95.96	97.20	96.80	96.66	96.47	96.73	± 0.53	N.S.	N.S.

* Standard error of treatment mean.

** Treatment means not underscored by the same line are significantly different at the 5% level of probability (Tukey, 1953).

growth when given in large amounts.

Since glycine has been reported to be less effective when used as a nitrogen source than ammonium salts and L-glutamic acid (Rose et al., 1949), it is still possible that rat growth coulb be improved by using either ammonium salts or glutamic acid to increase the dietary nitrogen level.

Carcass protein retention showed the 0.75% lysine barley diet with 0.20% added threonine, and an amino acid mixture not to be significantly different from the barley-soybean control. This was similar to the results obtained in Rat Experiment II. The former diet had the best value for protein retained as a percentage of protein intake.

The addition of glycine did not improved the carcass protein retention and the value for protein retained as a percentage of protein intake. It also appeared to depress the values of the above criteria when compared with values obtained with similar diets without glycine.

The carcass data further confirmed that glycine nitrogen cannot replace the nitrogen of an amino acid mixture for optimal rat growth. The total carcass fat content produced on the glycine-added diets was higher than that produced on the corresponding amino acid supplemented diets, supporting the fact that imbalanced diets tend to deposite more fat than balanced diets.

C. CONCLUSION

Experiment III further indicated that 0.75% lysine was adequate for the growing rat . The barley-lysine diet containing 0.75% lysine showed an improvement in average daily gain, feed conversion efficiency and nitrogen retention and nitrogen retained/nitrogen intake when graded levels of threonine were added. The supplementation with amino acid mixtures to the 0.20% threonine-added barley-lysine diets gave rat performance equivalent to that obtained with the barley-soybean control diet. This confirmed the result of Rat Experiment II.

When glycine was used as a nitrogen source, it failed to satisfactorily replace the amino acid mixture as a supplement to the barley-lysine-threonine diet. The depression of rat growth and nitrogen retention indicated that glycine at this level cannot be ultilized effectively as a nitrogen source for the growing rat. Amino acid 'imbalance' might have been created when this level of glycine was added. It is possible that either supplementation of one or more other essential amino acids instead of this total amino acids mixture or raising the total nitrogen level with another source of nitrogen could give better performance. This, however, was not investigated in this experiment.

VIII. GENERAL CONCLUSIONS

These experiments in the pigs and the rats have shown threonine to be the second limiting amino acid in barley, with lysine being the first. The trend showed that the addition of graded levels of L-threonine to the barley-lysine diet (0.75% total lysine) progressively improved the rate of growth, feed conversion efficiency, carcass quality and nitrogen retention for the growing-finsihing pigs to a level of 0.10% threonine. Threonine addition at a level of 0.10% to the diet (0.47% total threonine) produced the best mean daily gain, feed conversion efficiency, eye muscle index and eye muscle area. Further addition of threonine and threonine plus methionine did not improve the above criteria. However, all amino acid supplemented diets gave growth rates which were inferior to that obtained with the soybean control diet prior 45 Kg liveweight but not significantly different over 45 Kg liveweight. The nitrogen balance study showed that 0.15% threonine addition gave nitrogen retention insignificantly different from other threonine added diets and from the control diet although the control still gave greater nitrogen retention. The barley-lysine diet with no threonine addition gave significantly poorer performance in growht rate, feed conversion efficiency, carcass quality and nitrogen retention than the threonine supplemented diets and the control diet. The overall results of the pig experiments suggested that 0.10% threonine added to an 0.75% lysine-barley diet was comparable to the control diet after 45 Kg live-weight. However, a slight increase in the total lysine and threonine levels beyond 0.75% and 0.47%, respectively, or provision of additional nitrogen may be able to reduce the difference between the barley-amino acid and barley-soybean meal diets. More work is needed to clarify this point.

The rat experiments showed that 0.75% lysine was adequate for the growing rat since no significant improvement was obtained by further addition of lysine (to 0.90% total lysine). Similar to the results of the pig experiment, the mean daily gain, feed conversion efficiency, protein efficiency ratio and nitrogen retention improved progressively with the addition of graded threonine levels. The 0.10% added threonine (0.47% total threonine) apparently provided an adequately balanced diet for rat growth. However, 0.20% threonine had to be added to the diet (0.57% total threonine) when the diet was further supplemented with a purified essential amino acid mixture in order to obtain optimum rat performance equivalent to that obtained on the barley-soybean diet. Glycine could not be used as the nitrogen source to replace the amino acid mixture.

The evidence of the present study strongly indicates that purified amino acids are as effective as natural protein concentrates when added to barley for the finishing pig and the growing rat. Fine adjustment of the balance among amino acids, especially between the first and second limiting amino acids is needed to achieve optimal growth.

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X. APPENDIX

Table 1A. Average feed intake (kg) per pig per day for each diet on dry matter basis in Pig Experiment I .

Treatment	Rep 1	Rep 2	Rep 3
1	1.986	1.854	1.988
2	1.815	1.768	1.880
3	1.863	1.754	1.899
4	1.886	1.822	2.024
5	1.824	1.755	1.889
6	1.765	1.910	1.894

Table 1B. Analysis of variance of average daily feed intake in Pig Experiment I .

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.)	
					P=0.05	P=0.01
Total	17	0.1103				
Treatment	5	0.0389	0.0078	1.8616	3.20	5.32
Covariable starting weight	1	0.0264	0.0264	6.3247*	4.82	9.65
Residual	11	0.0460	0.0042			

*P < 0.05

Table 2A. Mean daily gain (Kg) from start to 45 Kg body weight of 108 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	0.7500	0.6071	0.7194	0.7898	0.7143	0.6684
	0.6760	0.6653	0.5833	0.6735	0.6449	0.5748
	0.5561	0.6403	0.5893	0.6505	0.5310	0.6378
2	0.6122	0.4949	0.4216	0.4595	0.4738	0.4274
	0.3036	0.4821	0.3857	0.4216	0.5281	0.4770
	0.4658	0.4506	0.5867	0.5214	0.4390	0.4512
3	0.6714	0.4949	0.6551	0.5612	0.5714	0.4645
	0.5136	0.4609	0.5274	0.5500	0.5485	0.5561
	0.4680	0.4797	0.4913	0.3866	0.5095	0.5918
4	0.6755	0.5383	0.4879	0.5434	0.6148	0.5289
	0.5561	0.5357	0.5643	0.5286	0.6490	0.5578
	0.6071	0.5012	0.7296	0.5459	0.5655	0.5714
5	0.5060	0.5893	0.5833	0.5262	0.5017	0.5689
	0.5536	0.5765	0.4727	0.5405	0.5689	0.4869
	0.5442	0.5578	0.6276	0.3583	0.4524	0.5408
6	0.5782	0.5013	0.5867	0.5052	0.6857	0.5740
	0.5405	0.5000	0.5167	0.5119	0.4900	0.5493
	0.6490	0.6327	0.5910	0.6020	0.5281	0.5918

Table 2B. Analysis of variance of mean daily gain from start to 45 Kg body weight of 108 pigs with starting weight as covariable in Pig Experiment I

Source	D.F.	S.S.	M.S.	F-value	Variance ratio (F nec.) P=0.05	Variance ratio (F nec.) P=0.01
Total	107	0.7442				
Treatment (a)	5	0.3222	0.0644	19.96**	2.35	3.29
Sex (b)	1	0.0043	0.0043	1.32	3.98	7.01
Block (c)	2	0.0117	0.0058	1.81	3.13	4.92
a × b	5	0.0136	0.0027	0.84	2.35	3.29
a × c	10	0.0334	0.0033	1.04	1.97	2.59
b × c	2	0.0020	0.0010	0.31	3.13	4.92
a × b × c	10	0.0257	0.0026	0.80	1.97	2.59
Starting weight	1	0.1023	0.1023	31.60**	3.98	7.01
Residue	71	0.2292	0.0032			

** $P < 0.01$

Table 3A. Mean daily gain (Kg) from 45 Kg body weight to finish of 108 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	0.8002	0.7917	0.6976	0.6345	0.7310	0.7071
	0.8257	0.7333	0.6186	0.6964	0.7071	0.6917
	0.7619	0.7214	0.6173	0.7738	0.8648	0.7440
2	0.6372	0.6247	0.4645	0.5125	0.6952	0.6359
	0.5434	0.6299	0.6344	0.4283	0.7262	0.5169
	0.7083	0.5710	0.5071	0.6368	0.7310	0.6567
3	0.8129	0.5263	0.5939	0.5656	0.8206	0.5701
	0.6797	0.7476	0.6881	0.5015	0.7345	0.5474
	0.6750	0.6074	0.5100	0.4710	0.5020	0.5900
4	0.7993	0.7107	0.8112	0.8248	0.8078	0.6024
	0.8112	0.7524	0.7058	0.6632	0.6976	0.7440
	0.8197	0.6301	0.7296	0.6883	0.7942	0.8019
5	0.8112	0.6905	0.7857	0.6494	0.8291	0.7655
	0.7631	0.5539	0.7279	0.6463	0.6909	0.6709
	0.7369	0.5658	0.5558	0.4408	0.8172	0.6571
6	0.8070	0.5560	0.6881	0.6395	0.6502	0.7152
	0.6048	0.7452	0.7107	0.6845	0.7645	0.6385
	0.7039	0.6821	0.7131	0.7131	0.6455	0.6848

Table 3B. Analysis of variance of mean daily gain from 45 Kg body weight to finish of 108 pigs with no covariable in Pig Experiment I .

Source		D.F.	S.S.	M.S.	F-value	Variance ratio (F nec.)	
						P=0.05	P=0.01
Total		107	1.0421				
Treatment	(a)	5	0.2904	0.0581	10.07**	2.35	3.29
Sex	(b)	1	0.1021	0.1021	17.69**	3.98	7.01
Block	(c)	2	0.0909	0.0455	7.88**	3.13	4.92
a × b		5	0.0381	0.0076	1.32	2.35	3.29
a × c		10	0.0701	0.0070	1.22	1.97	2.59
b × c		2	0.0117	0.0058	1.01	3.13	4.92
a × b × c		10	0.0235	0.0024	0.41	1.97	2.59
Residue		72	0.4153	0.0058			

** $P < 0.01$

Table 4A. Mean daily gain (Kg) from start to finish of 108 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	0.7812	0.7333	0.7026	0.7028	0.7276	0.6957
	0.7736	0.7173	0.6194	0.6702	0.6933	0.6411
	0.6837	0.7079	0.6446	0.7536	0.6988	0.6995
2	0.6508	0.5882	0.4897	0.5337	0.5778	0.5548
	0.3747	0.5830	0.5344	0.4587	0.6322	0.4993
	0.5994	0.5185	0.5627	0.6318	0.5899	0.5774
3	0.7687	0.4942	0.5903	0.5791	0.7146	0.5521
	0.6315	0.6162	0.6409	0.5226	0.6747	0.5739
	0.5805	0.5774	0.5197	0.4116	0.5203	0.6088
4	0.7724	0.6577	0.6775	0.6928	0.7581	0.6077
	0.7235	0.6679	0.6607	0.6267	0.7080	0.6850
	0.7301	0.5518	0.5795	0.6210	0.7190	0.7203
5	0.6768	0.6528	0.7141	0.6177	0.6859	0.6852
	0.6681	0.5860	0.6098	0.6098	0.6544	0.5603
	0.6615	0.5797	0.6038	0.3983	0.6342	0.5995
6	0.6962	0.5505	0.6533	0.6122	0.6626	0.6412
	0.5949	0.6134	0.6231	0.6465	0.6183	0.5973
	0.6151	0.6781	0.6796	0.7036	0.5749	0.6381

Table 4B. Analysis of variance of mean daily gain from start to finish of 108 pigs with starting weight as covariable in Pig Experiment I .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) P=0.05	Variance Ratio F(nec.) P=0.01
Total	107	0.6734				
Treatment (a)	5	0.2772	00.0554	17.549**	2.35	3.29
Sex (b)	1	0.0306	0.0306	9.688**	3.98	7.01
Block (c)	2	0.0019	0.0010	0.315	3.13	4.92
a × b	5	0.0299	0.0597	1.891	2.35	3.29
a × c	10	0.0256	0.0026	0.811	1.97	2.59
b × c	2	0.0036	0.0018	0.577	3.13	4.92
a × b × c	10	0.0173	0.0017	0.548	1.97	2.59
Covariable	1	0.0347	0.0347	10.995**	3.98	7.01
Residual	71	0.2243	0.0032			

** P < 0.01

Table 5A. Average feed conversion efficiency ratio per pig (D.M. feed intake (kg)/weight gain (kg)) in Pig Experiment I .

Treatment	Rep 1	Rep 2	Rep 3
1	2.762	2.787	2.943
2	3.414	3.466	3.341
3	3.222	3.289	3.341
4	2.890	2.924	3.040
5	2.971	3.049	3.035
6	2.919	3.048	3.093

Table 5B. Analysis of variance of feed conversion efficiency ratio in Pig Experiment I .

Source	D.F.	S.S.	M.S.	F-value	Variance ratio F(nec.)	
					P=0.05	P=0.01
Total	17	0.7699				
Treatment	5	0.7014	0.1403	23.4865**	3.20	5.32
Covariable starting weight	1	0.0007	0.0007	0.1128	4.82	9.65
Residual	11	0.0657	0.0060			

** $P < 0.01$

Table 6A. Total back fat (mm) of 107 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	91	80	90	82	84	62
	96	88	83	81	103	76
	87	78	83	84	90	77
2	104	95	70	93	91*	101
	79	82	100	78	100	95
	103	96	104	97	120	108
3	85	81	97	89	100	109
	106	86	120	95	104	87
	92	84	109	75	105	92
4	98	91	104	82	106	79
	95	94	96	99	102	79
	85	66	90	95	99	102
5	81	82	101	64	100	86
	87	88	103	75	85	79
	97	100	115	55	97	91
6	86	89	92	86	103	98
	81	100	86	95	91	68
	94	87	105	83	84	96

* Calculated missing value (Snedecor and Cochran, 1967).

Table 6B. Analysis of variance of total back fat thickness with final body weight, average body weight gain and body length as covariables in Pig Experiment I .

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	10860.683				
Treatment (a)	5	1157.6	231.53	3.207**	2.35	3.29
Sex (b)	1	656.35	656.35	9.090**	3.98	7.01
Block (c)	2	40.553	20.276	0.281	3.13	4.92
a × b	5	358.44	71.687	0.993	2.35	3.29
a × c	10	838.37	83.837	1.161	1.97	2.59
b × c	2	153.20	76.599	1.061	3.13	4.92
a × b × c	10	1190.0	119.00	1.648	1.97	2.59
Final body wt.	1	947.86	974.86	13.501**	3.98	7.01
A.D.G.	1	25.24	25.24	0.350	3.98	7.01
Body length	1	556.17	556.17	7.703**	3.98	7.01
Residual	68	4909.9	72.204			

** $P < 0.01$

Table 7A. Back fat 40 mm from mid line (mm) of 107 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	21	14	16	15	19	12
	22	17	16	9	17	12
	17	14	20	12	20	9
2	20	22	12	16	17*	17
	13	21	23	11	24	19
	20	18	23	21	25	22
3	18	11	20	16	20	19
	23	21	21	20	21	14
	14	15	23	12	25	21
4	16	16	18	14	24	14
	23	17	18	17	22	13
	20	12	17	17	22	20
5	15	13	17	13	22	18
	15	15	17	15	15	17
	15	17	28	6	17	19
6	17	16	19	14	19	17
	13	22	22	21	16	8
	22	21	22	16	13	21

* Calculated missing value (Scedecor and Cochran, 1967).

Table 7B. Analysis of variance of back fat 40 mm from mid line with final body weight, average daily weight gain and body length as covariables in Pig Experiment I .

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	1348.190				
Treatment (a)	5	154.03	30.807	3.698**	2.35	3.29
Sex (b)	1	48.793	48.793	5.858**	3.98	7.01
Block (c)	2	7.407	3.7035	0.445	3.13	4.92
a × b	5	55.658	11.132	1.336	2.35	3.29
a × c	10	150.61	15.061	1.808	1.97	2.59
b × c	2	30.150	15.075	1.810	3.13	4.92
a × b × c	10	49.043	4.9043	0.589	1.97	2.59
Final body wt.	1	138.575	138.575	16.636**	3.98	7.01
A.D.G.	1	21.131	21.131	2.537	3.98	7.01
Body length	1	126.343	126.343	15.167**	3.98	7.01
Residual	68	566.45	8.330			

* $p < 0.05$ ** $p < 0.01$

Table 8A. Eye muscle area (mm^2) of 107 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	2430	2900	2700	2650	2750	3080
	2510	2830	2960	3200	2750	3660
	2550	3060	2690	3220	3080	3440
2	2190	2680	2560	2820	2333*	2540
	2080	2600	2500	2910	2250	2800
	2390	2580	2420	2580	2170	2670
3	2410	3030	2540	2780	2250	2890
	2880	2810	2520	2400	2660	3290
	2620	2740	2660	2910	2360	2540
4	2900	2910	2280	2390	2890	2770
	2410	2880	2810	3320	2500	2750
	2540	2450	2880	2690	2590	2680
5	2760	3010	2900	2880	2490	2740
	3120	3000	2770	2750	2580	1980
	2480	2520	2320	2560	2470	2420
6	2760	2690	2550	2880	2330	3330
	2850	2470	2440	2600	2630	3010
	2320	2440	2400	2710	2710	2730

* Calculated missing value (Snedecor and Cochran, 1967).

Table 8B. Analysis of variance of eye muscle area with final body weight, average daily gain and body length as covariables in Pig Experiment I .

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	0.78515×10^7				
Treatment (a)	5	0.14274×10^7	0.28547×10^6	5.558**	2.35	3.29
Sex (b)	1	0.76233×10^6	0.76233×10^6	14.842**	3.98	7.01
Block (c)	2	36818.0	18409	0.358	3.13	4.92
a × b	5	0.46021×10^6	92043	1.792	2.35	3.29
a × c	10	0.93779×10^6	93779	1.826	1.97	2.59
b × c	2	70083	35042	0.682	3.13	4.92
a × b × c	10	0.26036×10^6	26036	0.507	1.97	2.59
Final body wt.	1	0.11576×10^6	0.11576×10^6	2.254	3.98	7.01
A.D.G.	1	0.22464×10^6	0.22464×10^6	4.374*	3.98	7.01
Body length	1	63524	63524	1.237	3.98	7.01
Residual	68	0.34926×10^7	51361.8			

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

Table 9A. Eye muscle index (length 'A' × width 'B') (mm^2) of the 107 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	3195	3927	3696	3840	3840	4717
	3750	3840	4345	4592	4056	5187
	3476	4312	3854	4536	4480	5162
2	2982	3871	3483	4050	3254*	3724
	3280	3619	3465	3864	3634	3876
	3600	3476	3300	3822	3358	3713
3	3234	4361	3600	4100	3212	4160
	4088	3850	3572	3577	3700	4346
	3854	3850	3555	3773	3168	3619
4	4250	4345	3337	3431	4100	3984
	3408	4081	4067	4644	3969	4080
	3564	3690	4080	3950	3750	3920
5	3672	4346	4182	4539	3528	3960
	4582	4592	3978	4018	4067	3139
	3724	3577	3225	3696	3650	3572
6	3927	4018	3431	4104	3360	4720
	3822	3672	3588	3936	3569	4316
	3192	3384	3397	3772	3840	3634

* Calculated missing value (Snedecor and Cochran, 1967).

Table 9B. Analysis of variance of eye muscle index (length × width) with final body weight, average daily gain and body length as covariables in Pig Experiment I .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	106	0.17293×10^8				
Treatment (a)	5	0.31403×10^7	0.62807×10^6	5.718**	2.35	3.29
Sex (b)	1	0.17253×10^7	0.17253×10^7	15.707**	3.98	7.01
Block (c)	2	0.29667×10^6	0.14834×10^6	1.351	3.13	4.92
a × b	5	0.61179×10^6	0.12236×10^6	1.114	2.35	3.29
a × c	10	0.22437×10^7	0.22437×10^6	2.043*	1.97	2.59
b × c	2	94287	47144	0.429	3.13	4.92
a × b × c	10	0.56122×10^6	56122	0.511	1.97	2.59
Final body wt.	1	0.33189×10^6	0.33189×10^6	3.022	3.98	7.01
A.D.G.	1	0.57957×10^6	0.57957×10^6	5.276*	3.98	7.01
Body length	1	0.23945×10^6	0.23945×10^6	2.180	3.98	7.01
Residual	68	$0.74690 \quad 10^7$	0.10984×10^6			

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

Table 10A. Days on trials of 108 pigs in Pig Experiment I .

Treatment	Rep 1		Rep 2		Rep 3	
	Male	Female	Male	Female	Male	Female
1	70	91	98	91	84	98
	91	91	119	98	91	105
	98	98	119	98	105	98
2	98	112	140	113	119	119
	113	105	140	140	105	119
	119	133	119	119	119	119
3	77	126	98	112	91	133
	112	105	112	133	98	119
	119	126	140	140	133	105
4	84	98	112	91	91	105
	91	98	105	119	91	105
	98	98	110	112	105	91
5	105	105	105	119	91	98
	98	112	112	119	105	105
	105	112	119	119	105	105
6	98	119	98	112	91	105
	119	105	112	112	112	112
	105	98	105	105	112	105

Table 10B. Analysis of variance of days on trials with initial body weight as covariable in Pig Experiment I .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) P=0.05	Variance Ratio F(nec.) P=0.01
Total	107	21601.74				
Treatment (a)	5	8072.284	1614.457	26.6750**	2.35	3.29
Sex (b)	1	268.3129	268.3129	4.4332*	3.98	7.01
Block (c)	2	134.3030	67.1515	1.1095	3.13	4.92
a × b	5	605.5736	121.1147	2.0011	2.35	3.29
a × c	10	588.8364	58.8836	0.9729	1.97	2.59
b × c	2	95.29601	47.6480	0.7873	3.13	4.92
a × b × c	10	274.5964	27.4596	0.4537	1.97	2.59
Covariable	1	4620.856	4620.856	76.3485**	3.98	7.01
Residual	71	4297.144	60.5232			

*P < 0.05 **P < 0.01

Table 11A. D.M. feed intake (g)/wk. in Pig Experiment II .

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1	12580.375	14140.000	13166.950	14990.225	12767.850	12900.675
2	11212.620	13109.890	11204.960	12840.660	11165.510	11465.850
3	11478.130	123821230	11865.790	13845.210	12656.450	13978.210
4	11870.400	12939.050	12250.950	14297.700	11515.750	12355.900
5	10855.400	12309.730	12093.620	13764.010	12466.520	13911.380
6	12779.450	14392.600	11454.800	11752.350	11735.550	11350.850

Table 11B. Analysis of variance of D.M. feed intake (g)/wk. in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	35	4.021×10^7				
Treatment	5	8.369×10^6	1.674×10^6	1.577^{NS}	2.53	3.70
Residual	30	3.185×10^7	1.0615×10^6			

NS : non-significance

Table 12 . Analysis of variance of N intake (g)/wk. in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) P=0.05	P=0.01
Total	35	13290.6				
Treatment	5	120407.6	24081.51	57.81**	2.53	3.70
Residual	30	12496.99	416.57			

** P < 0.01

Table 13A. Percentage D.M. digestibility in Pig Experiment II .

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1	79.26	78.91	77.59	76.58	78.41	79.85
2	78.98	75.19	78.41	75.58	80.51	77.86
3	78.07	75.93	78.34	76.04	74.57	74.59
4	79.36	78.96	78.20	77.06	78.22	75.92
5	78.90	77.89	77.65	75.64	77.55	78.08
6	72.70	73.20	79.02	78.96	80.58	78.40

Table 13B. Analysis of variance of dry matter digestibility in percent in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	35	127.679				
Treatment	5	16.943	3.389	0.9180^{NS}	2.53	3.70
Residual	30	110.74	3.691			

NS : Non-significance

Table 14A. Nitrogen retention (g)/wk. in Pig Experiment II .

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1	129.70	143.52	123.82	115.01	146.59	140.97
2	99.89	102.48	79.86	95.33	72.09	77.00
3	87.40	91.74	106.73	121.46	103.43	111.17
4	109.65	107.84	98.75	120.17	103.97	105.40
5	104.80	121.54	103.81	118.90	109.56	128.29
6	101.48	115.68	98.40	99.99	105.82	91.36

Table 14B. Analysis of variance for nitrogen retention in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	35	10351.80				
Treatment	5	6857.99	1371.60	11.7773**	2.53	3.70
Residual	30	3493.81	116.46			

** P < 0.01

Table 14C. Analysis of co-variance of nitrogen retention with feed intake as covariable in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) P=0.05	P=0.01
Total	35	10351.800				
Treatment	5	3818.843	763.769	8.668**	2.55	3.73
Covariable feed intake	1	938.654	938.654	10.653**	4.18	7.60
Residual	29	2555.155	88.109			

** P < 0.01

Table 14D. Analysis of co-variance for nitrogen retention with nitrogen intake as covariable in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) P=0.05	P=0.01
Total	35	10351.800				
Treatment	5	1665.369	333.074	3.3897*	2.55	3.73
Covariable nitrogen intake	1	644.268	644.268	6.5568**	4.18	7.60
Residual	29	2849.541	98.260			

* P < 0.05 ** P < 0.01

Table 15A. Nitrogen absorbed as a percentage of nitrogen intake in Pig Experiment II .

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1	78.07	78.24	75.99	75.15	75.59	77.61
2	68.89	64.00	68.45	67.92	74.49	70.91
3	69.45	66.20	69.84	68.33	62.22	60.84
4	71.37	71.14	69.87	70.44	68.96	67.67
5	72.74	70.84	69.29	66.76	67.01	70.60
6	59.05	59.91	75.66	74.73	78 08	71.19

Table 15B. Analysis of variance for nitrogen absorbed as a percentage of nitrogen intake in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	35	891.128				
Treatment	5	369.320	73.864	4.247**	2.53	3.70
Residual	30	521.808	17.394			

** $P < 0.01$

Table 16A. Nitrogen retained as a percentage of nitrogen intake in Pig Experiment II .

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1	36.65	36.09	33.09	27.16	40.82	38.85
2	50.60	44.39	40.48	42.16	36.67	38.14
3	41.88	40.75	49.47	48.25	44.95	43.74
4	50.83	45.86	44.35	46.25	49.68	46.93
5	53.29	54.50	47.38	47.69	48.51	50.91
6	43.51	44.03	47.06	46.61	49.40	44.10

Table 16B. Analysis of variance for nitrogen retained as a percentage of nitrogen intake in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.)	
					P=0.05	P=0.01
Total	35	1195.433				
Treatment	5	791.771	158.354	11.769**	2.53	3.70
Residual	30	403.663	13.455			

** P < 0.01

Table 17A. Nitrogen retained as a percentage of nitrogen absorbed in Pig Experiment II .

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
1	46.97	46.12	44.00	36.30	54.00	50.06
2	73.45	69.37	59.13	62.08	49.23	53.79
3	60.30	61.55	70.84	70.61	72.24	71.90
4	71.22	64.46	63.48	65.65	72.04	69.35
5	73.27	76.94	68.39	71.42	72.40	71.11
6	73.67	73.51	62.21	62.37	63.27	61.95

Table 17B. Analysis of variance of nitrogen retained as a percentage of nitrogen absorbed in Pig Experiment II .

Source	D.F.	S.S.	M.S.	F-value	Variance Ratio F(nec.) P=0.05	Variance Ratio F(nec.) P=0.01
Total	35	3565.118				
Treatment	5	2544.151	508.802	14.951**	2.53	3.70
Residual	30	1020.967	34.032			

** P < 0.01

Table 18 Analysis of covariance for average daily gain and feed conversion efficiency with initial weight and daily feed intake as covariables in Rat Experiments I, II and III.

Experiment	Source	DF	Mean Square: Average daily gain, week 1	2	3	4	overall	Feed efficiency 1	2	3	4	overall
I	Treatments	5	2.8962**	1.4948**	0.4414	0.4165	1.1954**	1.4702**	0.8876**	0.3294	0.2658	0.6117**
	Initial weight	1	1.6993**	1.6769*	0.0009	1.5153	0.4853**	0.8956**	0.7978*	0.0453	0.7881	1.7953*
	Daily feed intake	1	11.9174**	5.2256**	3.6885**	4.4858*	5.3272**	1.1758**	0.2282	0.5174	0.3543	1.7057*
	Residual	28	0.1396	0.2499	0.2720	0.4196	0.0630	0.0978	0.1605	0.3204	0.2975	0.0298
II	Treatments	11	3.0793**	1.0924**	1.0618**	1.1032**	1.4758**	1.8036**	0.6671**	0.8411**	1.5654**	0.9539**
	Initial weight	1	0.7948**	0.2835	1.9857**	2.4942**	0.3330**	0.8460	0.1672	1.5175**	3.7148**	0.1896**
	Daily feed intake	1	13.0126**	3.1398**	9.0803**	13.2613**	4.0303**	2.8256**	0.0046	0.9732*	7.2121**	0.0990*
	Residual	34	0.0960	0.1932	0.1894	0.2534	0.0293	0.2482	0.1093	0.1395	0.3732	0.0159
III	Treatments	9	2.8886**	1.0166**	1.0979**	0.9896*	1.2320**	1.9398**	0.6131**	0.8235**	1.1636	0.9381**
	Initial weight	1	1.0608**	0.2580	1.5302**	2.4655*	0.2132*	2.0862**	0.1295	0.9242**	3.5582*	0.1446*
	Daily feed intake	1	9.8727**	6.2404**	8.2364**	6.6900**	3.9260**	2.9883**	0.4623	1.2065**	3.8612*	0.1169*
	Residual	28	0.1115	0.2091	0.1812	0.3553	0.04755	0.1020	0.1360	0.1101	0.5217	0.02324

* $P < 0.05$

** $P < 0.01$

Table 19 Analysis of Covariance for PER with initial weight as a covariable in Rat Experiments I, II and III.

Source	Mean Square		
	Rat Experiment I PER	Rat Experiment II PER	Rat Experiment III PER
Treatment	(5)* 0.4564**	(11)* 0.3021**	(9)* 0.2419**
Initial weight	(1)* 0.0450	(1)* 0.0807**	(1)* 0.0142
Residual	(29)* 0.0199	(35)* 0.0092	(29)* 0.0479

* Degree of freedom .

** P < 0.01 .

Table 20. Analysis of covariance for carcass characteristics of rats in Rat Experiments I, II and III.

Experiment	Source	DF	MEAN SQUARE: Total carcass protein	Protein retention	Protein retained / Protein intakt	Protein % in dry carcass	Protein % in fat free carcass	Total carcass fat	Fat % in dry carcass	Total ash	Ash % in dry carcass	Ash % in fat free carcass	Protein+fat+ash / Dry carcass wt.
	Treatments	5	26.670*	26.658*	130.78**	105.00**	1.6265	85.464**	144.61**	0.3846	2.1107**	0.3526	0.4640
I	Average daily gains	1	17.2980	17.3055	31.0380	81.935*	0.1379	168.53**	146.31	0.2765	5.039 **	0.5558	0.0448
	Residual	29	8.1787	8.1784	29.392	13.665	1.7171	12.974	15.382	0.1729	0.4064	0.1615	0.8599
	Treatments	11	7.1153*	7.1153*	173.80**	63.485**	2.8013	46.360**	88.043**	0.1493*	1.2674**	0.7986**	0.4577
II	Average daily gains	1	84.275**	84.2752**	0.6210	41.167	0.0000	135.41**	61.029*	1.2933**	3.3327**	1.0066**	0.1453
	Residual	35	3.1826	3.1826	7.1922	11.850	1.7418	10.894	12.680	0.0716	0.3530	0.0821	0.8086
	Treatments	9	9.1631**	9.2307**	111.21**	80.424**	2.3840	76.464**	116.67**	0.1926*	2.2518**	0.2757	0.5970
III	Average daily gains	1	62.279**	61.609**	0.0575	128.11**	0.0227	399.38**	289.25**	0.7570**	10.8075**	1.4870*	0.0813
	Residual	29	1.8261	1.8490	6.0226	11.418	2.4095	10.002	11.492	0.0849	0.3168	0.2045	1.1074

* $P < 0.05$

** $P < 0.01$