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ESTIMATION AND EXPRESSION OF AMINO ACID REQUIREMENTS OF GROWING PIGS

YUJI KAJI

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肥育豚におけるアミノ酸要求量の推定ならびに表示法

梶　雄次

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SUMMARY

To enhance the production efficiency of pork, a technology to properly supply protein in diets is necessary. At present, supply of protein is recognized as supply of amino acids which constitute protein. As excess amino acids are rapidly deaminized, it is important to supply amino acids in proper quantities and for this purpose, amino acid requirements must be defined. However, as is known, amino acid requirements expressed as a percentage of the diet are influenced by various factors including factors due to animals (body weight and productivity of lean meat), those due to feed (amino acid digestibility and energy content), and environmental factors (environmental temperature). It is desirable that amino acid requirements be expressed by a method which is free from the influence of these factors. Consideration of amino acid digestibility is important in precisely determining amino acid requirements and the requirements should be expressed in terms of digestible amino acids.

I. Lysine Requirements of Growing Pigs Estimated under Practical Feeding Conditions

In common cereal based diets for pigs, lysine is known to be almost always the first limiting amino acid and there are many reports on the lysine requirements of growing pigs. However, lysine requirements expressed as a percentage of the diet vary considerably depending upon the reports. Energy content of diets and the rate of body weight gain are considered to be the factors affecting lysine requirements. Moreover, it is well known that lysine requirements which are generally expressed as the total amount of lysine are influenced by its digestibility. Accordingly, requirements for lysine and other amino acids should be measured under feeding conditions as practical as possible and it is desirable that amino acid requirements be expressed in a method free from the influence of the above affecting factors. Thus, lysine requirement of growing pigs weighing 5 to 60 kg was estimated by using practical diets in which lysine was the first limiting amino acid under the conditions of *ad libitum* group feeding in four experiment periods (first period: 5 to 10 kg; second period: 10 to 20 kg; third period: 20 to 35 kg; and fourth period: 45 to 60 kg). Lysine requirements were estimated for daily body weight gain (DG), feed conversion ratio and plasma urea nitrogen by the method of least squares. The mean lysine requirements (% of diet) of the three criteria for the first, second, third and fourth periods was 1.43, 1.42, 0.86 and 0.73%, respectively. Lysine requirements (g per day) for the first, second, third and fourth periods were 6.5, 11.1, 15.3 and 19.6, respectively, and the estimated DG for each requirement were 0.338, 0.583, 0.834 and 0.912 kg, respectively. Lysine requirements expressed in terms of g/kg of body weight gain calculated on these values for period 1 to 4 were 19.4, 19.1, 18.4 and 21.5 g, respectively, and the average was 19.6. It was shown that when lysine requirements were expressed as a percentage of the diet, they greatly varied depending upon the body weight of growing pigs but that lysine requirements expressed as g/kg of body weight gain were almost constant at about 20.

II. Lysine Requirements of Finishing Pigs Estimated under Practical Feeding Conditions

Lysine requirements for finishing pigs weighing 58 to 92 kg were estimated under the practical feeding conditions of *ad libitum* group feeding. Nitrogen retention, daily body weight gain and feed conversion ratio were used as response criteria. Nitrogen retention was determined by the potassium indicator method using urinary nitrogen and potassium excretion rates. As the result of fitting a broken line model to the data of nitrogen retention, daily body weight gain and feed conversion ratio, the estimated values of lysine requirements were 0.75, 0.75 and 0.72%, respectively. The lysine requirement expressed as g/kg of body weight gain was estimated to be 19.5.

III. Lysine Requirements of Growing Pigs in Cold Conditions

When environmental temperature falls below lower critical temperature, it is necessary to increase the energy supply for the maintenance of body temperature. On the other hand, daily requirement for protein and amino acids undergoes almost no influence of environmental temperature. Therefore, if amino acid requirements are expressed as a percentage of the diet, the requirements under cold conditions are expected to be less than those in the thermoneutral conditions if weight gain is invariable. However, measurement of amino acid requirements is carried out generally in thermoneutral conditions and there are few reports on the amino acid requirements in cold conditions. Thus, in the present study, lysine requirements were estimated in cold conditions. At an average temperature during the test period of 2.2 ℃, lysine requirement of pigs weighing 35 to 50 kg was estimated to be 0.71% using weight gain as a criterion. This value was lower than the lysine requirements of 0.86% and 0.73% for pigs weighing 20 to 35 kg and 45 to 60 kg, respectively, in thermoneutral conditions. Under the conditions of this experiment, energy intake was considered to increase by about 15% for the maintenance of body temperature. Taking this into account, lysine requirement of 0.71% in cold conditions was calculated to be 0.82% in thermoneutral conditions. Moreover, the lysine requirement expressed as g/kg of body weight gain was 21.5, similar to that in thermoneutral conditions.

IV. Digestible Lysine Requirements of Growing Pigs

In common pig diets, lysine is generally the first limiting amino acid.

For this reason, there have been many studies directed to the lysine requirement. Requirement obtained as the result is generally expressed as total amount of lysine without taking into account of digestibility. Various feedstuffs have been used to supply lysine in such experiments: Some feedstuffs such as crystalline lysine and casein have almost 100% digestibility, but others are poorly digestible. Accordingly, lysine requirement estimated as total lysine may possibly differ according to the feedstuffs used. To make the feed formulation more precise and to make the use of protein more effective, it is necessary to obtain amino acid requirements as digestible amount. True digestibility of lysine in the two basal diets (weanling and growing diets) used for the experiment of Chapter I was determined using pigs fitted with a cannula at the end of their ileum. Based on the determined digestible lysine content of the experimental diets used in Chapter I, digestible lysine requirements were estimated to be 1.24% for the first period (5 to 10 kg), 1.38% for the second period (10 to 20 kg), 0.72% for the third period (20 to 35 kg) and 0.63% for the fourth period (45 to 60 kg) and the digestible lysine requirements as g/kg of body weight gain were estimated to be 17.8, 17.5, 15.6 and 18.2 for the respective periods, the average being 17.3. From the results, it was suggested that the digestible lysine requirements as g/kg of body weight gain were almost constant regardless of body weight or age and that the requirement for maintenance was negligibly small.

V. Plasma Urea Nitrogen as a Response Criterion for Determining the Amino Acid Requirements of Growing Pigs

Estimation of protein and amino acid requirements has been carried out according to the methods including feeding experiments, carcass analysis and nitrogen balance experiments. Recently, a method in which oxidation of particular amino acids is used as a criterion has been proposed. However, any of these methods requires long period of time or remarkable labor and facility. Fuller *et al.* has reported that in the experiments using growing pigs, amount of urea excreted shows very rapid response against the change of amino acid balance in diets. Accordingly, the plasma urea nitrogen concentration (PUN) is expected to sharply reflects the change in the quality of diets and may possibly be used effectively as a criterion to allow the estimation of amino acid requirements. First, the rapidity of the response of PUN to the change in dietary protein level was studied. As the result, it was found that PUN quickly changed according to protein level in supplied diets and reached a new equilibrium within two days after changing diets. Then, diets prepared by adding graded levels of L-lysine HCl to a basal diet were given to growing pigs weighing 22 kg and the blood was taken two days after feeding to measure PUN and plasma free lysine. The lysine requirement was estimated to be 0.8 to 0.9% based on the responses of PUN and plasma free lysine as criteria. This value corresponded to that obtained from the feeding experiment in Chapter I. From these results, PUN was shown to be an effective criterion for the short term estimation of amino acid requirements.

VI. Requirements of Other Essential Amino Acids Estimated by Plasma Urea Nitrogen

In this study, requirements for threonine, methionine (sulfur amino acid), tryptophan, isoleucine and valine which are regarded to be liable to be deficient next to lysine in practical pig diets were examined using the concentration of plasma urea and nitrogen (PUN) as an criteria and discussed the validity of the amino acid pattern in the ideal protein proposed by the ARC (1981). In Experiment 1, 18 pigs weighing 21 kg were used and the effect of addition of 0.4% of lysine, 0.1% of threonine and 0.1% methionine to a corn–soybean meal basal diet (CP 10.2%) on PUN was observed. All contents of lysine, threonine and sulfur amino acid (methionine + cystine) in the basal diet were 0.47% and that of tryptophan was 0.15% including that added to the feed. In the absence of a mixture of three amino acids , PUN (mg/100 ml) was 9.79, while PUN was reduced to 2.79 when the mixture lacking only methionine was added. The value was 10.28 in case only lysine was not added, 7.48 in the absence of threonine and 2.88 when all these amino acids were added. From these results, it was suggested that in the basic diet supplemented with tryptophan, lysine and threonine were the first and the second limiting amino acids, respectively. In Experiment 2, 16 growing pigs weighing 22 kg and the same basal diet as in Experiment 1 were used. The response of PUN was observed to the addition of 0.1% threonine, 0.05% tryptophan, 0.1% isoleucine and 0.1% valine to the basal diet with 0.4% lysine and 0.1% threonine. The response of PUN was not observed by the addition of either threonine, isoleucine or valine but PUN was significantly reduced by the addition of tryptophan. From the results, it was indicated that addition of lysine, threonine and tryptophan to the corn–soybean meal diet of CP 10.2% enhanced nutritive value of protein and that the amino acid pattern of the ideal protein proposed by the ARC was reasonable for growing pigs under practical feeding conditions.

VII. Effects of Amino Acid Supplementation of Corn–Soybean Meal Diet and Threonine Requirement of Growing Pigs

Effect of addition of lysine, threonine, methionine, isoleucine and valine to a corn–soybean meal diet estimated using the plasma urea nitrogen concentration as a criterion was ascertained by feeding experiments. At the same time, threonine requirement was studied using growth performance as a criterion. In Experiment 1, 24 growing pigs weighing 25 kg were used and the effect of the addition of 0.5% L–lysine HCl (0.4% as lysine), 0.1% L–threonine and 0.1% DL–methionine to the basal diet was examined. The amino acid composition of the basal diet was 0.44% lysine, 0.39% threonine, 0.49% sulfur amino acid (methionine + cystine), 0.15% tryptophan and 10.7% crude protein (CP). Daily body weight gain (DG) and feed conversion rate (FC) were 762 g and 2.5, respectively when a mixture of three amino acids was added and 738 g and 2.6, respectively when methionine was excluded from the mixture, thus almost no difference being observed. In case a mixture without lysine was added, DG and FC were 524 g and 3.2 ,respectively, resulting in inferior growth. When threonine was absent from the mixture, the values were 619 g and 2.8, which come in the middle. In either of Experiments 2 and 3, 20 growing pigs weighing about 24 kg were used and threonine requirements was estimated. To the basal diet supplemented with 0.5% of L-lysine hydrochloride, 0, 0.05, 0.1 and 0.15% L-threonine was added in Experiment 2 and 0, 0.1, 0.15 and 0.2% L-threonine was added in Experiment 3 and the effect of the addition on growth was observed. DG and FC changed linearly by the addition of L-threonine up to 0.1 to 0.15% but no effect of further addition was observed. Threonine requirements were estimated to be 0.51 and 0.49% for DG and FC, respectively. In Experiment 4, 16 growing pigs weighing about 29 kg were used and the effect of addition of 0.1% L-isoleucine and 0.1% L-valine to the basal diet supplemented with 0.5% L-lysine HCl and 0.15% L-threonine was investigated. No effect of addition was observed as the result. From the experimental results, it was suggested that lysine and threonine were the first and the second limiting amino acids, respectively, and that sulfur amino acids, isoleucine and valine were not deficient in the 10.7% CP corn-soybean meal diet supplemented with tryptophan.

VIII. Conclusion

The following conclusion was reached from the data obtained.

1. The lysine requirement expressed as a percentage of the diet greatly varied depending upon the body weight or age of growing pigs but the lysine requirement expressed as g/kg of body weight gain was almost fixed with an average of 19.6.

2. Lysine requirements for finishing pigs were estimated to be 0.75% and 19.5 g/kg of body weight gain.

3. The lysine requirement as a percentage of the diet measured under cold conditions was lower than that measured under thermoneutral conditions but the lysine requirement as g/kg of body weight gain was almost equal to that under thermoneutral conditions.

4. The digestible lysine requirements expressed as g/kg of body weight gain were shown to be almost fixed regardless of body weight or age. The average was 17.3 g/kg of body weight gain. Also, the digestible lysine requirement for maintenance was suggested to be negligibly small.

5. Plasma urea nitrogen concentrations were revealed to be an effective criterion to estimate amino acid requirements in a short period of time.

6. From the change of plasma urea nitrogen concentration, it was estimated that when the corn-soybean meal diet of CP 10.2% was given to pigs weighing about 20 kg, lysine, threonine and tryptophan are deficient but sulfur amino acid, isoleucine and valine satisfy their requirements and that the amino acid pattern in ideal protein proposed by the ARC was reasonable in the case of pigs under practical feeding conditions.

7. The foregoing results were confirmed using growth performance as a criterion. The threonine requirement of growing pigs weighing 25 to 40 kg was estimated to be 0.50% and its ratio to that of lysine was 60%, in good agreement with the ratio proposed by the ARC.

GENERAL INTRODUCTION

There are two significant aspects of supplying protein to livestock efficiently. One is the economical aspect as feedstuffs that are used as the sources of protein supply are generally expensive. The other relates to the reduction of nitrogen excretion to the environment. Undigested protein is excreted in feces while excess protein not utilized within the body after digestion and absorption is excreted in urine as urea. Nowadays, supply of protein to pigs and other monogastric animals is recognized as supply of amino acids which constitute protein. For pigs, ten amino acids are essential dietary components. Some amino acids are liable to be and some are free from being deficient and others in-between. The difference in the extent of liability to deficiency is caused by the difference between the amino acid composition required by pigs and that of the protein in the diet given to pigs. In most pig diets, lysine is the most liable to be deficient, that is, to be the first limiting amino acid. For most typical diet in Japan mainly comprising corn and soybean meal, next to lysine, threonine, tryptophan, sulfur amino acids (methionine + cystine), valine and isoleucine are liable to be deficient and histidine, phenylalanine + tyrosine, leucine and arginine are hard to be deficient. To economically supply protein, presently, diets are formulated : Crude protein (CP) level in diet is reduced to the level at which only lysine becomes deficient and crystalline lysine is supplemented to the diet. However, in this case, amino acids other than lysine are supplied in excess so that there is still room to reduce nitrogen excretion from pigs. According to the estimation of Nishio, the nitrogen excretion of all the livestock in Japan is 579 thousand tons per year and the nitrogen load when this amount is uniformly spread to all the agricultural land is 109 kg/ha. Although this value is almost equal to the upper limit of optimum application as an organic substance to ordinary crops of 100 kg/ha, since the number of livestock varies depending upon regions, it sometimes corresponds to 3 to 4 times the optimum level in some regions (Furuya, 1992). Judging from the circumstances, it is likely that the necessity to further reduce nitrogen excretion from pigs will be more emphasized in the future. In this case, the method presumably taken is to reduce dietary CP level and supplement two or more amino acids which become deficient as the result, thereby to reduce the amount of excreted nitrogen without damaging the productivity.

To supply protein efficiently, amino acid requirements should be defined. According to the Agricultural Research Council (ARC, 1981), requirement is defined as follows: "The requirement for any given nutrient is the amount of that nutrient which must be supplied in the diet to meet the requirement (Net). The requirement (Net) is the quantity of a nutrient that should be absorbed by a normal healthy animal given a completely adequate diet in an environment compatible with good health in order to meet its needs for maintenance, including the obligatory losses, and for a stated rate of production or for reproduction." Growing pigs are generally fed *ad libitum* and "a stated rate of production" generally means "maximum growth". Thus, unless specifically noted, the term "requirement" is used for growing pigs to mean the amount of nutrient which must be supplied to the diet to meet a maximum growth. There are several methods to express amino acid requirements as shown below and each method has its own characteristics (Lewis, 1992).

a. Percentage of the Diet

In North America, the most common method of expressing the amino acid requirements of animals is to list them as a percentage of the diet, and this is the primary method used in most of the NRC tables. The method is satisfactory when animals are allowed to eat *ad libitum*, and there are only small differences in the energy density of common diets. Values need to be expressed on a dry matter basis if there is much variation in the dry matter content. This is relatively unimportant in most nonruminant diets, because the dry matter percentage of most diets is approximately 90%. The primary advantage of expressing amino acid requirements as a percentage of the diet is that diets can be formulated directly without the inclusion of any other factors such as the expected feed intake of the animals. The method is generally satisfactory for growing young animals, but less satisfactory for older animals. Adjustments may be necessary when intakes vary, either because of deliberate restriction or as a result of environmental factors.

b. Gram per Day

Expressing amino acid requirements as grams per day is particularly appropriate when feed intake is restricted in some manner, either deliberately or because of other factors such as physical bulk of the diet or high environmental temperature. Thus, the method is commonly used for adult animals such as gestating gilts and laying hens. An adjustment should be made for differences in body weight if these are substantial. This is the usual method of expressing amino acid requirements of adult humans.

c. Grams per Unit of Energy

Grams per unit of energy may be the best method of expressing amino acid requirements when animals are given *ad libitum* access to diet, and when they tend to eat to a constant energy intake regardless of the energy density of the diet. A common example of this method is a term such as grams of lysine per megacalorie of digestible energy. The method is especially appropriate when the energy density of the diet may vary widely, and is the main factor that influences feed intake. The method is used in poultry nutrition, but has not been widely adopted in other species. One problem limiting widespread application is the limited information on the utilizable energy content (digestible energy, metabolizable energy, or net energy) of many diets.

d. Relative to Another Amino Acid

Amino acid requirements may be expressed relative to another amino acid (usually the first limiting). This method, in essence, constructs the optimum pattern of amino acids one to another (also known as "ideal protein"). The method is valuable in making comparisons of the results of amino acid experiments that use protein sources of different qualities, and for extrapolating amino acid requirements from one phase of the life cycle to another, but has little application in practical diet formulation. The method was described in detail by the Agricultural Research Council (1981).

Amino acid requirements of growing pigs have been expressed mainly as percentages of diet. They can be obtained according to the following equation:

Amino acid requirement (%)
= Amino acid requirement (g/d) / Feed intake (g/d) x 100
= Amino acid requirement (g/d) x Energy content in feed (kcal/g)
/ Energy requirement (kcal/d) x 100

When dietary energy content is increased, amino acid requirement (%) increases since energy requirement and amino acid requirement (g/d) are not influenced by the change of dietary energy content. Where the environmental temperature is below the lower critical temperature, energy requirement increases to maintain the body temperature (NRC, 1981), however, the amino acid requirement (%) decreases because the amino acid requirement (g/d) does not undergo an influence (Filmer and

Curran, 1977). Further, it is known that the amino acid requirements (%) of growing pigs decrease with increase of body weight and high lean genotypes have high amino acid requirements (%). According to the method of "Grams per Unit of Energy", influence of dietary energy content can be excluded but that of cold conditions can not be. According to the method of "Gram pre Day", influence of dietary energy content and cold conditions can be excluded but it is not possible to quantitatively express the increase of requirement with increase of body weight or such relationship that pigs having high growth rates have higher amino acid requirements than those having lower ability. Traditionally, amino acid requirements have been expressed in terms of the total amount of amino acids that must be provided in diet. However, it has been recognized that not all of most amino acids present in diets are digested, absorbed and utilized. Thus, digestibility of amino acids influences "total" amino acid requirements. As described above, amino acid requirements are influenced by various factors. Accordingly, amino acid requirements should be measured under feeding conditions as practical as possible. In the present studies, experimental diets were formulated using practical feedstuffs and amino acid requirements were measured under the conditions of *ad libitum* group feeding. First, the requirement of lysine ,which is likely to be the first limiting amino acid, was estimated according to different body weight periods of growing pigs. It is desirable that the expression of amino acid requirements be hard to be the subject of the above factors. Thus, we are proposing a new expression method of amino acid requirements based on the resulting lysine requirement (Chapters I, II and III).

Generally, amino acid requirements are expressed as the requirement for total amount of amino acids determined by chemical analyses of dietary protein. However, dietary protein ingested is digested to amino acids, absorbed and utilized for the synthesis of body protein. Digestibility of amino acids varies widely depending on feedstuffs from crystalline amino acids and casein, highly digestible, to meat and bone meal and feather meal, poorly digestible. Therefore, it is more reasonable to express amino acid requirements as requirements for digestible amino acids rather than for total amino acids. Thus, digestibility of amino acids in the experimental diets of Chapter I was measured and the digestible lysine requirements of growing pigs were calculated (Chapter IV).

The response criteria for the estimation of amino acid requirements are divided into two broad classes: production traits and metabolic responses (Lewis, 1992). Production traits are those that are economically important, such as weight gain, feed efficiency, and body composition in growing animals. For farm animals, these are the ultimate criteria, and therefore it is important that other response criteria be validated relative to these traits. The advantage of the production traits is that they are usually easy to measure and do not require any expensive equipment. However, since production traits require longer period to obtain the results as compared to metabolic responses, large amount of feed is required for the experiments to measure them. The experiments become expensive when several amino acids are added to experimental diets. Moreover, there are relatively few reports on the requirements for other amino acids except lysine. For these reasons, in determining the requirements for such amino acids that are liable to be deficient next to lysine, it was decided to estimate the requirements by metabolic responses and then confirm the results by production traits rather than to employ production traits as criteria from the beginning. Metabolic responses include nitrogen balance, urinary urea excretion, plasma urea concentrations, plasma amino acid concentrations and amino acid oxidation. Since plasma urea nitrogen concentrations (PUN) can be measured easily and with pigs fed under practical feeding conditions, it was selected as a response criterion. Two experiments were carried out to find out the rapidity of the response of PUN to the change in dietary protein level and to estimate the lysine requirement of growing pigs by PUN as a criterion (Chapter V).

It requires a great deal of expenditure and labor to measure the amino acid requirements of growing pigs with respect to all the amino acids throughout the growth stages. ARC (1981) published an ideal protein that has an ideal amino acid pattern expressed as ratios of amino acids to lysine. Chung and Baker (1992) indicates the reason why the method of expressing an ideal pattern of amino acids as ratios to lysine is rational as follows: 1) protein synthesis represents the only need for dietary lysine, although endogenous lysine (trimethyl lysine) is used for carnitine biosynthesis; 2) lysine is almost always the most limiting AA in commercial pig diets; 3) the lysine requirement of pigs has been most extensively studied and firmly established; 4) lysine analysis in feeds is relatively accurate and easy, particularly compared with sulfur amino acids and tryptophan analyses; and 5) it is unlikely that any indispensable amino acid for growing pigs is needed in greater concentration than lysine. It is assumed that if lysine requirement increases (or decreases) due to environment, diets or the state of animals, the ratios of requirements for other amino acids to lysine requirement remain constant. If the ideal amino acid pattern is defined, requirements for other amino acids can be calculated. The ARC (1981) estimated the ideal amino acid pattern by examining data from a wide range of types of experiments with pigs in the weight range 15 to 90 kg. However, it is not clear whether or not this amino acid pattern is applicable under the practical feeding conditions of *ad libitum* and group feeding because the referenced data were derived from the experiments in which pigs were housed in metabolism cage and subjected to restricted feeding. Two experiments were conducted to find out if the ideal amino acid pattern of the ARC (1981) was reasonable under practical feeding conditions with respect to five amino acids which are liable to be deficient next to lysine (Chapters VI and VII).

EXPERIMENTAL STUDIES

I. Lysine Requirements of Growing Pigs Estimated under Practical Feeding Conditions

Abstract

The lysine requiremrents of pigs weighing 5 to 10 kg (Period 1), 10 to 20 kg (Period 2), 20 to 35 kg (Period 3) and 45 to 60 kg (Period 4) were estimated from responses to crude protein (CP) supplementation of CP-deficient diets on the basis of the assumption that in experiments where CP level is raised progressively, the response to protein which is measured, is really a response to the first limiting amino acid. Casein and soybean meal were added to a commercial weanling diet and to a corn-alfalfa meal diet for Periods 1 and 2 and for Periods 3 and 4, respectively, to raise the CP level of diets. Five and three to four pigs for Period 1 and Period 2, respectively, were group-fed one of the five diets containing 14 to 26 % CP (0.96 to 1.93 % lysine), and three pigs for both Periods 3 and 4 were group-fed one of the five diets containing 11 to 23 % CP (0.60 to 1.32 % lysine), and 8 to 20 % CP (0.42 to 1.14 % lysine), respectively. For all the experimental diets, lysine was the first limiting amino acid when the amino acid balance in each diet was examined on the basis of the proportions proposed as the ideal protein. Feed and water were available *ad libitum*. From the daily gain, the feed conversion ratio and the plasma urea nitrogen responses, the estimated lysine requirements were 1.36, 1.34 and 1.58 %, respectively, for Period 1, 1.49, 1.39 and 1.37 %, respectively, for Period 2, 0.84, 0.88 and

0.86 %, respectively, for Period 3 and 0.75, 0.78 and 0.67 %, respectively, for Period 4. The mean lysine requirement for Periods 1, 2, 3 and 4 was 1.43, 1.42, 0.86 and 0.73 % of the diet, respectively, and the lysine requirement presented in terms of g/kg body weight gain was 19.4, 19.1, 18.4 and 21.5, respectively. These results indicate that although lysine requirements expressed as a percentage of the diet differ markedly between live weight periods, lysine requirements expressed as g/kg body-weight gain remain relatively constant regardless of their weight or age, the mean value being approximately 20 g/kg body weight gain. On the basis of this value, the lysine requirements (% of diet) of pigs kept under various feeding conditions can be calculated according to their expected daily gain and feed intake. It is possible that lysine requirements estimated in this way may be sufficiently precise for most practical circumstances.

Introduction

The primary purpose of protein supply to monogastric animals is the supply of essential amino acids. Many researchers have, therefore, studied requirements for lysine which is known to be almost always the first limiting amino acid in practical pig diets, but lysine requirements expressed as a percentage of the diet largely differ between studies. Lysine requirements of starting pigs weighting 5 to 10 kg, for instance, are 0.95% recommended by the National Research Council (NRC, 1979) but 1.41% by the Agricultural Research Council (ARC, 1981), showing a large difference between the two (Lewis, 1984). The Japanese Feeding Standard (JFS, 1975) recommends 1.27%, which is the approximate median of the two, whereas the Institut National de la Recherche Agronomique (INRA, 1984) recommends 1.40%, an estimate very similar to the ARC's requirement. Such differences in lysine requirements between feeding standards are partly explained by the difference in the energy contents of experimental diets and the difference in the rate of body weight gain. Also pointed as a factor to cause discrepancy in lysine requirements is the dietary protein level (Baker *et al.*, 1975). Furthermore, lysine requirements, which are usually expressed as the total amount of lysine determined by chemical procedures, are of course affected by the digestibility of lysine. Requirements of lysine and other amino acids, therefore, should be measured under feeding conditions as practical as possible, and amino acid requirements should desirably be expressed by a method free from the influences of the above affecting factors.

This study was designed to estimate lysine requirements of growing pigs weighing 5 to 60 kg under practical feeding conditions, *i.e. ad libitum* group feeding with practical diets, and to propose a new method of expressing lysine requirements on the basis of the result.

Materials and Methods

Four experiments were conducted to estimate the lysine requirements of pigs weighing 5 to 10 kg (Period 1), 10 to 20 kg (Period 2), 20 to 35 kg (Period 3) and 45 to 60 kg (Period 4). The pigs were fed on of five diets with graded levels of lysine in each experiment, and lysine requirements in each body weight period were estimated by using daily body weight gain (DG), feed conversion ratio (FC) and plasma urea nitrogen (PUN) as response criteria.

Experimental Diets

Table 1-1 shows the formulation and chemical compositions of the basal diets used in the four experiments. For Periods 1 and 2, experimental diets containing 14 to 26% crude protein (CP) in 3% increments were formulated by mixing casein and cornstarch to the basal diet based on a commercial weanling diet with vitamins and minerals (Table 1-2). For Periods 3 and 4, soybean meal and cornstarch were mixed with corn basal diet for experimental diet to provide a range of CP levels from 11 to 23% and 8 to 20%, respectively, in 3% increments (Table 1-2).

Animals and General Procedures

Seventy two crossbred growing pigs were allocated to five blocks to contain five pigs for Period 1, three or four pigs for Period 2 and three pigs each for Periods 3 and 4, considering sex and litter. The pigs were housed in groups in a concrete-floored pens (room temperature; 23 ± 1℃) and allowed *ad libitum* access to feed and water.

After weaning at 4 weeks of age, the pigs used in Period 1 were given

Table 1-1. Formulation of the basal diets for the estimation of lysine requirements

	Basal diet	
	Weanling*	Growing**
Ingredient, %		
Corn	54.91	83.20
Wheat	4.90	—
Alfalfa meal	—	8.08
Wheat flour	3.92	—
Soybean meal	13.93	—
Roasted soybean flour	1.96	—
Fish meal	3.92	4.85
Skim milk	4.90	—
Glucose	4.90	—
Fancy tallow	1.37	—
Calcium carbonate	0.44	—
Dicalcium phosphate	1.23	—
Tricalcium phosphate	1.29	2.42
Sodium chloride	0.47	0.81
L-lysine HCl	0.27	—
DL-methionine	0.15	—
Vitamin and mineral mixture***	1.44	—
Vitamin and mineral mixture****	—	0.64

* For periods 1 and 2.

** For periods 3 and 4.

*** Supplied (per kg of diet) : Vitamin A, 18000 IU; Vitamin D_3, 2800 IU; Vitamin E, 32 IU; thiamin, 3.5 mg; riboflavin, 20 mg; pyridoxine HCl, 3 mg; pantothenic acid, 40 mg; nicotinic acid, 48 mg; choline, 500 mg; Vitamin B_{12}, 0.04 mg; folic acid, 0.54 mg; biotin, 0.1 mg; ascorbic acid, 100 mg; Vitamin K_3, 2.2 mg; Mn, 90 mg; Zn, 180 mg; Cu, 130 mg; Fe, 250 mg; I, 1.2 mg; Co, 0.2 mg.

**** Supplied (per kg of diet) : Vitamin A, 16000 IU; Vitamin D_3, 3200 IU; Vitamin E, 16 IU; thiamin, 3.2 mg; riboflavin, 22.4 mg; pyridoxine HCl, 1.6 mg; pantothenic acid, 35 mg; nicotinic acid, 19 mg; choline, 184 mg; Mn, 80 mg; Zn, 96 mg; Cu, 16 mg; Fe, 80 mg; I, 1.6 mg.

a commercial weanling diet for 1 to 2 weeks until they reached 5 kg of body weight on an average. Body weight and feed consumption were measured between 14:00 and 15:00 twice a week with an interval of three to four days in every experiment, and blood samples were collected at the same time. Blood sample collection was started 1 week after the initiation of each experiment. About 1 ml of blood from anterior vena cave was collected into a heparinized tube, and the blood was centrifuged to obtain plasma at 3,000 rpm for 10 min. The plasma was stored in a freezer until measurement of PUN. Gross energy digestibility was determined by the chromic oxide indicator method (Morimoto, 1971) in the final week of each experiment. The test periods, which were defined by body weight ranges, was 21, 14, 18 and 17 days for Periods 1, 2, 3 and 4, respectively.

Essential Amino Acid Compositions of the Experimental Diets

Amino acid compositions of the experimental diets for each period are shown in Table 1–2. The traditional method for estimation of amino acid requirements is that to observe the response of animals to the addition of graded amounts of crystalline amino acids increased stepwise to the basal diet lacking in only the test amino acid. In the present study, however, which aimed to conduct experiments under as practical conditions as possible, graded levels of dietary lysine were made by the addition of casein or soybean meal instead of crystalline lysine. The precondition in this method is that lysine is the first limiting amino acid in any of the experimental diets. The ratio of each essential

Table 1-2. **Formulation and chemical and essential amino acid compositions of the experimental diets**

	Periods 1 and 2					Periods 3 and 4					
Crude protein level, %	14	17	20	23	26	8	11	14	17	20	23
Ingredient, %											
Basal diet*	77.30	77.30	77.30	77.30	77.30	61.90	61.90	61.90	61.90	61.90	61.90
Casein	—	3.53	7.06	10.59	14.12	—	—	—	—	—	—
Soybean meal	—	—	—	—	—	1.30	8.00	14.80	21.60	28.40	35.20
Corn starch	22.60	19.07	15.54	12.01	8.48	36.70	30.00	23.20	16.40	9.60	2.80
Chromic oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Crude protein, %	13.78	16.72	19.66	22.60	25.54	8.51	11.28	14.23	16.73	20.50	22.91
Gross energy, kcal/g	3.79	3.82	3.92	3.96	4.01	3.74	3.80	3.82	3.85	3.87	3.90
Digestible energy, kcal/g**	3.24	3.26	3.39	3.41	3.41	3.06	3.60	3.06	2.99	2.89	3.00
Essential amino acids***, %											
Arginine	0.76	0.88	1.01	1.13	1.26	0.39	0.62	0.85	1.09	1.32	1.55
Histidine	0.33	0.43	0.52	0.62	0.71	0.19	0.27	0.35	0.42	0.50	0.58
Isoleucine	0.55	0.71	0.88	1.04	1.21	0.29	0.44	0.59	0.74	0.89	1.04
	(104)	(108)	(111)	(112)	(114)	(126)	(133)	(138)	(140)	(142)	(143)
Leucine	1.14	1.44	1.73	2.03	2.32	0.78	1.03	1.27	1.52	1.77	2.01
	(119)	(120)	(120)	(120)	(120)	(186)	(172)	(163)	(158)	(155)	(152)
Lysine	0.96	1.20	1.44	1.69	1.93	0.42	0.60	0.78	0.96	1.14	1.32
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Methionine + cystine	0.58	0.69	0.80	0.90	1.01	0.30	0.38	0.47	0.57	0.65	0.74
	(121)	(115)	(111)	(107)	(105)	(143)	(127)	(121)	(119)	(114)	(112)
Phenylalanine + tyrosine	1.09	1.44	1.80	2.15	2.51	0.65	0.92	1.20	1.48	1.77	2.05
	(118)	(125)	(130)	(133)	(135)	(160)	(160)	(160)	(161)	(162)	(162)
Threonine	0.60	0.74	0.88	1.02	1.16	0.30	0.43	0.56	0.69	0.82	0.95
	(104)	(103)	(102)	(101)	(100)	(119)	(119)	(120)	(120)	(120)	(120)
Tryptophan	0.15	0.19	0.22	0.26	0.29	0.06	0.10	0.13	0.17	0.20	0.24
	(106)	(104)	(103)	(102)	(101)	(100)	(107)	(112)	(115)	(117)	(119)
Valine	0.68	0.88	1.09	1.30	1.51	0.38	0.53	0.68	0.83	0.99	1.14
	(101)	(105)	(108)	(110)	(112)	(129)	(126)	(125)	(124)	(124)	(123)

* See Table 1-1.

** The values are means of determined values in periods 1 and 2 and periods 3 and 4.

*** The ratios of each essential amino acid to lysine calculated on the basis of amino acid balance in ideal protein are shown in parentheses.

amino acid to lysine (sufficiency ratio) calculated on the basis of amino acid balance in the ideal protein proposed by the ARC (1981) is also shown in Table 1-2. A balance with higher lysine and lower sulfur amino acid is recommended for the ideal protein as compared to the Japanese Feeding Standard (1975) or NRC (1979).

The sufficiency ratio for any other essential amino acid than lysine in the experimental diets given in Periods 1 and 2 was 100% or higher as shown in Table 1-2. It was therefore confirmed that lysine was the first limiting amino acid in the diets. In the diet containing 14% CP and that containing more than 17% CP, sufficiency ratios of valine and threonine were comparatively low, respectively, showing that these amino acids were the second limiting amino acids, in the respective diets. In the diets given in Periods 3 and 4, too, any other essential amino acid than lysine showed higher than 100% sufficiency ratio, and the second limiting amino acids in these diets were tryptophan in the diet containing 17% or lower CP and sulfur amino acid in the diets containing 20% and 23% CP. Lysine was thus the first limiting amino acid in any of the diets used in this study. Consequently, the measurements of DG, FC and PUN can be interpreted as the response to dietary lysine level.

Analytical and Statistical Procedures

CP content in diet was assayed by the routine method (Morimoto, 1971). Gross energy contents of feed and feces were measured in an automatic bomb calorimeter (CA-3, Shimadzu Seisakusho Ltd.) and chromic oxide was determined by the method of Yoshida *et al.* (1967). For amino acid quantification, the method previously described (Furuya *et al.*, 1986) was employed. PUN were measured by the urease–indophenol method with an analyzer for laboratory test (RaBA 3010, Chugai Pharmaceutical Co., Ltd.). Lysine requirements were estimated as inflection points given by fitting a broken line model (Ohtsuka and Yoshihara, 1975) to each set of data (DG, FC and PUN) in each experiment.

Results and Discussion

Growth performance and PUN are shown in Tables 1–3 and 1–4 with dietary CP and lysine levels. Feed consumption was nearly constant for any period, not affected by CP level. DG increased linearly with dietary CP level up to a certain level of CP, after that DG did not increase. At the highest level of CP, DG tended to decrease except for that in Period 4. FC was improved as CP level increased, but no further improvement was achieved after CP reached a certain level. PUN remained low at low CP levels, but increased rapidly after CP exceeded a certain level.

Since lysine was the first limiting amino acid in all the diets given in this study, the response of pigs to dietary CP level, therefore, can be regarded as the response to lysine. Lysine requirements were thus estimated by fitting the broken line model (Ohtsuka and Yoshihara, 1975) to the data of DG, FC and PUN on dietary lysine level given in

Table 1–3. Effects of crude protein and lysine levels on growth performance a nd plasma urea nitrogen (PUN) during periods 1 and 2

	Diet				
Crude protein, %	14	17	20	23	26
Lysine, %	0.96	1.20	1.44	1.69	1.93
Lysine, mg/kcal DE*	2.96	3.68	4.25	4.96	5.66
Period 1 (5–10 kg body weight)					
Consumption					
Feed, g/d	492	484	501	465	409
Lysine, g/d	4.72	5.81	7.21	7.86	7.89
Daily gain, g	239	298	354	357	303
Feed conversion ratio	2.06	1.62	1.42	1.30	1.35
PUN, mg/dl	10.0	9.8	10.3	10.7	12.2
Period 2 (10–20 kg body weight)					
Consumption					
Feed, g/d	756	733	750	768	744
Lysine, g/d	7.26	8.80	10.80	12.98	14.36
Daily gain, g	433	488	572	602	564
Feed conversion ratio	1.75	1.50	1.31	1.28	1.32
PUN, mg/dl	7.1	5.6	7.0	12.0	14.2

* Digestible energy

Table 1–4. **Effects of crude protein and lysine levels on growth performance and plasma urea nitrogen (PUN) during periods 3 and 4**

	Diet					
Crude protein, %	8	11	14	17	20	23
Lysine, %	0.42	0.60	0.78	0.98	1.14	1.32
Lysine, mg/kcal DE*	1.37	1.96	2.55	3.21	3.94	4.40
Period 3 (20–35 kg body weight)						
Consumption						
Feed, g/d	—	1650	1781	1795	1748	1657
Lysine, g/d	—	9.90	13.89	17.23	19.93	21.87
Daily gain, g	—	628	780	852	895	756
Feed conversion ratio	—	2.63	2.28	2.11	1.95	2.19
PUN, mg/dl	—	9.7	11.2	11.8	20.3	21.2
Period 4 (45–60 kg body weight)						
Consumption						
Feed, g/d	2589	2821	2397	2565	2690	—
Lysine, g/d	10.87	16.93	18.70	24.62	30.67	—
Daily gain, g	716	823	912	912	912	—
Feed conversion ratio	3.62	3.43	2.63	2.81	2.95	—
PUN, mg/dl	9.6	9.0	12.0	16.0	20.5	—

* Digestible energy

Tables 1-3 and 1-4 as previously reported (Furuya *et al.*, 1985), and the results are shown in Table 1-5. The lysine requirements estimated for DG was similar to those for FC in any period. The lysine requirements estimated for PUN tended to be a little bit lower than those estimated for DG and FC in any period except Period 1. The mean lysine requirements of these three estimates were 1.43%, 1.42%, 0.86% and 0.73% for Period 1 (5 to 10 kg), Period 2 (10 to 20 kg), Period 3 (20 to 35 kg) and Period 4 (45 to 60 kg), respectively. These values are higher than the requirements recommended by the JFS (1975) and the NRC (1979), and significantly high in particular when compared with the lysine requirements recommended by the NRC for pigs weighing 5 to 10 kg (0.95%) and 10 to 20 kg body weight (0.79%). The requirements estimated in this study were similar to the recommendations by the ARC (1981) and the INRA (1984). Fig. 1-1 indicates representative lysine requirements estimated in the last decade using DG as a criterion (Brown *et al.*, 1973; Wahlstrom and Libal, 1974; Lunchick *et al.*, 1978; Campbell, 1978; Easter and Baker, 1980; Lewis *et al.*, 1980; Aherne and Nielsen, 1983; Williams *et al.*, 1984; Rosell and Zimmerman, 1984; Asche *et al.*, 1985; Parsons *et al.*, 1985). In comparison with the data from the literature, the lysine requirements in this study also proved to be high especially for starting pigs. Such high lysine requirements estimated in this study is probably due to the high rate of weight gain. Amino acid requirements have been estimated under rather different conditions from practical ones such as supply of specifically formulated diet, restricted feeding, and housing in metabolic cages, resulting in

Table 1–5. Lysine requirements estimated for daily gain, feed conversion ratio and plasma urea nitrogen (PUN) responses

	Period (body weight, kg)			
	5–10	10–20	20–35	45–60
Requirement, % of diet				
Daily gain	1.36	1.49	0.84	0.75
Feed conversion ratio	1.34	1.39	0.88	0.78
PUN	1.58	1.37	0.86	0.67
Mean	1.43	1.42	0.86	0.73
Requirement, mg/kcal DE*				
Daily gain	4.16	4.43	2.76	2.45
Feed conversion ratio	4.38	4.27	2.88	2.55
PUN	4.64	4.03	2.97	2.11
Mean	4.39	4.24	2.87	2.37
Requirement, g/d				
Daily gain	6.5	11.1	15.3	19.6
Estimated daily gain, kg	0.338	0.583	0.834	0.912
Requirement, g/kg body weight gain**	19.4	19.1	18.4	21.5

* Digestible energy.

** These values were obtained by dividing the estimated lysine requirement (g/d) by the estimated daily gain (kg/d).

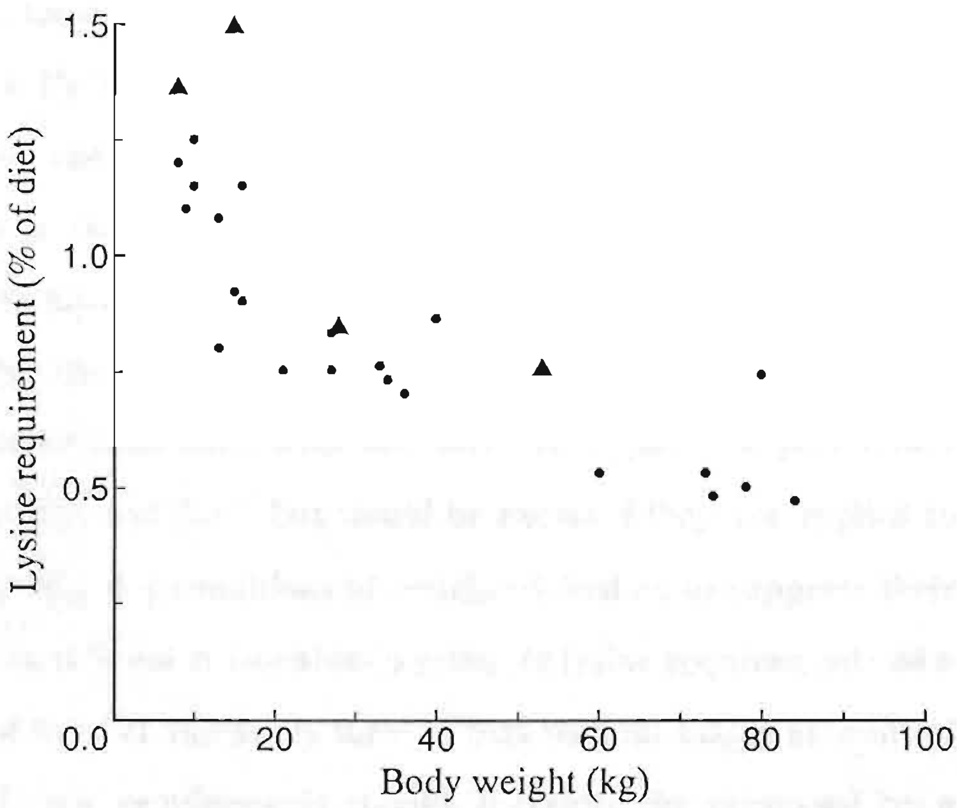

Fig. 1–1. Relationship between body weight and requirement of lysine expressed as a percentage of diet on data from literature ● (see text) and from the present study ▲ .

comparatively low DG. In the present study, on the contrary, which was conducted with diets for practical use under *ad libitum* group feeding conditions, DG was greater than the level shown in the JFS's table (1975). Lysine absorbed in the body is used for the production of lean meat in its most part, if lysine supply is close to its requirement, and used for maintenance in a very small part. Energy, on the other hand, is consumed in its substantial part for maintenance. Therefore, the proportion of energy required for maintenance to the total energy requirement is reduced corresponding to the increase in lean meat production, requiring more lysine expressed as a percentage of the diet. Consequently, lysine supply would be deficient if its requirements estimated under conditions with low DG are applied to pigs under practical feeding conditions, but would be excess if they are applied to developing gilts under conditions of restricted feeding to suppress their growth. Thus, it is not reasonable to estimate lysine requirements as a percentage of the diet and apply them to pigs without taking account of their DG. Lysine requirements should, therefore, be expressed by a method that excludes the effect of DG, though having been expressed as a percentage of the diet. With this view, daily lysine requirements (g) and expected DG were estimated by fitting the broken line model (Ohtsuka and Yoshihara, 1975) to the data of DG on daily lysine consumption in each experiment, and the amount of lysine required for body weight gain of 1 kg was calculated from the estimates (Table 1-5). In this way, the lysine requirements expressed as grams per kg of body weight gain (g/kg BWG) were obtained and 18.4 to 21.5 from Period 1 through Period 4 with the mean of four periods of 19.6, indicating that the lysine requirements (g/kg BWG) almost remain constant regardless of the body weight. Fig.1-2 shows the lysine requirements (g/kg BWG) estimated from the same data as those in Fig.1-1. They also are almost constant at about 20 g/kg BWG, showing good agreement with the result of the present study. This suggests that the lysine requirement of growing pigs can be regarded as about 20 g/kg BWG regardless of body weight.

Baker *et al.* (1975) found that the lysine requirement of growing pigs increases 0.02% of the diet for each 1% increase in the level of dietary CP on the basis of their study that lysine requirements are 0.69% and 0.77% when CP levels are 12% and 16%, respectively. This report is a basis of the common knowledge that amino acid requirements increase with the level of dietary CP. The data by Bakers *et al.*, however, also demonstrated that pigs fed 12% CP diet gain slower and eat more than pigs fed 16% CP diet, suggesting that lysine requirements (g/kg BWG) are rather higher for pigs fed the low protein diet. This can be reasonably interpreted as that other limiting factors than lysine in the 12% CP diet suppressed body weight gain, resulting in less lysine requirements expressed as a percentage of the diet. This can be regarded as a case to show that the influence of DG affected lysine requirements as percentage of the diet. Influence of dietary CP level on lysine requirements is denied recently by Asche *et al.* (1985).

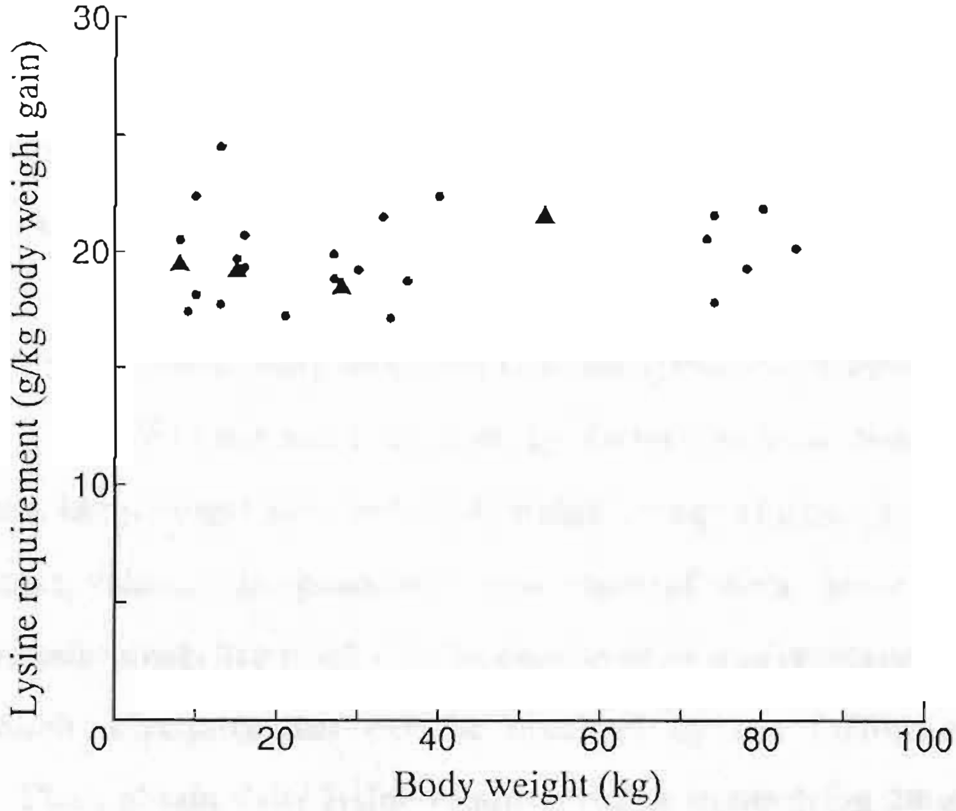

Fig. 1–2. Relationship between body weight and requirement of lysine expressed as g/kg body weight gain on data from literature ● (see text) and from the present study ▲.

Lysine requirements of the ARC are expressed as grams per unit of digestible energy (DE). The lysine requirements (mg/kcal DE) estimated in this study were shown also in Table 1-5. The lysine requirements of the ARC are 3.51 and 2.51 mg/kcal DE for pigs weighing 15 to 50 kg and 50 to 90 kg, respectively. These requirements are similar to the results of the present study, though direct comparison is difficult because of the different weight ranges. The influence of dietary energy content can be excluded in expressing lysine requirements as grams per unit of DE, but the influence of DG cannot.

The results of the present study indicated that the lysine requirements expressed as g/kg BWG are not influenced by factors such as dietary energy content, body weight gain and body weight or age of pigs, giving almost constant values. In practical formulation of diets, however, amino acid requirements are needed to be expressed as a percentage of the diet. Such a requirement can be obtained by the following procedures: First, obtain daily lysine requirement by multiplying 20 g lysine/kg BWG by expected or predicted DG (kg). Second, calculate daily energy requirement (kcal DE) for the expected DG by an appropriate method, for instance, from a feeding standard. Third, calculate daily feed supply or feed consumption (g) by dividing the daily energy requirement by dietary energy content (kcal DE/g) , which can be decided depending on feedstuff supply conditions. Last, lysine requirement as a precentage of the diet can be calculated from daily lysine requirement and daily feed supply or feed consumption. This method would exclude the influences of dietary energy content and DG among other factors affecting amino acid requirements, so that individual farm could determine their own lysine requirements most appropriate to their economical situations, types of feed and seasonal factors such as temperature.

Another important factor in determining lysine requirements is bioavailability of lysine, in other words, digestibility and utilization in the body. Crystalline amino acids to be mixed in diet are almost completely absorbed in the body, but the digestibility of amino acids are reported to vary remarkably (Furuya *et al.*, 1986; Tanksley and Knabe, 1984; Furuya and Kaji, 1987; Yamazaki and Kamata, 1986) between feedstuffs. Amino acid requirements are traditionally estimated as the total amounts of the amino acids, not taking digestibility into account. In the present study, too, lysine requirements were expressed as the total amount of lysine. More reasonably, however, they should be expressed as the requirements for digestible amino acids, like protein requirements which are expressed as digestible crude protein, because different feedstuffs have different amino acid digestibilities. A study with the aim of expressing lysine requirements as the digestible lysine requirements is described in Chapter IV.

II. Lysine Requirements of Finishing Pigs Estimated under Practical Feeding Conditions

Abstract

The lysine requirements of pigs weighing 58 to 92 kg which were kept in practical *ad libitum* group feeding conditions were determined by using nitrogen retention, daily weight gain (DG) and feed conversion ratio (FC) as the response criteria. Nitrogen retention was determined by the potassium indicator method using urinary nitrogen and potassium excretion rates. Graded levels of L-lysine and 0.1 % L-threonine and 0.1 % DL-methionine were added to a rice-soybean meal basal diet containing 12.8 % crude protein, 0.61 % lysine and 3,143 cal digestible energy per gram; lysine levels ranged from 0.61 to 0.85 % in 0.08 % increments. For all the experimental diets except the basal diet, lysine was the first limiting amino acid. A 5 x 5 latin square design was used, with five groups of two female pigs in each initially averaging 58 kg, five diets including the basal diet, and five 7-day periods. The lysine requirement was determined by the method of least squares for each criterion, showing a broken line response. The results obtained were as follows: 1) There was an improvement in nitrogen retention, DG and FC as lysine level in the diet was increased from 0.61 to 0.77 % but an increase from 0.77 to 0.85 % tended to decrease these performance criteria. 2) From nitrogen retention, DG and FC, the estimated lysine requirements were 0.75, 0.75 and 0.72 %, respectively. 3) The lysine

requirement expressed in terms of g/kg of body weight gain was estimated to be 19.5. 4) The results obtained confirmed that nitrogen retention determined by the potassium indicator method could be used efficiently as a response criterion for the determination of amino acid requirements.

Introduction

Recently, formulation of pig diets is based on amino acid composition in feedstuffs instead of protein. However, the requirement of lysine, the first limiting amino acid in most diets for pigs, largely differ among researchers or feeding standards. For example, the lysine requirements expressed a percentage of the diets for pigs weighing 20 to 35 kg in the Japanese Feeding Standard (JFS, 1975) and the National Research Council (NRC, 1979), most commonly applied in Japan, are 0.74 and 0.70, respectively. They are much lower than the lysine requirement, 1.10 %, for pigs weighing 15 to 50 kg recommended by the Agricultural Research Council (ARC, 1981). We had previously estimated the lysine requirement of growing pigs under practical *ad libitum* group-feeding conditions and obtained similar results to the ARC's (Chapter I; Kaji and Furuya, 1987a).

In the present study, the lysine requirements of finishing pigs were estimated by a combination of a nitrogen balance examination by the potassium indicator method (Furuya *et al.*, 1970a,b) and feeding examination (Furuya *et al.*, 1985).

Materials and Methods

Animals and General Procedures

Ten female pigs weighing 58 kg on an average were divided into 5 groups of 2 pigs each and housed in concrete-floored pigpens. A basal diet (Table 2-1) containing rice and soybean meal as the principal ingredients and other 4 experimental diets with the addition of 0.1% L-threonine, 0.1% DL-methionine, or graded levels (0.1, 0.2% or 0.3%) of L-lysine HCl to the basal diet were prepared (Table 2-2). The ratios of other essential amino acids to lysine were all higher than those of the amino acid pattern recommended by the ARC (1981) even at the highest lysine level of 0.85%, confirming that lysine was the first limiting amino acid in the diets. The pigs were allowed *ad libitum* access to feed and water. A 5x5 latin square design was used with five 7-day experimental periods. On the last 4 days in each experimental period, urine samples for all pigs were collected between 14:00 and 15:00 and also fecal samples for the pigs fed the basal diet were collected to determine digestibilies (Morimoto, 1971). For urine sampling, 5 to 10 ml/head of urine were collected in a test tube containing 0.2 ml of 1 N hydrochloric acid through a catheter (TU-554, No. 5, Takei Ika Koki Seisakusho, Co., Ltd.) inserted into the urinary bladder using a lubricant, and stored in a refrigerator until analysis. Body weight and feed intake were measured on the last day of each experimental period to calculate daily body

Table 2–1. Ingredients and composition of the basal diet (% or cal/g)

Ingredients	
Rice	81.6
Soybean meal	13.0
$CaCO_3$	1.2
$CaHPO_4$	0.8
KH_2PO_4	1.9
$MgSO_4$	0.5
NaCl	0.5
Vitamin and mineral mixture*	0.4
Chemical composition (by analysis)	
Dry matter	85.6
Crude protein	12.8
Potassium	0.83
Gross energy, cal/g	3565
Digestible energy, cal/g	3043
Amino acid composition (by analysis)	
Lysine	0.61
Threonine	0.55
Methionine + cystine	0.43
Tryptophan	0.16
Histidine	0.31
Leucine	1.07
Isoleucine	0.57
Valine	0.69
Phenylalanine + tyrosine	1.13

* Providing (per kg diet): Vitamin A, 16000 IU; Vitamin D, 3200 IU; Vitamin E, 16 IU; thiamin, 3.2 mg; riboflavin, 16 mg; Vitamin B_6, 3.2 mg; pantothenic acid, 7 mg; nicotinic acid, 3.2 mg; choline, 221 mg; folic acid, 1.6 mg; Zn, 48 mg; Fe, 40 mg; Mn, 40 mg; Cu, 8 mg; I, 0.8 mg.

Table 2-2. Amount of amino acids added to the basal diet in each experimental diet (%)

	Basal diet	Lysine level (%)			
	with Cr_2O_3	0.61	0.69	0.77	0.85
Basal diet	99.9	99.8	99.7	99.6	99.5
L-lysine HCl	–	–	0.1	0.2	0.3
L-threonine	–	0.1	0.1	0.1	0.1
DL-methionine	–	0.1	0.1	0.1	0.1
Cr_2O_3	0.1	–	–	–	–

weight gain (DG) and feed conversion ratio (FC). Nitrogen retention (a percentage of absorbed nitrogen and daily amount) was calculated by the method of Furuya *et al.* (1985). The digestibilities of the added amino acids were assumed to be 100%.

Analytical and Statistical Procedures

Analyses for dry matter and crude protein (CP) were by a method based on the AOAC's procedures (Morimoto, 1971). Gross energy content was measured in automatic bomb calorimeter (CA-3, Shimadzu Seisakusho Ltd.) and chromic oxide was quantified by the method of Brisson (1956). The samples for potassium analysis were prepared by the method of Furuya *et al.* (1970) and analyzed with an atomic absorption flame emission spectrophotometer (AA 8500 Mark II, Nippon Jarrell Ash Ltd.). The samples for amino acids quantification were prepared by hydrolyzing the diet with 6 N hydrochloric acid at 110℃ for 24 hours and analyzed by a fluorescence derivatization method with o-phthalaldehyde with a high performance liquid chromatograph (Nihon Waters Ltd.). Among amino acids, tryptophan was analyzed by the method of Ikumo *et al.* (1983) and cystine by the method of Spencer and Wold (1969). Lysine requirements were estimated as a inflection points by fitting a broken line model to the data obtained (Ohtsuka and Yoshihara, 1975).

Table 2-3. Effects of lysine level in diets on means for growth performance and nitrogen retention

	Basal diet	Lysine level (%)			
	with Cr_2O_3	0.61	0.69	0.77	0.85
Daily gain, g/day	943	886	979	1086	1009
Feed intake, g/day	2826	2849	2771	2842	2822
Feed conversion ratio	3.00	3.22	2.83	2.62	2.80
Lysine intake, g/day	17.2	17.4	19.1	21.9	24.0
N intake, g/day	57.7	58.7	57.4	59.3	59.2
N digested, %	76.1	76.4	76.5	76.7	76.9
N absorbed, g/day	43.9	44.8	43.9	45.5	45.5
N retained, % of absorbed	42.2	40.2	43.8	54.7	50.1
N retained, g/day	18.7	18.4	19.6	25.2	23.3

Results and Discussion

The results of the present study are shown in Table 2-3. The percentage and daily amount of nitrogen retained increased with dietary lysine levels in diet from 0.61% to 0.77%, but did not increase further at 0.85%. DG and FC showed the same tendency as nitrogen retention, though their results are of a short period of only 1 week and might have the effect of the previous diets because of employing a latin square design. Inflection points where each response reached the plateau were estimated by a broken line model (Ohtsuka and Yoshihara, 1975) (Fig. 2-1 and Fig. 2-2). The estimates were 0.75% for the percentage and daily amount of nitrogen retained, 0.75% for DG and 0.72% for FC. Based on the results of nitrogen retention and DG, the lysine requirement for pigs weighting 58 to 92 kg was estimated to be 0.75%. In our previous experiment (Furuya *et al.*, 1985), the protein requirement was determined for pigs initially weighing about 53 kg fed the diet containing rice and soybean meal as the principal ingredients and estimated to be 15.2% CP. The lysine level was estimated to be 0.76% at the CP level. When this estimate, 0.76%, was regarded as the lysine requirement because lysine was the first limiting amino acid, the result of the present study were well consistent with that of the previous study.

However, the lysine requirement estimated by the method above has some problems. In the conventional method, graded levels of a test

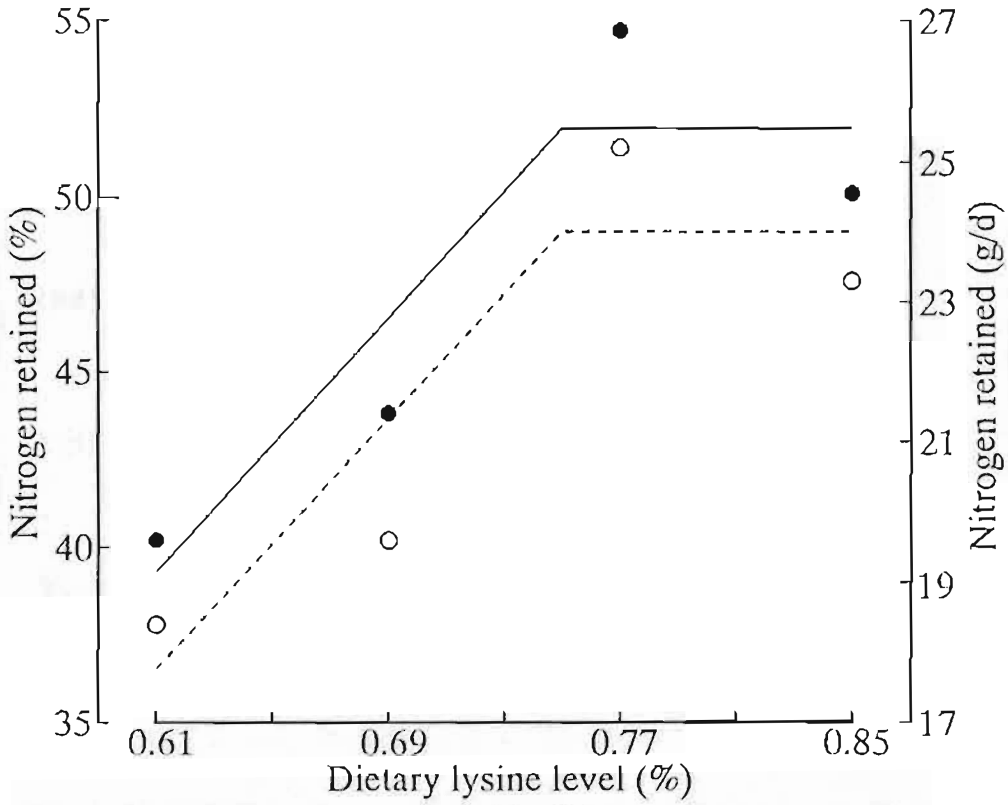

Fig. 2–1. Effect of lysine level in diet on nitrogen retention. Each point represents the mean value for ten pigs. ●——● nitrogen retained (%); ○-----○ nitrogen retained (g/d).

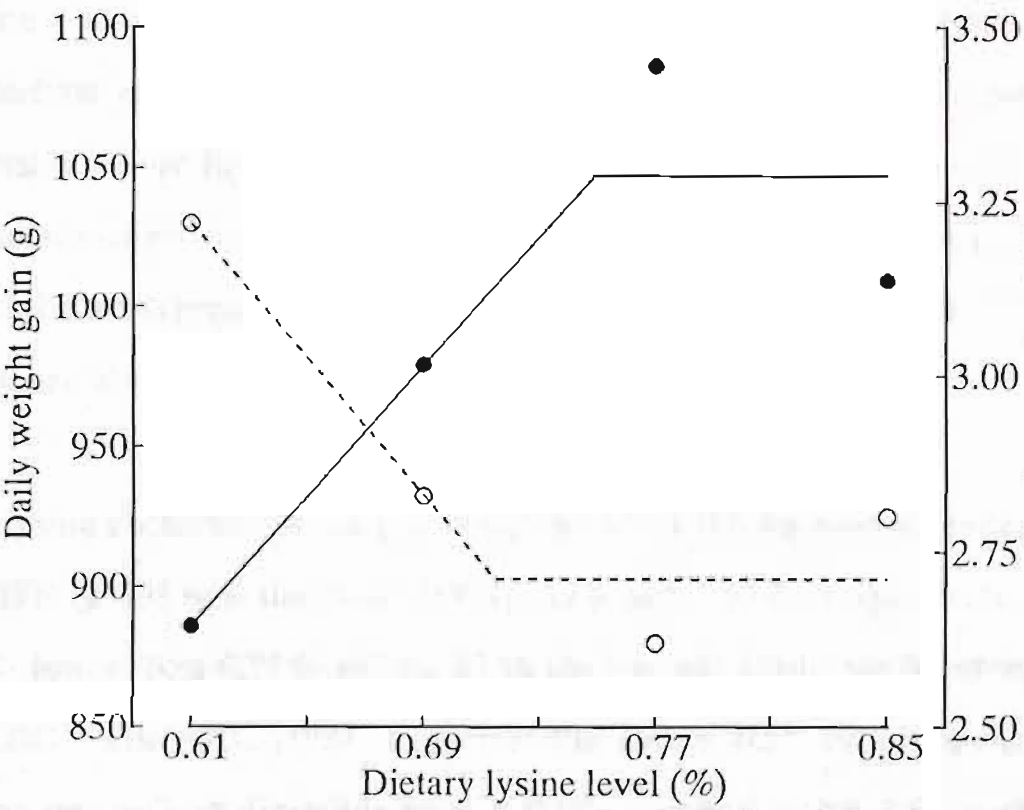

Fig. 2–2. Effect of lysine level in diet on daily gain and feed conversion ratio. Each point represents the mean value for ten pig. ● —— ● daily weight gain; ○ - - - - ○ feed conversion ratio.

amino acid are added to a basal diet deficient in only the test amino acid and the response of animals fed the diets are observed, and then a point where a response reached a plateau is regarded as the requirement of the animals for the response criterion. The present study also followed these procedures to estimate lysine requirements, but the pigs tended to respond adversely at the highest lysine level (Fig. 2-1 and Fig. 2-2). Batterham *et al.* (1985) found the same responses. If such responses generally occur in animals, the requirements estimated based on the conventional method assuming that animals' response reaches a plateau could be underestimated. The lysine requirements obtained in this study are also possibly to be underestimated.

The lysine requirement for pigs weighing 60 to 100 kg recommended by the JFS (1975) and the NRC (1979), 0.60 and 0.57%, respectively, are much lower than 0.75% estimated in the present study for N retention and DG. The ARC (1981) expresses the amino acids requirements as grams per unit of digestible energy (DE), recommending 2.51 mg/kcal DE of lysine requirement for pigs weighing 50 to 90 kg. Since the basal diet used here had 3,043 kcal/g DE (Table 2-1), the lysine requirement was calculated to be 2.46 mg/kcal DE. This two values are in very good agreement.

Lysine is an extremely important amino acid for the lean meat production. Lysine requirement almost depends on lean gain. Lysine is necessary also for maintenance but it is negligible when pigs are normally growing (Chapter IV; Furuya and Kaji, 1987). We indicated that the amount of lysine needed is almost proportional to body weight gain and the lysine requirement expressed as grams per kg of body weight is 19.6 in the study of growing pigs (Chapter I; Kaji and Furuya, 1987a). Therefore, a broken line model was fitted to the data of DG on daily lysine intake (Table 2-3), showing that body weight gain reached a plateau at 20.4 g of daily lysine intake and DG was estimated to be 1,047g. From these values, the lysine requirement as grams per kg of body weight was calculated to be 19.5g, very close to 19.6g obtained in the study with growing pigs (Chapter I; Kaji and Furuya, 1987a). Shields *et al.* (1983) investigated the change in body composition of growing pigs with the body weight. According to their study, the fat contents increased from 11 to 30%, while the protein contents remained to be about 15%, in the range from 6 to 109 kg of body weight, supporting the finding that the lysine requirement expressed as grams per unit of body weight gain remains constant regardless of body weight of pigs.

The amount of lysine required is directly related with the lean gain or body weight gain, but considerable portion of energy requirements are for maintenance (Kametaka *et al.*, 1984). Therefore, the slower the pigs gain, the lower the lysine requirement is as a percentage of the diet. We have tried to estimate the amino acids requirements under practical *ad libitum* group feeding conditions (Chapter I; Kaji and Furuya, 1987a; Furuya *et al.*, 1985) and obtained body weight gain similar to that in practical farms. In the present study, DG exceeded 1 kg when dietary lysine levels met or exceeded the requirement (Table 2–3). On the other hand, the JFS (1975) and the NRC (1979) set their DG for pigs weighing 60 to 100 kg at relatively low levels of 700 and 800 g, respectively. Such differences in body weight gain seems partly responsible for the differences between the lysine requirement estimated in the present study and those recommended by the two feeding standards. In the JFS's table, the expected body weight gain and feed intake are 700 g and 2.80 kg, respectively, for pigs weighing 70 kg. Supposing the lysine requirement per kg body weight gain is 19.5g based on the result of the present study, it gives 13.7g of the daily lysine requirement at 700 g of DG and 0.49% of the dietary lysine requirement from the daily feed amount 2.80 kg. The feed amount of the JFS has a margin of 15%. Even if the daily feed amount of 2.38 kg is sufficient, the lysine requirement is calculated to be 0.58%, lower than 0.60% recommended by the JFS (1975). The amino acid requirements of the JFS (1975) is probably enough at such low DG about 700g. However, DG of pigs in pig farms is much higher than that of the JFS and the lysine requirement should be increased accordingly.

In the present study, we estimated the lysine requirements of finishing pigs under practical *ad libitum* group feeding conditions by a combination of a nitrogen balance experiment by the potassium indicator method and a feeding experiment. The lysine requirement estimated from nitrogen retention was in good agreement with that estimated from the growth performance, confirming nitrogen retention by the potassium indicator method can be used efficiently to estimate amino acid requirements. However, this method have some problems such as a difficulty of applying it for pigs fed restrictedly because of the variation of the ratio between nitrogen and potassium in urine with sampling time and a disadvantage that pigs used for the study are limited to females owing to convenience in urine sampling through a catheter (Furuya *et al.*, 1985). Recent reports describe that lysine requirements differ between the feeding methods (*ad libitum* feeding and restricted feeding) or the sexes e.g., male, female, castrated male (Batterham *et al.*, 1985; Yen *et al.*, 1986). Yen *et al.* (1986) estimated that the daily lysine requirements of pigs weighing 50 to 90 kg for females and castrated males are 21.2 and 18.6g, respectively, and suggested that this is caused by the difference of capacities for muscle synthesis between the two sexes. Under these circumstances, we tried to apply plasma urea nitrogen concentration as a response criterion which is applicable to both sexes under practical feeding conditions (Chapter V; Chapter VI; Kaji and Furuya, 1987b; Kaji and Furuya, 1987c).

III. Lysine Requirements of Growing Pigs in Cold Conditions

Abstract

In cold conditions, the lysine requirements of pigs weighing 45 to 60 kg were estimated for daily weight gain (DG) and feed conversion ratio (FC). Soybean meal was added at the expense of corn starch to a basal diet, and five experimental diets containing 8 to 20 % crude protein (0.42 to 1.14 % lysine) were formulated. Lysine was the first limiting amino acid for all experimental diets. Feed and water were available *ad libitum*. The average temperature during the experimental period was 2.2 ℃, about 15 ℃ below the lower critical temperature for the pigs in this study. From DG and FC, the estimated lysine requirements were 0.71 and 0.70 %, respectively. They were lower than 0.86 and 0.73 %, for pigs weighing 20 to 35 kg and 45 to 60 kg, respectively, estimated previously by us in thermoneutral conditions. However, the lysine requirement expressed as grams per kg body weight gain was 21.5, corresponded to those in thermoneutral conditions.

Introduction

In cold conditions, that is, where environmental temperature is below lower critical temperature, extra energy supply is required to maintain body temperature (Kametaka *et al.*, 1984). On the other hand, the daily requirements of protein and amino acids are hardly influenced by the environmental temperature (Filmer and Curran, 1977). Accordingly, daily body weight gain is constant, the dietary amino acid requirements expressed as a percentage of the diet in cold conditions are lower than those in thermoneutral conditions. However, since amino acid requirements are generally determined in thermoneutral conditions, there are little reports on amino acid requirements in cold conditions. In the present study, we estimated the requirements of lysine, the first limiting amino acid in most pig diets, for growing pigs in cold conditions.

Materials and Methods

Five experimental diets were formulated by adding soybean meal at the expense of cornstarch to a basal diet (Table 3−1), containing 8, 11, 14, 17 or 20% crude protein (CP) with 0.42, 0.60, 0.78, 0.96 or 1.14% lysine) (Table 3−1). As shown in Table 3−2, lysine was the first limiting amino acid in each of the diets on the basis of amino acid pattern in ideal protein proposed by the Agricultural Research Council (ARC, 1981). Forty growing pigs weighing 35 kg on an average were divided into 10 groups of 4 pigs, and two groups each were allotted to each dietary treatment. Feed and water were available *ad libitum*. The pigs were housed in a pig house of our experiment station, with all the doors and windows open. The experiment was conducted from January 10 to 28, 1986. Body weight and feed intake were measured twice a week with an interval of three to four days and daily body weight gain (DG) and feed

Table 3–1. Formulation of the basal diets for the estimation of lysine requirement for growing pigs in cold conditions

Ingredient, %	
Corn	83.20
Alfalfa meal	8.08
Fish meal	4.85
Tricalcium phosphate	2.42
Sodium chloride	0.81
Vitamin and mineral mixture*	0.64

* Supplied (per kg of diet): Vitamin A, 16000 IU; Vitamin D_3, 3200 IU; Vitamin E, 16 IU; thiamin, 3.2 mg; riboflavin, 22.4 mg; pyridoxine HCl, 1.6 mg; pantothenic acid, 35 mg; nicotinic acid, 19 mg; choline, 184 mg; Mn, 80 mg; Zn, 96 mg; Cu, 16 mg; Fe, 80 mg; I ,1.6 mg.

Table 3-2. Formulation and chemical and essential amino acid compositions of the experimental diets

Crude protein levels, %	8	11	14	17	20
Ingredient, %					
Basal diet*	61.9	61.9	61.9	61.9	61.9
Soybean meal	1.3	8.0	14.8	21.6	28.4
Corn starch	36.7	30.0	23.2	16.4	9.6
Chromic oxide	0.1	0.1	0.1	0.1	0.1
Crude protein, %	8.51	11.28	14.23	16.73	20.50
Gross energy, kcal/g	3.74	3.80	3.82	3.85	3.87
Digestible energy , kcal/g**	3.06	3.06	3.06	2.99	2.89
Essential amino acids***, %					
Arginine	0.39	0.62	0.85	1.09	1.32
Histidine	0.19	0.27	0.35	0.42	0.50
Isoleucine	0.29	0.44	0.59	0.74	0.89
	(126)	(133)	(138)	(140)	(142)
Leucine	0.78	1.03	1.27	1.52	1.77
	(186)	(172)	(163)	(158)	(155)
Lysine	0.42	0.60	0.78	0.96	1.14
	(100)	(100)	(100)	(100)	(100)
Methionine + cystine	0.30	0.38	0.47	0.57	0.65
	(143)	(127)	(121)	(119)	(114)
Phenylalanine + tyrosine	0.65	0.92	1.20	1.48	1.77
	(160)	(160)	(160)	(161)	(162)
Threonine	0.30	0.43	0.56	0.69	0.82
	(119)	(119)	(120)	(120)	(120)
Tryptophan	0.06	0.10	0.13	0.17	0.20
	(100)	(107)	(112)	(115)	(117)
Valine	0.38	0.53	0.68	0.83	0.99
	(129)	(126)	(125)	(124)	(124)

* See Table 3-1.

** See Table 1-2.

*** The ratios of each essential amino acid to lysine calculated on the basis of amino acid balance in ideal protein (ARC, 1981) are shown in parentheses.

conversion ratio (FC) were calculated. The requirements of CP and lysine were estimated for DG and FC by the method of least squares (Ohtsuka and Yoshihara, 1976). Dietary CP was analyzed by a method based on the AOAC's procedures (Morimoto, 1971). Amino acids were quantified by a fluorescence derivatization method with o-phthalaldehyde using a high performance liquid chromatography (Nihon Waters Ltd.). The diets were hydrolyzed with 6N hydrochloric acid at 110 ℃ for 24 hours before the measurement of amino acids. Among amino acids, tryptophan was analyzed by the method of Ikumo *et al.* (1983), and cystine by the method of Spencer and Wold (1969).

Results and Discussion

During the experiment, the daily mean temperature in the pig house was 5.9 ℃ at the maximum and −1.3 ℃ at the minimum. The mean temperature throughout the experiment was 2.2 ℃. The environmental temperature was much below the lower critical temperature ,which is 16 to 17 ℃, for pigs with the same body weight as the pigs used in this study (Kametaka *et al.*, 1984). Figure 3−1 shows the effect of dietary CP or lysine level on DG and FC. DG was 479 g at 8% CP and increased up to 952g at 17% CP, but no further increase was observed at 20% CP. The CP requirement for DG was estimated to be 12.8% by using a broken line model (Ohtsuka and Yoshihara, 1975). Since lysine was the first limiting amino acid in every diet, 0.71% lysine contained in the 12.8% CP diet could be regarded as the lysine requirement for DG. FC was improved as dietary CP level increased, but no further improvement was observed beyond 17% CP. The lysine requirement for FC was estimated to be 0.70%. These estimates were lower than 0.86 and 0.73%, for pigs weighing 20 to 35 kg and 45 to 60 kg, respectively, obtained in our previous feeding experiments in thermoneutral conditions (23 ± 1 ℃) (Chapter I; Kaji and Furuya, 1987). It is probably due to increased feed intake to maintain body temperature in cold conditions. The mean environmental temperature in the present study was about 15 ℃ below the lower critical temperature for the pigs used here. In such conditions, feed supply should be increased by 10 to 15% according to the Japanese Feeding Standard (JFS, 1975). For instance, if feed intake is increased by 15%, 0.71% of the lysine requirement obtained from DG in the present study will be equivalent to 0.82% in thermoneutral conditions. This value correspond to that obtained in thermoneutral conditions in our previous study (Chapter I; Kaji and Furuya, 1987), considering the body weight (35 to 50 kg) of the pigs used in this study.

We reported previously that the lysine requirement expressed in terms of grams per kg of body weight gain (g/kg BWG) is almost constant regardless of body weight of pigs in thermoneutral conditions and that the requirement is estimated to be about 20 g/kg BWG (Chapter I; Chapter II; Kaji and Furuya, 1987). The lysine requirement (g/kg BWG) was calculated to be 21.5 from the data of this study, a little higher than, but not so far from, that in thermoneutral conditions.

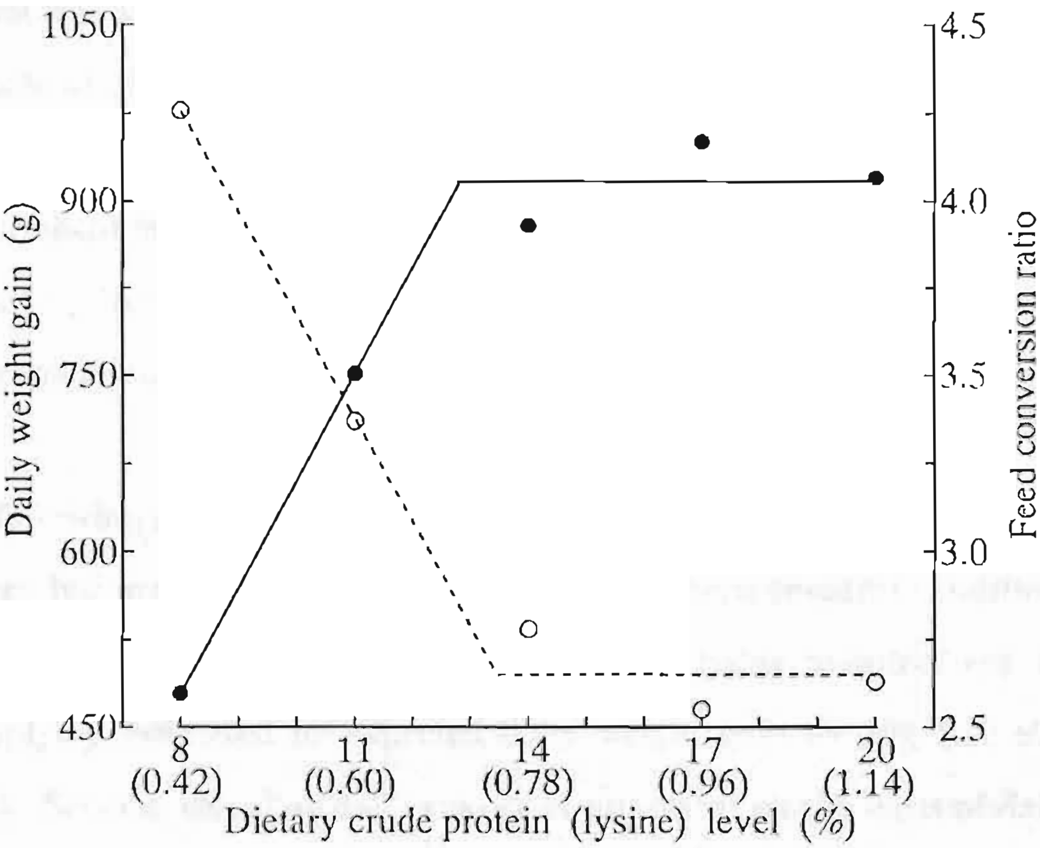

Fig. 3-1. Effects of crude protein and lysine level in diets on daily weight gain (●) and feed conversion ratio (○).

Therefore, the amount of lysine required for 1 kg of body weight gain may be constant regardless of environmental temperature, though the lysine requirement expressed as a percentage of the diet is lower in cold conditions than that in thermoneutral conditions, .

As shown in Figure 3-1, DG and FC changed quadratically with increase of dietary CP level. It may be unreasonable to fit a broken line model to the data of DG and FC in this study. The estimated lysine requirement might have been lower than the true value, and, if so, CP or lysine requirement would have been higher in cold conditions than those in thermoneutral conditions. Further studies are required on this point.

The following procedures are recommended for the determination of the dietary lysine requirement of growing pigs in thermoneutral conditions and in cold conditions. First, obtain daily lysine requirement by multiplying estimated or expected daily weight gain by 20g lysine/kg BWG. Second, calculate daily energy requirement on the basis of daily weight gain, body weight and environmental temperature or obtain it from feeding standards. For developing gilts, we published an equation of energy requirement considering body weight, daily weight gain and environmental temperature (Kaji and Furuya, 1985). Third, calculate daily feed intake or daily feed supply by dividing daily energy requirement by dietary energy content. Finally, from the daily feed intake or supply and daily lysine requirement, obtain the dietary lysine requirement as a percentage of the diet for the expected daily gain in the thermal conditions.

The lysine requirement expressed as a percentage of the diet is affected by many factors such as dietary energy content, body weight gain, environmental temperature. The above procedures make it possible to determine an appropriate lysine requirement as a percentage of the diet according to individual feeding conditions.

IV. Digestible Lysine Requirements of Growing Pigs

Abstract

The digestible lysine requirements of pigs weighing 5 to 10 kg (Period 1), 10 to 20 kg (Period 2), 20 to 35 kg (Period 3) and 45 to 60 kg (Period 4) under *ad libitum* group feeding conditions were estimated for daily gain by the method of least squares. For each period, pigs were allotted to each of the five diets which were formulated to ensure that all essential amino acids except lysine would meet or exceed the ARC amino acid balance recommendation. The true ileal digestibilities of amino acids in basal diets were determined with pigs fitted with a simple cannula and the digestibilities for casein and soybean meal added to the basal diets were based on literature. The dietary digestible lysine levels ranged from 0.83 to 1.81 % for Periods 1 and 2, from 0.51 to 1.13 % for Period 3 and from 0.35 to 0.97 % for Period 4. The results obtained were as follows: 1) There was a significant improvement in daily gain as the digestible lysine level in the diet was increased, but at the highest level, daily gain tended to decrease for all periods except Period 4. 2) The dietary digestible lysine requirement expressed as a percentage of the diet for Periods 1, 2, 3, and 4 was estimated to be 1.24, 1.38, 0.72, and 0.63, respectively, and the digestible lysine requirement expressed as g/kg of body weight gain was 17.8, 17.5, 15.6 and 18.2, respectively, the mean value being 17.3. 3) The data indicate that the digestible lysine requirements, expressed as g/kg of body weight gain, remain relatively

constant regardless of their daily gain, body weight or age and that the digestible lysine requirement for maintenance is negligible.

Introduction

Lysine is almost always the first limiting amino acid in common pig diets. There are many studies on lysine requirements, but the estimates are generally expressed as the total amounts of lysine without taking account of its digestibility. Various feedstuffs have been used to supply lysine in experiments: Some feedstuffs such as crystalline lysine and casein have almost 100% digestibility, but others are poorly digestible (Tanksley and Knabe, 1984). It is suggested that digestibilities of amino acids are different between grains and particle sizes of ground grains (Sauer *et al.*, 1977; Taverner et at., 1981; Owsley *et al.*, 1981; Furuya and Kaji, 1987). Total lysine requirements estimated without considering its digestibility is probably different depending on feedstuffs formulated. To formulate diets more accurately and supply dietary protein more efficiently, it is reasonable to estimate amino acid requirements in terms of digestible amino acids and determine amino acid composition also in terms of digestible amino acids.

In the present study, we intended to estimate the digestible lysine requirements of growing pigs, based on the results of our previous study on the total lysine requirements of growing pigs (Chapter I; Kaji and Furuya, 1987), by determining the digestibilities of amino acids in the basal diets used in the study.

Materials and Methods

In the previous study (Chapter I), the total lysine requirements of pigs weighing 5 to 10 kg, 10 to 20 kg, 20 to 35 kg and 45 to 60 kg were determined. First, experimental diets containing graded levels of lysine were formulated by adding casein at the expense of cornstarch to a basal diet (weanling diet) of a commercial weanling diet for the first two periods and by adding soybean meal at the expense of cornstarch to a basal diet (growing diet) of corn, fish meal and alfalfa meal for the latter two periods. Then, the total lysine requirements were estimated from the responses of the growing pigs fed the experimental diets.

In the present study, the digestibilities of crude protein (CP) and amino acids in the weanling and growing basal diets were determined. From the results and the lysine digestibilities of casein and soybean meal from literature, the digestible lysine levels in each experimental diets were calculated and digestible lysine requirements were estimated for weight gain reported in Chapter I.

Determination of the Ileal Digestibilities of Amino Acids

Five crossbred pigs with initial mean body weight of 30 kg were fitted with a simple 'T' cannula, 12 mm internal diameter, at the terminal ileum, about 30 cm anterior to the ileo-caecal junction, according to the procedures reported by Furuya *et al.* (1974). The pigs were housed and fed in individual concrete-floored pens without bedding.

Experimental diets were a cornstarch diet, a weanling diet and a growing diet formulated by adding cornstarch, the weanling basal diet and the growing basal diet in Chapter I, respectively, to a basal diet of corn and alfalfa meal (Table 4-1). The 5 pigs were used repeatedly for 3 periods, 4 days per period. The pigs were given randomly each of the 3 diets for each period, not to repeat the same diet. The diets were given 1200g daily, divided into 400g each at 17:00, 01:00 and 09:00 h. Water was available *ad libitum*. The pigs ate all amount of experimental diets offered normally within 1 h. Samples of ileal digesta were collected about 50 g each between 13:00 and 14:00 h (on day 3, that is, 45 to 46 h after the initial feeding) and between 16:00 and 17:00 h on days 3, 4 and 5 and immediately freeze-dried. The freeze-dried samples over three days for each sampling period on each pig were combined and ground with a ball mill before chemical analyses.

The digestibilities of CP and amino acids in each of the experimental diets were determined by the indicator method of chromic oxide. Then, according to the procedures by Furuya and Kaji (1987), their true digestibilities of CP and amino acids in the weanling and growing basal diets used in Chapter I were calculated.

Table 4-1. Formulation and crude protein and some essential amino acid compositions of diets used for the digestion trial (%)

	Diet		
	Cornstarch	Weanling	Growing
Ingredient			
Corn	32.0	32.0	32.0
Alfalfa meal	4.0	4.0	4.0
Cornstarch	60.0	–	–
Weanling diet*	–	60.0	–
Growing diet*	–	–	60.0
Calcium carbonate	1.0	1.0	1.0
Tricalcium phosphate	2.0	2.0	2.0
Sodium chloride	0.5	0.5	0.5
Vitamin and mineral mixture**	0.4	0.4	0.4
Chromic oxide	0.1	0.1	0.1
Crude protein (N x 6.25)	3.2	13.7	9.0
Essential amino acid			
Lysine	0.15	0.80	0.50
Methionine	0.08	0.30	0.21
Threonine	0.12	0.33	0.29

* See Table 1-1 for the formulation.

** Supplied (per kg of diet): Vitamin A, 10000 IU; Vitamin D_3, 2000 IU; Vitamin E, 10 IU; thiamin, 2 mg; riboflavin, 14 mg; pyridoxine HCl, 1 mg; pantothenic acid, 22 mg; nicotinic acid, 12 mg; choline, 115 mg; Mn, 50mg; Zn, 60 mg; Cu, 10 mg; Fe, 50 mg; I, 1 mg.

Analytical and Statistical Procedures

Chemical and amino acid analyses were by the procedures described by Furuya and Kaji (1987). The digestible lysine requirements were estimated by the method of least squares (Ohtsuka and Yoshihara, 1975).

Results and Discussion

Table 4–2 shows the true ileal digestibilities of amino acids and CP in the weanling and growing basal diets used for the estimation of total lysine requirements in Chapter I. The digestibilities of lysine in the weanling and growing basal diets were 87.3% and 84.2%, respectively, almost equivalent to the average of all the essential amino acids and higher than that of CP. The digestibility of threonine tended to be lower than those of other essential amino acids, as shown in the previous paper (Furuya and Kaji, 1987; Furuya *et al.*, 1986). The lysine digestibilities in the experimental diets used in Chapter I were calculated from the true digestibilities of lysine in the weanling and growing basal diets determined here, and those in casein and soybean meal, 100% (Furuya *et al.*, 1986) and 86% (Furuya and Kaji, 1987), respectively. Tables 4–3 and 4–4 show digestible lysine levels in the experimental diets in Chapter I, and the daily digestible lysine intake. From these values and daily body weight gain, digestible lysine requirements were obtained as a percentage of the diet, grams per day and grams per kg body weight gain (g/kg BWG) by the method of least squares (Table 4–5).

Table 4-2. The true ileal digestibilities of amino acids and crude protein in the basal diets* used for the estimation of digestible lysine requirements (%)

	Diet	
	Weanling	Growing
Amino acid		
Essential		
Arginine	89.8	87.0
Histidine	84.3	79.3
Isoleucine	84.7	84.2
Leucine	87.1	84.7
Lysine	87.3	84.2
Methionine	88.1	85.3
Phenylalanine	87.4	85.0
Threonine	81.6	79.6
Valine	83.6	82.2
Average	86.0	83.5
Nonessential		
Alanine	85.9	81.7
Aspartic acid	81.8	80.1
Glutamic acid	87.9	81.6
Glycine	77.0	73.1
Serine	84.6	79.9
Tyrosine	87.3	82.3
Average	84.1	79.8
Average of all amino acids	85.2	82.0
Crude protein	83.1	79.0

* Weanling diet and growing diet were used for periods 1 and 2 and periods 3 and 4, respectively.

Table 4–3. Effects of digestible lysine levels on growth performance during periods 1 and 2*

	Digestible lysine (% of diet)				
	0.83	1.08	1.32	1.57	1.81
Period 1 (5–10 kg body weight)					
Intake					
Feed, g/d	492	484	501	465	409
Digestible lysine, g/d	4.1	5.2	6.6	7.3	7.4
Daily gain, g	239	298	354	357	303
Period 2 (10–20 kg body weight)					
Intake					
Feed, g/d	756	733	750	768	744
Digestible lysine, g/d	6.3	7.9	9.9	12.1	13.5
Daily gain, g	433	488	572	602	564

* See Table 1–2 for the formulation of each diet.

Table 4–4. Effects of digestible lysine levels on growth performance during periods 3 and 4*

	Digestible lysine (% of diet)					
	0.35	0.51	0.66	0.82	0.97	1.13
Period 3 (20–35 kg body weight)						
Intake						
Feed, g/d	—	1650	1781	1795	1748	1657
Digestible lysine, g/d	—	8.4	11.8	14.8	17.0	18.7
Daily gain, g	—	628	780	852	895	756
Period 4 (45–60 kg body weight)						
Intake						
Feed, g/d	2589	2821	2397	2565	2690	—
Digestible lysine, g/d	9.1	14.4	15.8	21.0	26.1	—
Daily gain, g	716	823	912	912	912	—

* See Table 1–2 for the formulation of each diet.

Table 4–5. Digestible lysine requirements estimated for daily gain for the four experimental periods

	Period (body weight, kg)			
	5–10	10–20	20–35	45–60
Requirement, % of diet	1.24	1.38	0.72	0.63
Requirement, g/d	6.0	10.2	13.0	16.6
Weight gain estimated, g/d	338	583	834	912
Requirement, g/kg weight gain*	17.8	17.5	15.6	18.2

* These values were obtained by dividing the estimated digestible lysine requirement (g/d) by the estimated daily gain (kg/d).

The dietary digestible lysine requirement for Periods 1 and 2 was estimated to be 1.24 and 1.38%, respectively, but for Periods 3 and 4, much lower values were obtained. In general, the digestible lysine requirement expressed as a percentage of the diet decreased as body weight and age increased. However, the digestible lysine requirements expressed as g/kg BWG were relatively constant regardless of the body weight range of the pigs: The value for Period 1 was 17.8 g and that for Period 3 was 15.6 g , a little lower than that for Period 1, but that for Period 4 was 18.2 g, a little higher than that for Period 1. These values generally changed less remarkably than the dietary digestible lysine requirements with the body weight of the pigs, and the mean value of the four periods was 17.3g.

As illustrated in Figure 4-1, very close correlation was detected between the daily digestible lysine requirement and estimated daily weight gain for each period. The regression crossing the point close to the origin indicated that, when the growth rate of pigs is at nearly highest, most of digestible lysine ingested is used for body weight gain or lean gain and extremely little for maintenance.

We (Chapter I; Kaji and Furuya, 1987) demonstrated that the requirements of amino acids, conventionally expressed as a percentage of the diet, are affected by daily body weight gain and dietary energy content. To remove these influences, we suggested that the requirements of amino acids should be expressed as g/kg BWG. In the

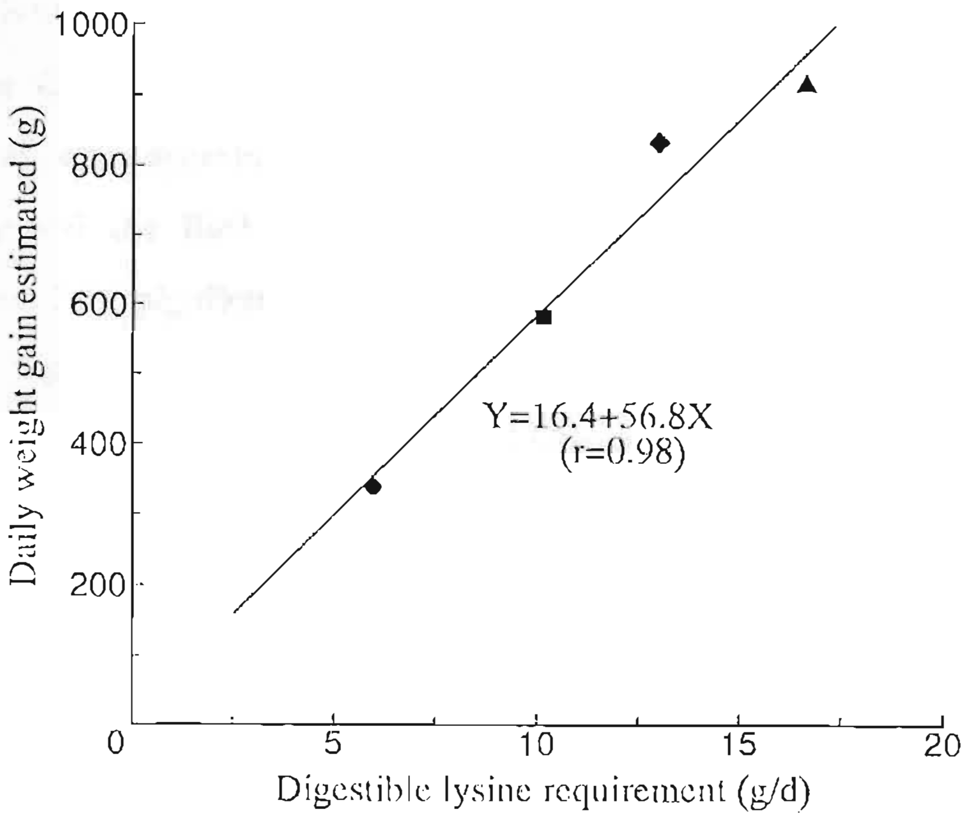

Fig. 4–1. Regression of estimated daily weight gain on daily digestible lysine requirement ; ● period 1 (5–10kg), ■ period 2 (10–20kg), ◆ period 3 (20–35kg) and ▲ period 4 (45–60kg).

present study, we obtained the digestible requirements of lysine, the first limiting amino acid in most pig diets, taking into account the digestibilities of lysine in the diets that is one of factors affecting total lysine requirements: The digestible lysine requirement expressed as g/kg BWG was 17.3, relatively constant regardless of daily gain, body weight or age. This value was about 88% of the total lysine requirement, 19.6 g, obtained in Chapter I. This means that the average of the lysine digestibilities in the experimental diets used in Chapter I was about 88%. We determined the ileal digestibilities of amino acids for various feedstuffs used for pig diets, and the mean value is about 85% (Furuya and Kaji, 1987). Compared with this value, the mean digestibility obtained in the present study was a little higher. In the present study, the pigs used in Periods 1 and 2 were fed the diets made of casein and the weanling basal diet based on a commercial weanling diet. The digestibilities of lysine were 87.3% for the weanling basal diet (Table 4-2) and 100% for casein (Furuya *et al.*, 1986),due to the high mean digestibility of lysine.

To apply the digestible lysine requirement (g/kg BWG) obtained here to diet formulation, it have to be expressed as a percentage of the diet. The procedures are follows: First, obtain daily digestible lysine requirement by multiplying expected or estimated DG by 17.3 g/kg BWG. Second, calculate daily feed supply or daily feed intake by dividing daily energy requirement, which can be obtain from feeding standards or other reports, according to DG by dietary energy content. Finally, digestible lysine requirement as a percentage of the diet is calculated by dividing daily digestible lysine requirement by feed supply or estimated feed intake.

Digestible amino acid requirements obtained in this way, however, cannot be practically applied, until the digestibilities of amino acids in feedstuffs are obtained. Recently, the ileal digestibilities of amino acids in feedstuffs used for pig diets have been determined intensively (Tanksley and Knabe, 1984; Sauer *et al.*, 1977; Taverner *et al.*, 1981; Owsley *et al.*, 1981; Furuya and Kaji, 1987; Furuya *et al.*, 1986), but the information is not enough yet. Since the cannulated pigs are required, determination of ileal digestibility could be limited. Therefore, there is an increased demand for a simple method for estimating digestibilities of amino acids without using such pigs.

V. Plasma Urea Nitrogen as a Response Criterion for Determining the Amino Acid Requirements of Growing Pigs

Abstract

Two experiments were conducted to evaluate the use of plasma urea nitrogen (PUN) as a response criterion for determining the amino acid requirements of growing pigs kept under a practical *ad libitum* group feeding conditions. 1) In experiment 1, involving 20 pigs weighing approximately 12 kg each, we measured the response of PUN to crude protein (CP) levels, at the dietary levels of 14 % and 26 %. A new equilibrium of PUN was reached within 1–2 days of changing CP levels. This suggests that the period of metabolic adjustment necessary before PUN can be used to evaluate amino acid requirements is less than 2 days. 2) In experiment 2 we measured the response of PUN and plasma free lysine to the graded addition of L-lysine to a rice-soybean meal basal diet (12.7% CP and 0.6% lysine) with pigs weighing approximately 25 kg. A linear decrease in PUN was observed until reaching 0.9 % dietary lysine. Plasma free lysine was gradually increased up to 0.8 % dietary lysine, and after that increased rapidly as dietary lysine increased. Using PUN and plasma free lysine as the response criteria, the lysine requirement was estimated to be 0.8–0.9 %. 3) The results obtained in experiments 1 and 2 suggest that PUN could be used efficiently as a response criterion for the determination of amino acid requirements.

Introduction

The protein or amino acid requirements for pigs have been estimated conventionally by feeding experiments, carcass analysis, nitrogen balance experiment, etc. (the Agricultural Research Council; ARC, 1981). Recently, a method of using oxidation of a particular amino acid as a criterion is published(Kim *et al.*, 1983). However, these methods are quite time- or labor-consuming and require facilities.

Furuya *et al.* (1970; 1971) developed the potassium indicator method determining nitrogen retention. This method does not require the whole urine sampling or housing animals in individual metabolic cages, and so has an advantage that the experiment can be conducted under practical *ad libitum* feeding conditions. We reported the lysine requirement in growing pigs estimated by this method (Furuya *et al.*, 1985), but it has a disadvantage that its application is limited to female pigs due to the convenience of urine sampling.

It has been known that blood urea concentration changes depending on the quantity and quality of protein or amino acids contained in diets (Rose *et al.*, 1950). When dietary protein is oversupplied or dietary amino acid composition is disproportionate, excess amino acids are catabolized to be urea and it appears in the blood, being excreted into urine. Fuller *et al.* (1979) reported that urea excretion shows a rapid response to the change in amino acid composition in the diet as a result of the experiment with pigs. Therefore, blood urea concentration also should reflect the change in dietary protein quality rapidly and could be used as an effective criterion to estimate the amino acid requirements for pigs. Some researchers used blood urea as a criterion to estimate the amino acid requirements for pigs, (Brown and Cline, 1974; Russell *et al.*, 1983; Leonard and Speer, 1983; Kaji and Furuya, 1987) but few reports are available on the rapidity of the response in blood urea concentration to dietary protein quality (amino acid composition).

In the present study, the response of plasma urea nitrogen concentration (PUN) to the change of dietary protein levels was investigated (Experiment 1), showing the rapid response of PUN to dietary protein level. We tried to estimate the lysine requirement for growing pigs using PUN as a response criterion (Experiment 2).

Materials and Methods

Response of PUN to the change in dietary protein level (Experiment 1)

Daily change in PUN was investigated with pigs fed either of two experimental diet alternately. Two kinds of diet with different protein contents were given to growing pigs to investigate the daily PUN change. Twenty growing pigs initially weighing 12 kg were divided into two groups and housed in concrete-floored pens with the room temperature kept at 23 ± 1 ℃. The experimental diets were formulated by mixing a commercial weanling diet with casein or cornstarch to make a low protein diet of 14% crude protein (CP) and a high protein diet of 26% CP (Table 5-1). During a 15-day experimental period, either of these two diets was fed to the animals of two groups. The diet was switched to another on day 6, and switched on day 12 again. Feed and water were available *ad libitum*. During the experimental period, blood samples were taken about 1 ml from the anterior vena cava between 14:00 and 15:00 h on days 4, 6 to 8, 10, 12 to 14, and 15. They were collected in heparinized tubes and plasma separated by centrifugation (3,000 rpm, 10 min.) were used for PUN determination.

Determination of Lysine Requirements Using Plasma Urea and Free Lysine as Criteria (Experiment 2)

Twenty growing pigs initially weighing 22 kg on an average were divided into 5 groups of 4 pigs and fed *ad libitum* one of randomly allocated 5 diets of graded lysine levels. The experimental diets were formulated by adding graded amounts of L-lysine HCl to the basal diet containing 0.6% lysine (Table 5-2) to make the range of dietary lysine from 0.6 to 1.0%. To all the diets, 0.2% DL-methionine and 0.1% L-threonine were added to make lysine always the first limiting amino acid even in the diet containing the highest lysine level of 1.0%, based on the amino acid pattern in the ideal protein proposed by the ARC (1981). The tryptophan level in the basal diet was 0.16% and satisfied the ARC's requirement(1981) even at the maximum lysine level. Allowing

Table 5-1. Composition of the experimental diets used in experiment 1 (%)

	Protein level (%)	
	14	26
Ingredient		
Formula feed*	75.8	75.8
Cornstarch	22.6	8.48
Casein	—	14.12
Tricalcium phosphate	1.0	1.0
Sodium chloride	0.2	0.2
Vitamin and mineral mixture**	0.3	0.3
Chromic oxide	0.1	0.1
Chemical composition (by analysis)		
Dry matter	87.4	87.7
Crude protein	13.7	25.1

* A commercial feed commonly used for weanling pigs. See Table 1-1 for formulation.

** Providing (per kg diet): retinol, 3 mg; cholecalciferol, 50 μ g; DL- α -tocopheryl acetate, 10 mg; thiamin, 1 mg; riboflavin, 7 mg; pyridoxine HCl, 0.5 mg; pantothenic acid, 10.9 mg; nicotinic acid, 6 mg; choline, 57.6 mg; Mn, 50 mg; Zn, 60 mg; Cu, 10 mg; Fe, 50 mg; I, 1mg.

Table 5-2. Composition of the basal diet used in experiment 2 (%)

Ingredient	
Rice	85.0
Soybean meal	12.0
Tricalcium phosphate	0.8
Calcium carbonate	1.2
Sodium chloride	0.5
Vitamin and mineral mixture*	0.5
Chemical composition (by analysis)	
Dry matter	85.1
Crude protein	12.7
Amino acid composition (by analysis)	
Arginine	0.97
Histidine	0.30
Isoleucine	0.56
Leucine	1.05
Lysine	0.60
Methionine	0.26
Cystine	0.18
Phenylalanine	0.66
Tyrosine	0.45
Threonine	0.55
Tryptophan	0.16
Valine	0.69

* Providing (per kg diet): retinol, 3 mg; cholecalciferol, 50 μg; DL-α-tocopheryl acetate, 10 mg; thiamin, 2 mg; riboflavin, 14 mg; pyridoxine HCl, 1.0 mg; pantothenic acid, 21 mg; nicotinic acid, 12 mg; choline, 115 mg; Mn, 100 mg; Zn, 120 mg; Cu, 20 mg;; Fe, 100 mg; I, 2mg.

for a margin, however, 0.05% L-tryptophan was added to all the diets.

Between 14:00 and 15:00 h two days after the initial feeding of the experimental diets, blood samples were taken in the same manner as in Experiment 1 to determine PUN and plasma free lysine concentration. Other conditions were the same as those in Experiment 1. The experiment was repeated twice with the same 20 pigs. The experimental results were pooled and presented as those of 8 heads per dietary treatment.

Analytical and Statistical Procedures

Analyses for of dry matter and CP were by a method based on the AOAC's procedures (Morimoto, 1971). Gross energy content was measured in an automatic bomb calorimeter (CA-3, Shimadzu Seisakusho Ltd.) and chromic oxide was quantified by the method of Yoshida *et al.* (Morimoto, 1971). PUN was determined by the urease-indophenol method (Kanai, 1983) with a clinical analyzer (RaBA 3010, Chugai Pharmaceutical Co., Ltd.). Amino acids were quantified by a fluorescence derivatization method with o-phthalaldehyde using a high performance liquid chromatograph (Nihon Waters Ltd.). Before the quantification, the experimental diets were hydrolyzed with 6N hydrochloric acid at 110 ℃ for 24 h and plasma was deproteinized with sulfosalycilic acid. Among amino acids, tryptophan was analyzed by the method of Ikumo *et al.* (1983) and cystine by the method of Spencer and Wold (1969). Statistical significance between treatment means was tested by t-test (Yoshida, 1983).

Results

Response of PUN to the change in dietary protein level (Experiment 1)

Fig. 5-1 shows the daily PUN change by switching the protein levels in the experimental diets. PUN of the pigs fed the high protein diet was markedly higher than that of the pigs fed the low protein diet. On the following days of switching the experiments diets, PUN changed remarkably. Particularly on the following days of switching from the high protein diet to the low protein diet, *i.e.* on days 7 and 13, PUN decreased markedly. The PUN of the pigs given the low protein diet on day 12 was not significantly different from that of the pigs fed the switched high protein diet on day 13 , but significantly different from the that on day 14, *i.e.* 2 days after switching ($p<0.01$). These results indicate that PUN almost reaches a new equilibrium by two days after switching diets. PUN of pigs fed the low protein diet tended to increase gradually from day 7 to day 12.

Determination of Lysine Requirements Using Plasma Urea and Free Lysine as Criteria (Experiment 2)

Fig. 5-2 shows the effect of dietary lysine level on PUN and plasma free lysine concentrations. PUN decreased almost linearly with the increase

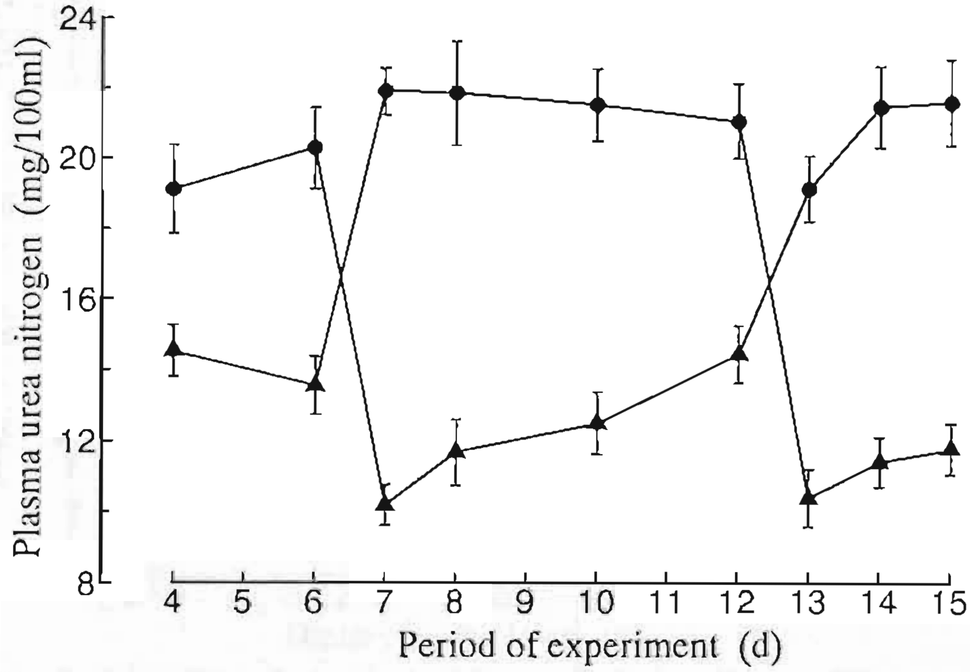

Fig. 5–1. Daily plasma urea nitrogen of pigs given a low protein (crude protein 14%) diet (▲) and a high protein (crude protein 26%) diet (●) in experiment 1. Each point is the mean for 10 pigs with the standard error.

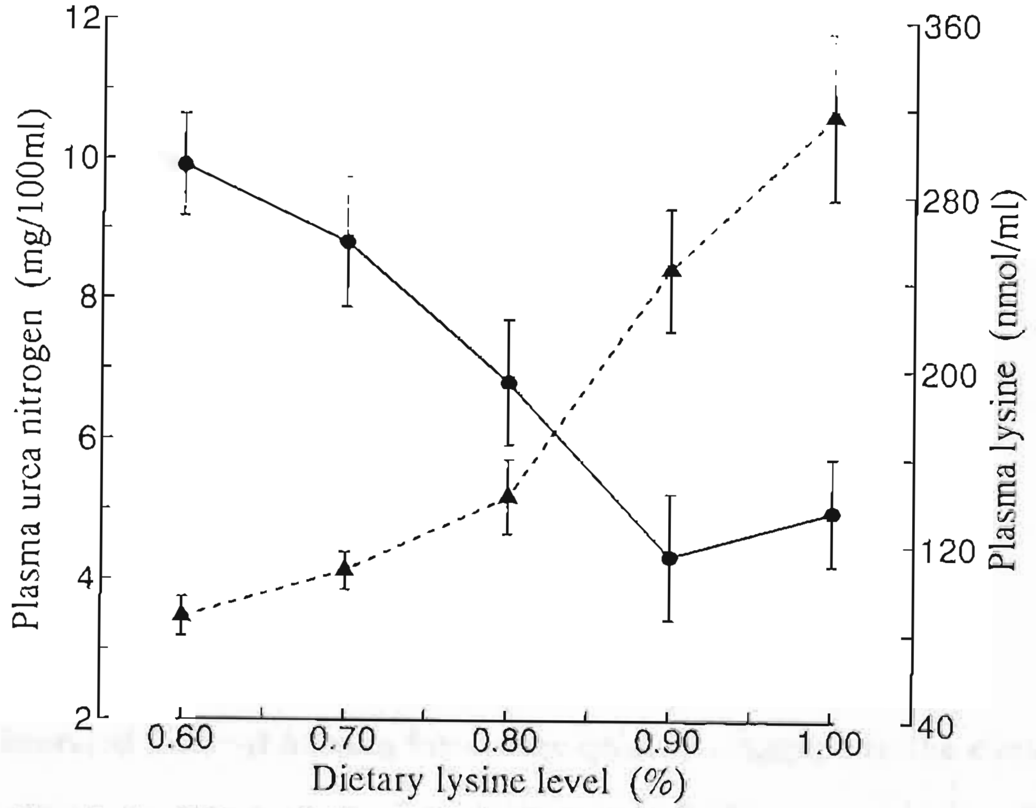

Fig. 5–2. Effect of dietary lysine supplementation on plasma urea nitrogen (●) and plasma free lysine (▲) in experiment 2. Each point is the mean for eight determinations with the standard error.

of dietary lysine level, reaching the minimum at 0.9% lysine and rather got higher at 1.0% lysine. PUN was high up to 0.8% lysine, presumably because the inadequate level of lysine, the first limiting amino acid, limited the use of the other amino acids, resulting in their deamination and the production of urea. PUN reached a low plateau at 0.9% lysine, suggesting that the lysine requirement was met by that point. On the other hand, plasma free lysine increased gradually with the increase of dietary lysine level from 0.6% to 0.8% and increased markedly after that. This indicated that lysine was oversupplied at 0.9% lysine.

The above results suggested that growing pigs weighing about 25 kg require 0.8 to 0.9% lysine.

Discussion

The excreted amount of urea into urine quickly responds to the change of protein or amino acids in diet. Fuller *et al.* (1979) reported that after adding amino acids to a diet a new equilibrium rate of urea excretion is reached within 2 to 3 days. Then, it was thought that PUN responds quickly to switching diets. In Experiment 1, two experimental diets with largely different CP levels of 14% and 26% were fed to pigs to investigate the daily PUN change. As the result, PUN showed a quick response to switching diets and reached an almost stable level 48 h after switching. This indicates that amino acid requirements can be estimated in an extremely short period by using PUN as a criterion. However, as shown in Fig. 5-1, PUN markedly decreased on the following day of switching to the low protein diet and then tended to increase gradually. It seems to be a phenomenon of the animals to adapt to the low protein diet. Further investigation will be required on the period from the initial feeding to blood sampling.

Mitchell *et al.* (1968) obtained the lysine requirement using plasma free lysine as a criterion, but reported that lysine requirement cannot be estimated by blood sampling within 1 day after the initial feeding because the animals cannot adapt to the lysine deficient diet. Thus, in Experiment 2, lysine requirement was estimated from blood samples taken 48 h after initial feeding using PUN and plasma free lysine as criteria. The results showed typical response patterns of PUN and plasma free lysine to dietary lysine level, as shown in Fig. 5-2. PUN responded very quickly to the change in dietary lysine level, confirming that PUN is an effective criterion to estimate amino acid requirements.

In Chapter I we estimated the lysine requirement, 0.86%, of pig weighing 20 to 35 kg as a result of a feeding experiment. This value was similar to the estimates, 0.8 to 0.9% lysine estimated using PUN and plasma free lysine as criteria for pigs weighing about 22 kg. These values are higher than 0.74% and 0.70% recommended by the Japan Feeding Standard (1975) and the National Research Council (1979), respectively, but much lower than the lysine requirement, 1.10%, recommended by the ARC (1981) for pigs weighing 15 to 50 kg. As described so far, lysine requirements largely differs among feeding standards. Considering that lysine is the first limiting amino acid in most pig diets, further study will be necessary to estimate quantitative lysine requirements.

VI. Requirements of Other Essential Amino Acids Estimated by Plasma Urea Nitrogen

Abstract

Two experiments were conducted to estimate the threonine, methionine, tryptophan, isoleucine and valine requirements for growing pigs kept under practical *ad libitum* group-feeding conditions. Plasma urea nitrogen (PUN) was used as a response criterion. 1)In experiment 1, involving 18 pigs weighing approximately 21 kg each, we measured the response of PUN to addition of 0.5% L-lysine HCl (0.4% free base), 0.1%L-threonine, 0.1% DL-methionine to a tryptophan supplemented corn-soybean meal basal diet, which contained lysine (0.47%), threonine (0.47%), methionine + cystine (0.47%) and tryptophan (0.15%). PUN (mg/100 ml) was 9.79 when the mixture of three amino acids was not added, and this value was decreased to 2.78 by adding the mixture devoid of methionine. The mixture lacking lysine drew no response, 10.82: while the mixture without threonine was intermediate, 7.48. With the addition of the three amino acids PUN decreased to 2.88. These results suggest that lysine and threonine are the first and second limiting amino acids in a basal diet supplemented with 0.05% L-tryptophan. 2) In experiment 2, involving pigs weighing approximately 22 kg each, we measured the response of PUN to the addition of L-threonine (0.1%), L-tryptophan (0.05%), L-isoleucine (0.1%) and L-valine (0.1%) to the basal diet supplemented with L-lysine HCl (0.5%) and L-threonine (0.1%). There was little response to the addition of

isoleucine or valine, or to the further addition of threonine. But the addition of tryptophan significantly reduced PUN. 3) It is concluded that the addition of three amino acids, lysine, threonine and tryptophan, can improve the nutritive value of protein in the corn–soybean meal diet and that the amino acid composition of the ideal protein proposed by the ARC is suitable for growing pig kept under practical conditions.

Introduction

Lysine is the first limiting amino acid in most pig diets, and, therefore, many studies have been conducted to estimate lysine requirement. There are, however, comparatively few reports about requirements for other essential amino acids. The Agricultural Research Council (ARC, 1981) has proposed the concept of ideal protein and recommended that the requirements for other essential amino acids than lysine should be estimated from lysine requirement and the amino acid pattern in the ideal protein. There still, however, remains substantial discrepancies between the essential amino acid pattern in the ideal protein and those recommended by the Japanese Feeding Standard (JFS, 1975) and the National Research Council (NRC, 1979). For instance, the ratio of sulfur amino acid, the third or fourth limiting amino acid in most pig diets to lysine is 50% in the ideal protein, whereas it is 73% and 64% in the JFS (1975) and the NRC(1979), respectively.

In the present study, we estimated the requirements of threonine, methionine (sulfur amino acid), tryptophan, isoleucine and valine which are limiting amino acids next to lysine in most pig diets, using plasma urea nitrogen (PUN) as a response criterion, and discussed the validity of the proposed amino acid pattern in the ideal protein.

Materials and Methods

Threonine and Methionine (Sulfur Amino Acid) Requirements (Experiment 1)

Eighteen growing pigs with initial body weight of 21 kg on an average were allocated to six groups of three pigs. The pigs were housed in concrete-floored pens in a room controlled at 23 ± 1 ℃ during a 16-day experimental period.

Five experimental diets were a basal diet of corn and soybean meal containing 0.47% lysine (Table 6-1) and four different diets added L-lysine HCl, L-threonine, DL-methionine or L-tryptophan to the basal diet (Table 6-2). In the first period, all 18 pigs were given the basal diet containing tryptophan with addition of neither lysine, threonine nor methionine (amino acid non-supplement diet) for four days. In the second period, the pigs of six groups were divided into three blocks of two groups, and given one of the three diets (diet lacking lysine, diet lacking threonine, diet lacking methionine) for four days. In the third period, all the pigs were given the diet added all three amino acids for four days. In the last period, all the pigs were given the amino acid non-

Table 6-1. Composition of the basal diet used in experiment 1 and 2 (% or cal/g)

Ingredient	
Yellow corn	87.0
Soybean meal	10.0
Tricalcium phosphate	0.8
Calcium carbonate	1.2
Sodium chloride	0.5
Vitamin and mineral mixture*	0.5
Chemical composition (by analysis)	
Dry matter	84.9
Crude protein	10.2
Gross energy, cal/g	3790
Digestible energy, cal/g	2960
Amino acid composition (by analysis)	
Arginine	0.67
Histidine	0.30
Isoleucine	0.50
Leucine	1.21
Lysine	0.47
Methionine	0.21
Cystine	0.26
Phenylalanine	0.61
Tyrosine	0.47
Threonine	0.47
Tryptophan	0.10
Valine	0.62

* Providing (per kg diet): retinol, 3 mg; cholecalciferol, 50 μ g; DL- α -tocopheryl acetate, 10 mg; thiamin, 2 mg; riboflavin, 14 mg; pyridoxine HCl, 1 mg; pantothenic acid, 21 mg; nicotinic acid, 12 mg; choline, 115 mg; Mn, 100 mg; Zn, 120 mg; Cu, 20 mg;; Fe, 100 mg; I, 2mg.

supplement diet again. Feed and water were available *ad libitum*.

Blood samples were collected in the same way in Chapter V between 14:00 and 15:00 h every day except the following day of switching diets. Body weight and feed intake were measured on the day of switching diets.

A digestion experiment was also conducted in Experiment 1 to estimate digestible energy content of the basal diet. In the fourth period, all the pigs were given amino acid non-supplement diet with 0.1% chromic oxide, and feces were collected on the last day of the experiment. The digestibility of gross energy was determined by the chromic oxide indicator method (Morimoto, 1971).

Threonine, Tryptophan, Isoleucine and Valine Requirements (Experiment 2)

Sixteen growing pigs with initial body weight of 22 kg were allocated to four groups of four pigs and the experiment was conducted for 9 days. In the first period, all 16 pigs were given a diet formulated by adding 0.5% L-lysine HCl, 0.2% L-threonine, 0.05% L-tryptophan, 0.1% L-isoleucine and 0.1% L-valine to the same basal diet used in Experiment 1 for 5 days. Blood sample was collected on day 3 to 5. In the second period, each groups of four pigs were given one of the four diets (0.1% threonine supplemented diet, diet lacking tryptophan, diet lacking isoleucine, diet lacking valine) for five days (Table 6-3). Blood samples

Table 6-2. Amount of amino acids added to the basal diet supplemented with 0.05% L-tryptophan, growth performance and plasma urea nitrogen (PUN) in each period in experiment 1

	Period 1	Period 2			Period 3	Period 4
		Lacking amino acid in mixture				
		Lysine	Threonine	Methionine		
No. of pigs	18	6	6	6	18	18
Added amino acids, %						
L-lysine	− (0.47) **	− (0.47)	0.4 (0.87)	0.4 (0.87)	0.4 (0.87)	− (0.47)
L-threonine	− (0.47)	0.1 (0.57)	− (0.47)	0.1 (0.57)	0.1 (0.57)	− (0.47)
DL-methionine	− (0.21)	0.1 (0.31)	0.1 (0.31)	− (0.21)	0.1 (0.31)	− (0.21)
Daily gain, g*	479^{a}	563^{ab}	688^{abc}	750^{bc}	799^{c}	569^{ab}
Daily feed intake, g	1643	1716	1690	1711	1831	1799
Feed conversion ratio	3.43	3.05	2.46	2.28	2.29	3.16
PUN, mg/100ml*	9.79^{ab}	10.82^{a}	7.48^{b}	2.78^{c}	2.88^{c}	9.02^{ab}

* Means in the same line not followed by the same letter are significantly different ($P<0.05$).

** The number in parenthesis indicates the total amino acid content.

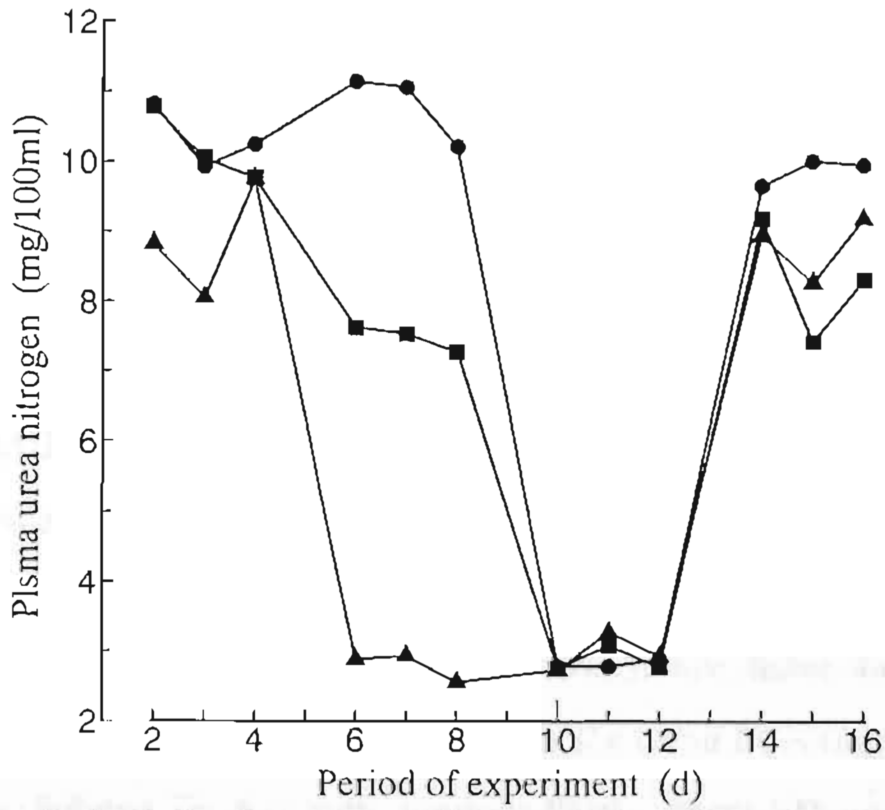

Fig. 6–1. Daily plasma urea nitrogen of pigs given a tryptophan supplemented corn–soybean meal basal diet (period 1), which was then supplemented with incomplete mixtures of amio acids (period 2): lacking in lysine (●), threonine (■) and methionine (▲). In period 3, the missing amino acid was added, and, then, the basal diet was given again (period 4) in experiment 1.

were collected on day 3 to 5. Other experimental conditions were the same as those of Experiment 1.

Analytical and Statistical Procedures

Chemical analyses were performed by the same method as that in Chapter V. Statistical significance between treatment means was tested by t-test (Yoshida, 1983).

Results

Threonine and Methionine (Sulfur Amino Acid) Requirements (Experiment 1)

In Experiment 1, PUN response was observed through four periods with the basal diet supplemented with 0.05% tryptophan and with further addition of lysine, threonine or methionine which are liable to be deficient in practical pig diets. Table 6-2 shows the mean PUN (mg/100 ml) in each period and Fig. 6-1 indicates daily PUN changes. The mean PUN was 9.79 in Period 1 when pigs were given the diet added neither of the three amino acids. However, PUN response was different depending on lacking amino acid in Period 2 when diets were changed to that lacking either of the three amino acids to be added. For the diet lacking lysine PUN was 10.82, almost similar to that in Period 1. However, PUN markedly reduced to 2.78 for the diet lacking methionine and to 7.48 for the diet lacking threonine, showing significant difference ($p<0.05$) between treatments. In Period 3 for the diet with addition of all three amino acids, PUN was 2.88, similar level to that for the diet lacking methionine in Period 2. In Period 4 for the diet with addition of neither amino acid, PUN was as high as that in Period 1. These results confirmed that lysine and threonine were the first and second limiting amino acids in the basal diet supplemented with tryptophan.

PUN responded very rapidly to the change of diet. An almost perfect equilibrium was reached in 2 days after the change of diet as shown in Fig. 6-1. This result is in agreement with that previously reported (Chapter V; Kaji and Furuya, 1987a).

Table 6-2 also shows growth performance of pigs. Daily body weight gain (DG) and feed conversion ratio (FC) changed practically corresponding to the change in PUN, though the results are not more than reference data because of the short experimental period of four days each. DG and FC in Periods 1 and 4 for the diet without amino acid addition were significantly lower than those in Period 3 for the diet added the complete mixture of amino acids. Growth performance for the diet lacking methionine were similar to that for the diet with all amino acids in Period 3. The growth performance for the diet lacking lysine was similar to those for the diet with no amino acids added (Periods 1 and 4).

Table 6–3. Changes in plasma urea nitrogen (PUN) when incomplete mixtures of amino acids were added to the basal diet in experiment 2

		Lacking amino acid in mixture		
	0.1% Threonine	Tryptophan	Isoleucine	Valine
No. of pigs	4	4	4	4
Added amino acids, %				
L–lysine	0.4	0.4	0.4	0.4
L–threonine	0.1	0.2	0.2	0.2
L–tryptophan	0.05	—	0.05	0.05
L–isoleucine	0.1	0.1	—	0.1
L–valine	0.1	0.1	0.1	—
PUN, mg/100ml				
Before (period 1)*	3.82	3.06**	2.51	2.73
After (period 2)	5.09	4.71	3.43	3.89

* The basal diet supplemented with 0.5% L–lysine HCl (0.4% free base), 0.2% L–threonine, 0.05% L–tryptophan, 0.1% L–isoleucine, 0.1% L–valine was given in period 1.

** $P<0.05$: between means in the same row.

Threonine, Tryptophan, Isoleucine and Valine Requirements (Experiment 2)

The results of Experiment 2 are shown in Table 6-3. The pigs were given the lysine and threonine supplemented basal diet with tryptophan, isoleucine, valine and threonine further added in Period 1. PUN in this period was 3.03 in the mean of the four groups. PUN in Period 2, when the diet lacking either tryptophan, isoleucine or valine or with addition of threonine reduced to 0.1% was given, tended to increase from the level in Period 1, but the difference was very slight. For tryptophan, among others, a significant difference ($p<0.05$) in PUN was observed between results with and without it.

Discussion

We have studied the requirement of lysine, the first limiting amino acid in most pig diets (Chapter I to IV; Kaji and Furuya, 1987a; Furuya and Kaji, 1985; Kaji and Furuya, 1987b; Furuya and Kaji, 1987; Kaji and Furuya, 1987c) and demonstrated that although reported lysine requirements expressed as a percentage of the diet considerably differ, the lysine requirements expressed as grams per kg of body weight gain (g/kg BWG) shows almost similar estimates (Fig. 1-1 and Fig 1-2). The lysine requirements are 19.6 g/kg BWG for total lysine (Chapter I; Kaji and Furuya, 1987b) and 17.3 g/kg for digestible lysine (Chapter IV; Furuya and Kaji, 1987). In the present study, we estimated the requirements of threonine, methionine (sulfur amino acid), tryptophan, isoleucine and valine.

There are some discrepancies in the requirements of other amino acids than lysine between feeding standards. The JFS (1975), for instance, recommends higher methionine (sulfur amino acid) and lower threonine requirements. Table 6-4 shows the requirements of individual essential amino acids estimated from 0.86%, the mean lysine requirement estimated in Chapter I, and on the basis of the amino acid pattern in the ideal protein proposed by the ARC (1981). The table also shows amino acid requirements recommended by the JFS (1975) and the NRC (1979). Comparison of these estimated amino acid requirements with the amino acid compositions in the basal diet and the supplemented basal diet used in Experiments 1 and 2 (Table 6-4) showed that 0.47% lysine in the basal diet was much lower than 0.86%, the requirement estimated previously (Chapter I; Kaji and Furuya, 1987a), or 0.74% and 0.70%, the lysine requirements of growing pigs weighing 20 to 30 kg recommended by the JFS (1975) and the NRC (1979), respectively. The level of sulfur amino acid in the basal diet was 0.47%, which was lower than the JFS requirement, 0.54%, but exceeded the requirement estimated ,0.43%, from lysine requirement (Chapter I; Kaji and Furuya, 1987a) and that recommended by the NRC (1979), 0.45%. For threonine, the level in the basal diet, 0.47%, did not reach the estimate ,0.51%, based on the lysine requirement, but satisfied the requirements, 0.45%, recommended by the two standards (JFS, 1975; NRC, 1979). Other essential amino acids are in the basal diet

Table 6–4. Amino acid compositions of diets used in experiments 1 and 2 and amino acid requirements estimated and recommended in Japanese Feeding Standard and NRC (%)

	Amino acid compositions		Amino acid requirements		
	Basal diet	Supplemented basal diet*	Estimated**	JFS***	NRC****
Arginine	0.67	0.67	—	0.20	0.20
Histidine	0.30	0.30	—	0.18	0.18
Isoleucine	0.50	0.60	0.47	0.50	0.50
Leucine	1.21	1.21	0.86	0.60	0.60
Lysine	0.47	0.87	0.86	0.74	0.70
Methionine+cystine	0.47	0.57	0.43	0.54	0.45
Phenylalanine+tyrosine	1.08	1.08	0.83	0.50	0.70
Threonine	0.47	0.67	0.52	0.45	0.45
Tryptophan	0.10	0.15	0.13	0.13	0.12
Valine	0.62	0.72	0.60	0.50	0.50

* Amino acid composition when the basal diet is supplemented with 0.5% L–lysine HCl (0.4% free base), 0.1% L–threonine, 0.1% DL–methionine, 0.05% L–tryptophan, 0.1% L–isoleucine, and 0.1% L–valine.

** Lysine requirement was determined in the study of chapter I and the requirements of the other amino acids were based on the recommended balance of essential amino acids in ARC (1981).

*** Japanese Feeding Standard for Swine (1975) for pigs weighing 20–35 kg.

**** NRC (1979) for pigs weighing 20–35 kg.

supplemented with 0.05% tryptophan were sufficient, though the margins of isoleucine and valine were small. The levels of arginine, histidine, leucine and Phenylalanine + tyrosine were much higher than the requirements recommended by the two feeding standards (JFS, 1975; NRC, 1979).

On the basis of above amino acid composition, PUN response was observed of pigs given the basal diet supplemented with 0.05% tryptophan with further addition of lysine, methionine and threonine. The results showed that the basal diet was apparently deficient in lysine (Table 6-2 and Fig. 6-1) and that addition of lysine to satisfy the requirement caused deficiency of threonine. This indicated that 0.47% threonine is insufficient in the diet with 0.87% lysine, . It is not clear whether or not the threonine requirement was satisfied in the diet with further addition of 0.1% threonine, 0.57% dietary threonine, because of no further addition of L-threonine in Experiment 1. However, the ratio of the threonine level, 0.57%, to the lysine requirement, 0.86%, is 66%, which is higher than the ratio, 60%, in the ideal protein proposed by the ARC (1981) or recommendations by the JFS(1975) and the NRC (1979) (61% and 64%, respectively),suggesting that the threonine requirement is in the range from 0.47 to 0.57%. This was further studied in Experiment 2. The threonine requirements recommended by the JFS (1975) and the NRC (1979) is rather low, but the ratio of threonine to lysine ratio is not so different from that of the ARC (1981) because the lysine requirements, too, are low in the former two standards.

PUN scarcely responded to the addition of methionine when the basal diet was supplemented with lysine and threonine, suggesting that 0.47% methionine content in the basal diet is sufficient. This means that the ratio of sulfur amino acid to lysine will not exceed 54%, in good agreement with the recommendation by the ARC (1981), 50%. The ratios of sulfur amino acid to lysine recommended by the JFS (1975) and the NRC (1979), on the other hand, are 73% and 64%, respectively. Such high ratio recommended by the JFS (1975) may be partly explained by the ground that the daily body weight gain of the growing pigs weighing 20 to 35 kg is estimated as low as 400 to 550 g by the standard and that, therefore, priority is placed on sulfur amino acid which is important for maintenance (Mitchell, 1950) rather than lysine, which is important particularly for the lean gain. Under such feeding conditions that daily body weight gain is as high as about 800 g (Table 6-2, Period 3 of Experiment 1), as in this study, and with priority placed on the lean gain, it is necessary to raise lysine level and, correspondingly, threonine level from the recommendation by the JFS (1975). The sulfur amino acid requirement, on the contrary, can be considerably reduced from the recommendation by the standard.

In Experiment 2, PUN response to the increased addition of threonine from 0.1% to 0.2% to the basal diet supplemented with 0.4% lysine and to the addition of 0.05% tryptophan, 0.1% isoleucine and 0.1% valine was investigated. A significant difference was shown only by the addition of tryptophan. This result suggests that isoleucine and valine levels in the basal diet, 0.50% and 0.62%, respectively, satisfy their requirements. These values are practically equal to their requirements estimated based on the amino acid pattern in the ideal protein, 0.47% and 0.60%, respectively (Table 6-4). The tryptophan level in the basal diet, 0.10%, seemed deficient and the requirement was estimated to be in the range from 0.10 to 0.15%. This result coincides the tryptophan requirement estimated from the amino acid pattern in the ideal protein (Table 6-4), 0.13%. In Experiment 2, the addition of threonine was increased to 0.2% because PUN significantly responded to the addition of 0.1% threonine, but no further significant response was observed. The threonine requirement, therefore, was estimated to be in the range from 0.47 to 0.57%. The threonine requirement estimated from the amino acid pattern in the ideal protein proposed by the ARC (1981), 0.51%, is the approximate median in the range.

It is concluded from the results of this study that the amino acid pattern in the ideal protein proposed by the ARC is reasonable so far as the six essential amino acids investigated here are concerned, provided that animals are fed under conditions comparatively close to practical *ad libitum* group feeding conditions. A different amino acid pattern may be required under different conditions.

VII. Effects of Amino Acid Supplementation of Corn-Soybean Meal Diet and Threonine Requirement of Growing Pigs.

Abstract

Four experiments were conducted 1) to measure the response of lysine, threonine, methionine, isoleucine and valine supplementation of a L-tryptophan 0.05% supplemented corn-soybean meal basal diet, and 2) to estimate the threonine requirement for growing pigs kept under practical *ad libitum* group feeding conditions. Daily gain (DG) and feed conversion ratio (FC) were used as response criteria. 1) In experiment 1, involving 24 pigs weighing approximately 25 kg, we measured growth response to the addition of 0.5% L-lysine HCl (0.4% free base), 0.1% L-threonine and 0.1% DL-methionine to the basal diet, which contained 0.44% lysine, 0.39% threonine, 0.49% sulfur amino acid (methionine + cystine), 0.15% tryptophan and 10.7% crude protein (CP). DG and FC were 762 g and 2.5, respectively, when the mixture of three amino acids was added, while DG was decreased to 524 g and FC increased to 3.2 by adding mixture devoid of lysine. A mixture lacking methionine drew no response, 738 g and 2.6, respectively, while a mixture without threonine was intermediate, 619 g and 2.8, respectively. 2) In experiments 2 and 3, we determined the threonine requirement for 20 pigs weighing approximately 24 kg each. We measured growth response to the addition of L-threonine, 0, 0.05, 0.1 and 0.15% for experiment 2 and 0, 0.1, 0.15 and 0.2% for experiment 3, to the basal diet supplemented with 0.5% L-lysine HCl. DG and FC changed linearly as the addition of L-threonine was increased up to 0.1 to 0.15%. Further increases in the addition of L-threonine had no effect. Using DG and FC as the response criteria, the calculated requirements for threonine were 0.51% and 0.49%, respectively. 3) In experiment 4, involving 16 pigs weighing approximately 29 kg, we measured growth response to the addition of 0.1% L-isoleucine and 0.1% L-valine to the 0.5% L-lysine HCl and 0.15% L-threonine supplemented basal diet, which contained 0.44% isoleucine and 0.54% valine. There was no response to the addition of isoleucine and valine. 4) The results of these experiments suggest that lysine and threonine are the first and second limiting amino acids in a 10.7% CP, tryptophan supplemented corn-soybean meal diet, which is not limited in sulfur amino acid, isoleucine and valine.

Introduction

A number of studies have been conducted on the lysine requirement in pigs, because lysine is the first limiting amino acid in most pig diets, but there are not so many reports of the requirement for the other essential amino acids. Threonine is one of the amino acids which are liable to be insufficient next to lysine. We estimated the threonine requirement by using plasma urea nitrogen (PUN) as a criterion and obtained the ratio of threonine to lysine of 57 to 63% (Chapter VI; Kaji and Furuya, 1987a). These values are almost coincident with the ratio, 60%, recommended by the Agricultural Research Council (ARC, 1981).

The present study was carried out to investigate the effects of adding some essential amino acids to a corn-soybean meal diet and to estimate threonine requirements by growth performance and compare them with that by PUN (Chapter VI; Kaji and Furuya, 1987a).

Materials and Methods

Supplementation of Lysine, Threonine and Methionine to a Low Protein Diet (Experiment 1)

Involving 24 pigs with initial weight of about 25 kg on an average, a feeding experiment was conducted for three weeks. The pigs were divided into four groups of six pigs and housed in concrete-floored pens with a room temperature of 23 ± 1 ℃.

A basal diet was based on corn and soybean meal (Table 7-1). One experimental diet was formulated by adding 0.4% L-lysine (0.5% as L-lysine HCl), 0.1% L-threonine, 0.1% DL-methionine and 0.05% L-tryptophan to the basal diet, and the other three diets were formulated by adding a mixture of amino acids lacking either lysine,

Table 7-1. Composition of the basal diet used in the present study

Ingredient (%)	
Yellow corn	87.0
Soybean meal	10.0
Tricalcium phosphate	0.8
Calcium carbonate	1.2
Sodium chloride	0.5
Vitamin and mineral mixture*	0.5
Chemical composition (by analysis)	
Dry matter, %	87.2
Crude protein, %	10.7
Gross energy, cal/g	3810
Digestible energy, cal/g	3130
Amino acid composition (by analysis, %)	
Arginine	0.63
Histidine	0.27
Isoleucine	0.44
Leucine	1.08
Lysine	0.44
Methionine	0.25
Cystine	0.24
Phenylalanine	0.51
Tyrosine	0.42
Threonine	0.39
Tryptophan	0.10
Valine	0.54

* Providing (per kg diet): retinol, 3 mg; cholecalciferol, 50 μg; DL-α-tocopheryl acetate, 10 mg; thiamin, 2 mg; riboflavin, 14 mg; pyridoxine HCl, 1 mg; pantothenic acid, 21 mg; nicotinic acid, 12 mg; choline, 115 mg; Mn, 100 mg; Zn, 120 mg; Cu, 20 mg;; Fe, 100 mg; I, 2mg.

threonine, or methionine to the basal diet. These four experimental diets were randomly allocated to the above four groups. To make crude protein (CP) level constant, casein was added to diets to the three amino acid lacking diets. This adjustment was made also in Experiments 2 to 4. The lysine level in diet was set to meet the lysine requirement ,0.84% , for pigs weighing 20 to 35 kg, estimated previously by daily body weight gain as a criterion (Chapter I; Kaji and Furuya, 1987b).

Threonine Requirement (Experiments 2 and 3)

In both of Experiments 2 and 3, 20 pigs initially weighing about 24 kg on an average were divided into four groups of five pigs, and the basal diet (Table 7-1) supplemented with 0.4% L-lysine and 0.05% L-tryptophan was used. In Experiment 2, the above diet was supplemented with 0, 0.05, 0.1 or 0.15% L-threonine, and in Experiment 3 with 0, 0.1, 0.15 or 0.20% L-threonine. The other conditions were similar to those in Experiment 1.

In Experiment 3, a digestion experiment was also done to determine digestible energy (DE) of the basal diet. In the last week (3rd week) of the test, the diet with 0.1% chromic oxide was given to pigs, and the digestibility of gross energy was calculated by the chromium oxide indicator method (Morimoto, 1971). DE of the basal diet was calculated, assuming that the digestibility of gross energy of amino acids and casein was 100%.

Supplementation of Isoleucine and Valine (Experiment 4)

The CP level of corn in the basal diet was 7.19%, considerably lower than 8.8% shown in the Standard Tables of Feed Composition in Japan (1987). Accordingly, the measured values of amino acids contained in the basal diet were lower than the calculated values. In particular, the level of isoleucine in the basal diet was as low as 0.44% and was short of the isoleucine requirement, 0.50%, recommended by the Japanese Feeding Standard (JFS, 1975). The level of valine in the basal diet was 0.54%, exceeding the requirement ,0.50%, of the JFS, but it might become insufficient under certain circumstances. Then, in Experiment 4, the effect of supplementation of isoleucine and valine was investigated. Two experimental diets were formulated with or without the addition of 0.1% L-isoleucine and 0.1% L-valine to the basal diet with 0.4% L-lysine, 0.15% L-threonine and 0.05% L-tryptophan. Sixteen pigs initially weighing about 29 kg on an average were divided into two groups and fed the diets with or without L-isoleucine and L-valine. To adjust dietary CP level in the diet, 0.2% casein was added to the diet without isoleucine and valine. By addition of casein, the level of valine in the diet was increased to 0.55%, but the level of isoleucine, 0.44%, remained unchanged. The other conditions were the same as those in Experiment 1.

Analytical and Statistical Procedures

Analyses of water and CP contents in these diets were made by the method based on the AOAC's procedures, (Morimoto, 1971). Gross energy content was measured in an automatic bomb calorimeter (CA-3, Shimadzu Seisakusho Ltd.), and chromic oxide was quantified by the method of Yoshida *et al.* (1967). Amino acids were quantified by a fluorescent derivatization method with o-phthalaldehyde with a high performance liquid chromatograph (Nihon Waters Ltd.). The diets were hydrolyzed with 6N hydrochloric acid at 110 C for 24 h before the quantification of amino acids. Among amino acids tryptophan was analyzed by the method of Ikumo *et al.* (1983) and cystine by the method of Spencer and Wold (1969), but proline was not determined. Statistical significance between treatment means was tested by Tukey's method (Yoshida, 1983).

Results and Discussion

In Experiment 1, the basal diet added with L-tryptophan in 0.05% was further supplemented with lysine, threonine and methionine, which are sometimes insufficient in pig diets, and the effect of their supplementation was investigated. According to the results shown in Table 7-2, all of daily weight gain (DG), daily feed intake (FI) and feed conversion ratio (FC) were markedly inferior in the group given the diet lacking lysine; there was a significant difference ($P<0.05$) in DG

Table 7-2. Amount of amino acids added to the basal diet and growth response of pigs in experiment 1

	Diet				
		Lacking amino acid in mixture			
	Supplemented with all the amino acids	Lysine	Threonine	Methionine	SE*
Added amino acids and casein, %					
L-lysine	0.4 (0.84)**	— (0.47)	0.4 (0.85)	0.4 (0.85)	
L-threonine	0.1 (0.49)	0.1 (0.52)	— (0.39)	0.1 (0.49)	
DL-methionine	0.1 (0.35)	0.1 (0.36)	0.1 (0.35)	— (0.25)	
L-tryptophan	0.05(0.15)	0.05(0.15)	0.05(0.15)	0.05(0.15)	
Casein	—	0.5	0.1	0.1	
Daily gain, g	762[a]	524[b]	619[b]	738[a]	28
Daily feed intake, g	1882	1659	1740	1895	
Feed conversion ratio	2.5	3.2	2.8	2.6	

* Standard error of the mean for six pigs.

** The number in parenthesis indicates the total amino acid content.

[a, b] Means for the daily gain with different superscripts differ ($P<0.05$).

from the groups given the diet with all amino acids and the diet lacking methionine. The next inferior group was the group given the diet lacking threonine; there was a significant difference ($P<0.05$) in DG from the groups given the diets with all amino acids and the diet lacking methionine. On the other hand, DG, FI and FC of the group given the diet lacking methionine was similar to those of the group given the diet with all amino acids.

These results showed that the low protein diet of corn and soybean meal supplemented with 0.05% tryptophan was apparently deficient in lysine, and that when lysine is supplemented to meet the requirement (Kaji and Furuya, 1987b) it became insufficient in threonine. Methionine (sulfur amino acid) requirement was probably satisfied at the methionine level in the basal diet. The level of sulfur amino acid (methionine + cystine) in the basal diet was 0.49% (Table 7-1), lower than the requirement, 0.54%, recommended by the JFS (1975) for pigs weighing 20 to 35 kg, but higher than that by the new JFS (1987) ,0.43%. According to the ARC (1981), the ideal ratio of sulfur amino acid to lysine is 50%. Therefore, as the L-lysine supplemented diet in the present study contained 0.84% lysine, the level of sulfur amino acid was enough at 0.42%. According to the NRC (1988), the requirement of sulfur amino acid for pigs weighing 20 to 50 kg is 0.41%, considerably lower. Judging from the results of the present study and the recently revised standards(ARC, 1981; JFS, 1987; NRC,

1988) the requirement for sulfur amino acid in the old JFS (1975) 0.54%, seemed too high.

Since Experiment 1 showed that the threonine level in the basal diet, 0.39%, was insufficient, the effect of diets supplemented with graded levels of L-threonine on growth performance was investigated in Experiments 2 and 3. According to the results shown in Table 7-3, DG increased with dietary threonine level up to 0.54%, but rather decreased at 0.59% threonine. FC reached a low plateau at 0.49% threonine. Fig. 7-1 shows the relative values of DG and FC at each threonine level to those (100%) at 0.39% threonine. A broken line model (Ohtsuka and Yoshihara, 1975) was fitted to these data and threonine requirements estimated for DG and FC were 0.51% and 0.49%, respectively.

Both of the JFS (1975) and the NRC (1979) recommend the threonine requirement of 0.45% for pigs weighing 20 to 35 kg, lower than the values obtained in the present study. However, the threonine requirements of the new JFS (1987) and NRC (1988) are 0.51% (for pigs weighing 20 to 35 kg) and 0.48% (for pigs weighing 20 to 50 kg), respectively, consistent with the results of the present study. Furthermore, the ARC (1981) proposes that the ideal ratio of threonine to lysine is 60%, and accordingly, if lysine requirement is 0.84%, threonine requirement is calculated to be 0.50%, which is in good

Table 7–3. Effect of amount of threonine added to the basal diet supplemented with 0.4% L–lysine and 0.05% L–tryptophan on the growth response of pigs in experiments 2 and 3

	Threonine level in diet (%)					
	0.39	0.44	0.49	0.54	0.59	SE*
Experiment 2						
Daily gain, g	608^{a}	661^{ab}	719^{ab}	779^{b}	—	41
Daily feed intake, g	1654	1697	1785	1922	—	
Feed conversion ratio	2.7	2.6	2.5	2.5	—	
Experiment 3						
Daily gain, g	564	—	703	719	690	66
Daily feed intake, g	1519	—	1594	1693	1576	
Feed conversion ratio	2.7	—	2.3	2.4	2.3	

* Standard error of the mean for five pigs.

a, b Means for the daily gain with different superscripts differ ($P<0.05$).

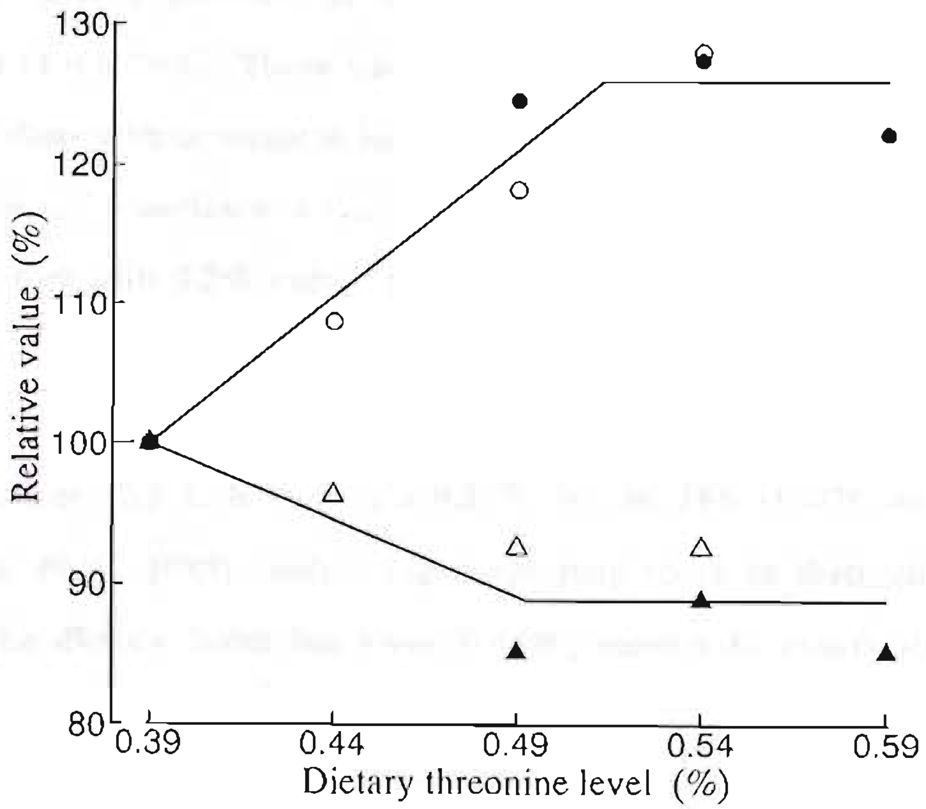

Fig. 7-1. Effects of threinine level in diet on daily gain (○ for experiment 2 and ● for experiment 3) and feed conversion ratio (△ for experiment 2 and ▲ for experiment 3). Each point represents the relative value compared with daily gain or feed conversion ratio for the basal diet containing 0.39 threonine.

agreement with the results of the present study.

In Experiment 4, lysine, threonine and tryptophan were added to the basal diet to meet the requirements for them, and the effect was observed of further addition of isoleucine and valine on growth performance (Table 7–4). There were no difference in DG, FI and FC between the diets with or without isoleucine and valine. This suggests that the levels of isoleucine and valine, 0.44% and 0.55%, respectively, in the basal diet with 0.2% casein satisfy the requirements for these amino acids.

The requirements for isoleucine are 0.47% in the JFS (1987) and 0.46% in the NRC (1988), both being lower than those in their old editions. The dietary isoleucine level, 0.44%, seemed to satisfy its requirement.

The requirement for valine is 0.50% in the JFS (1975) and the NRC (1979). However, in their new editions, it is raised to 0.59% in the JFS, (1987) but lowered to 0.48% in the NRC (1988), partially due to the alteration of weight range from 20 to 35 kg to 20 to 50 kg; there is a difference by about 0.1% between the two standards. In Experiment 4, the valine content was increased from 0.55% to 0.64%, but the increase had no effect on growth. This suggests that the valine requirement in the JFS (1987), 0.59%, may be a little higher. The requirements for all

Table 7–4. **Effect of added 0.1% L–isoleucine and 0.1% L–valine to the basal diet supplemented with 0.4% L–lysine, 0.15% L–threonine and 0.05% L–tryptophan on the growth response of pigs in experiment 4**

	Diet		
	Addition	No addition	SE*
Daily gain, g	746	760	35
Daily feed intake, g	2037	2070	
Feed conversion ratio	2.7	2.7	

* Standard error of the mean for eight pigs.

amino acids except lysine in the JFS (1987) are calculated from the ratio of them to lysine on the basis of the amino acid pattern recommended by the ARC (1981) and the ratio of valine to lysine is 70%. However, the NRC (1988) recommends that the ratio of valine to lysine is 64%, a little lower. Therefore, in case the lysine level is 0.84%, the valine requirement calculated on the basis of the above ratio is 0.54%, being equal to that in the basal diet used here . Russell *et al.* (1987) studied the valine requirement, using the corn-soybean meal diet containing 11% CP, and reported that the basal diet of 0.51% valine could not reach the highest DG. Accordingly, the valine requirement seemed satisfied in the present study but there were little margin.

We estimated the requirements for threonine, sulfur amino acid, tryptophan, isoleucine and valine by using PUN as a criterion (Chapter VI; Kaji and Furuya, 1987a). As a result, it was shown that the threonine requirement was 0.47 to 0.57% and that the requirements for sulfur amino acid, isoleucine and valine were satisfied at the levels in the basal diet, 0.47%, 0.50 and 0.62% respectively. These values are not conflict with the results obtained from the feeding experiment in the present study.

The tryptophan requirement was not investigated in this study, but we estimated it to be in the range between 0.10 to 0.15% by PUN as a criterion (Chapter VI; Kaji and Furuya, 1987a). Recommendations of the JFS (1987) and the NRC (1988) are both 0.12%. Moreover, from the ratio of tryptophan to lysine in the amino acid pattern of the ARC (1981), it is calculated to be 0.12%. These values are well coincident with each other. Accordingly, the tryptophan level in the basal diet used in the present study, 0.1% (Table 7-1), seemed to be insufficient.

From the results of the present study and the previous study (Chapter VI; Kaji and Furuya, 1987a) as well as the amino acid requirements of recently revised feeding standards (JFS, 1987; NRC, 1988), it is concluded that threonine and tryptophan are insufficient next to lysine in the corn-soybean meal diet, but that the requirements for sulfur amino acid and isoleucine are satisfied, even if the CP level is decreased to about 10.7%, which was its content in the basal diet used for the present study. The results of the present study and the value recommended by the NRC (1988) show that the valine requirement was satisfied in the basal diet used for the present study, but according to the JFS, (1987) it was insufficient. Russell *et al.* (1987) have suggested that valine may become the fourth limiting amino acid, and the valine requirement remains to be further studied.

GENERAL DISCUSSION

The amount of digestible lysine required for 1 kg of body weight gain (g/kg BWG) may be regarded as a method to express the lysine requirement that excludes affecting factors such as those due to diets (amino acid digestibility and energy content), environment (cold environment) and animals (growth rate). Shields *et al.* (1983) fed *ad libitum* corn–soybean meal diets formulated to meet minimum nutrient allowances (NRC, 1979) to pigs from 1.5 kg to 145 kg, and determined whole body composition. They reported that fat in the empty body increased from 11.5% of 36 kg body weight to 30.4% of 109 kg body weight but the change was offset against the change of water content so that the protein content is almost invariable from the starting period (6.4 kg) to 109 kg body weight (Fig. 8–1). In corn–soybean meal diets fortified with vitamins and minerals, lysine is the first limiting nutrient (NRC, 1988). Thus, the results of Shields *et al.* (1983) indicated that the content of protein in body weight gain is almost fixed regardless of the body weight if growing pigs under practical conditions are fed lysine according to lysine requirements. This supports the results of the present studies. These facts suggest that the following three assumptions are valid: 1) the lysine requirement for maintenance is negligibly small (Chapter IV); 2) protein synthesis represents the only need for dietary lysine (Chung and Baker, 1992); and 3) utilization efficiency of lysine after digestion and absorption is almost constant

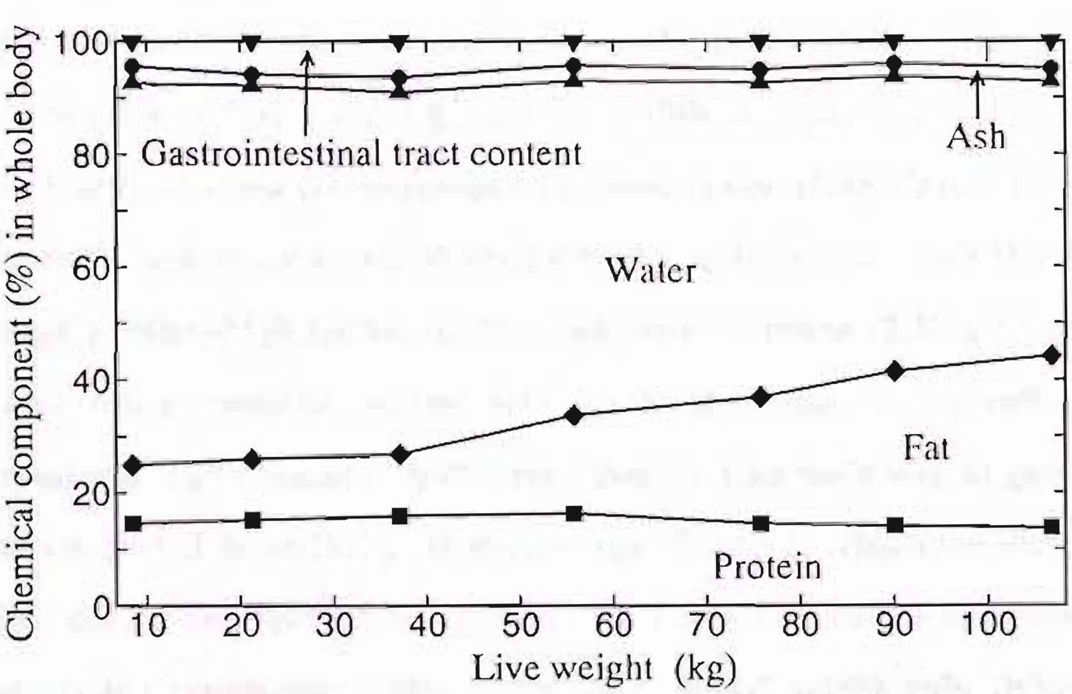

Fig. 8–1. Percentage composition of the whole body of growing pigs *ad libitum* fed corn–soybean meal diets formulated to meet minimum nutrient allowances (Shields et al., 1983).

after digestion and absorption is almost fixed regardless of the bogy weight of growing pigs.

Noblet *et al.* (1987) gave three different diets composed of corn and soybean meal at 120 g of feed/kg metabolic body weight (80 to 100% of *ad libitum* intake) to pigs weighing about 20 kg for seven weeks. Two diets (low protein–low lysine, or pl; and low protein–high lysine, or pL) supplied less protein than recommended by the INRA (1984). Diet pl was inadequate in lysine (2.00 g/Mcal DE) while diet pL was fortified with L–lysine HCl and DL–tryptophan to meet lysine (2.35 g/Mcal DE; INRA, 1984) and other essential amino acid requirements. The third diet (high protein–high lysine, or PL) had protein, lysine (2.53 g/Mcal DE) and other essential amino acid levels that met or exceeded requirements. The amount of lysine required for 1 kg body weight gain has been reported to be 19.7 g for each of the pL and PL (both the diets satisfied the lysine requirement) groups. This result corresponds to the average lysine requirement grams per kg of body weight gain (g/kg BWG) of growing pigs of 19.6 g shown in Chapter I. Since pigs of the pl group were poor in daily gain and daily muscle gain while they showed high daily adipose tissues gain, the amount of lysine g/kg BWG was significantly small as 18.0 g. However, the amount of lysine per unit of protein deposited (120 g/kg protein deposited) was independent of protein and lysine levels in the diet. The results of experiments by Noblet *et al.* (1987) show that the required amount of lysine (lysine requirement in the case of maximum growth) is proportional to the amount of protein deposited and thus show that the above assumptions are valid.

The lysine requirement g/kg BWG is an expression method on the assumption that the lysine requirement (g) is proportional to body weight gain, but does not directly show the relationship with the amount of protein accretion. According to the factorial method in which amino acid requirements are shown as the sum of the requirements for maintenance and for protein accretion, the relationship between the amount of protein deposited and amino acid requirements can be expressed directly. As to growing pigs, Fuller *et al.* (1989) for the first time conducted experiments to simultaneously measure the requirements for maintenance and those for the protein accretion. Since they conducted experiments using casein and crystalline amino acids as the sole protein source, the requirements obtained as the results were shown as the requirements for digestible amino acids. The requirement for digestible lysine (g/d) was calculated from the method of Fuller *et al.* and from the requirement of digestible lysine g/kg BWG, 17.3, and the resulting values were compared (Table 8–1). The values on which the calculation was based were quoted from the table of requirements of the NRC (1988) which had no relation with either of the methods. According to the NRC, growing pigs (5 to 110 kg) are divided into four stages based on the live weight. An average live weight of each of the live weight periods was calculated and the values of body weight gain shown in Tables 8–1 and 8–2 were quoted as from the NRC's table. The amount of protein deposited (g/d) was calculated by multiplying the body weight gain by 14.5% which was an average value of the protein content of the live weight of pigs weighing 6.4 to 109 kg reported by Shields *et al.* (1983). The following calculation was made using these as initial values. From the report of Fuller *et al.*(1989), the requirement for digestible lysine for maintenance (g/d) was calculated using 36 (mg/kg body weight$^{0.75}$ per d) and that for protein accretion (g/d) was calculated using 68 (mg/g body protein). The requirement for digestible lysine (g/d) according to the method of Fuller *et al.* was calculated as the sum of the two values. On the other hand, the requirement for digestible lysine (g/d) according to the present method was calculated by multiplying 17.3 (g/kg BWG) by body weight gain. When the thus obtained values were compared, the values obtained according to the method of Fuller *et al.* were apparently smaller and corresponded to 61 to 64% of the requirement of digestible lysine according to the present method. When total lysine requirement (g/d) was calculated from the digestible lysine requirement by the present method assuming the lysine digestibility to be 85%, the resulting values fully coincided with those of the NRC. Therefore, the requirement of digestible lysine for protein accretion of 68 (mg/g body protein) reported by Fuller *et al.* is considered to be too low. Provided that the average value of lysine content in body protein of 6.4% obtained from the values shown in literature (7.1%, Zhang *et al.*, 1986; 5.9%, Moughan and Smith, 1987; 6.5%, Campbell *et al.*, 1988; 6.4%, Batterham *et al.*, 1990a; 6.5%, Batterham *et al.*, 1990b; and 6.2%, Chung and Baker, 1992) is equal to the lysine content in the deposited protein, the accretion efficiency of digestible lysine of 68 (mg/g body protein) is 94%. Batterham *et al.* (1990a) measured the amount of lysine accretion by feeding pigs weighing 20 kg with eight diets containing 0.1 to 0.8 g lysine/MJ digestible energy in which lysine was the first limiting amino acid using soybean meal as the sole source of lysine. The diets were given to the pigs at three times maintenance and they were slaughtered when their body weight reached 45 kg. As the result of analyzing the relationship of lysine accretion (g/d) against digestible lysine intake (g/d), it was found that the relationship was linear until the values reach a plateau with the slope of 0.86, that is, the accretion efficiency of digestible lysine was 86%. This also suggests that the requirement of digestible lysine of 68 (mg/g body protein) is too low. Fuller *et al.* compared the amino acid requirements for protein accretion estimated for growing rats using the similar method by Dreyer (1975) with those of their own. They reported that though the estimated values of Dreyer showed some general similarities as the results obtained by Fuller *et al.*, remarkable difference was observed in the lysine requirement (122 mg/g body protein) and no obvious explanation on such discrepancy could be found. Thus, re-calculation was tried of the results shown in Table 8-1 using the lysine requirement for protein accretion of 122 (mg/g body protein). The results are shown in Table 8-2. The values obtained by calculation according to the factorial method and those according to the present method almost coincided with each other. The factorial method is regarded as an ideal method because it can directly show the

Table 8-1. Comparison of digestible lysine requirements estimated by a factorial method of Fuller *et al.* (1989) and the method of the present study and the total lysine requirements recommended by NRC.

Live weight	5–10	10–20	20–50	50–110
A) Mean body weight, kg	7.5	15	35	80
B) Body weight gain, kg/d	0.25	0.45	0.70	0.82
C) Body protein accretion, g/d	36.3	65.3	102	119
D) Requirement for maintenance ,g/d	0.16	0.27	0.52	0.96
E) Requirement for body protein accretion ,g/d (by the equation of Fuller et al.)	2.5	4.4	6.9	8.1
D + E	2.6	4.7	7.4	9.0
F) Requirement for maintenance and body protein accretion, g/d (by the method of this study)	4.3	7.8	12.1	14.2
(D + E) / F	0.61	0.61	0.61	0.64
G) Total lysine requirement estimated from F, g/d	5.1	9.2	14.2	16.7
Total lysine requirement recommended by NRC (1988), g/d	5.3	9.0	14.3	18.7

A)Mean body weight for each stage of live weight. B)Expected body weight gain given by NRC. C) = B × 0.145 × 1000(Assuming that 14.5 % of body weight gain is protein accretion). D) = 36 × $A^{0.75}$ ÷ 1000 (36 mg/kg $W^{0.75}$/d). E) = 68 × C ÷ 1000 (68 mg／g body protein accretion). F) = 17.3 × B (Digestible lysine requirement of 17.3 g/kg body weight gain). G) = F ÷ 0.85 (Assuming that the lysine digestibility is 85 %).

Table 8-2. Comparison of digestible lysine requirements estimated by a factorial method of Dreyer (1975) and the method of the present study and the total lysine requirements recommended by NRC.

Live weight	5–10	10–20	20–50	50–110
A) Mean body weight, kg	7.5	15	35	80
B) Body weight gain, kg/d	0.25	0.45	0.70	0.82
C) Body protein accretion, g/d	36.3	65.3	102	119
D) Requirement for maintenance ,g/d	0.16	0.27	0.52	0.96
E) Requirement for body protein accretion ,g/d (by the equation of Dreyer)	4.4	8.0	12.4	14.5
D + E	4.6	8.2	12.9	15.5
F) Requirement for maintenance and body protein accretion, g/d (by the method of this study)	4.3	7.8	12.1	14.2
(D + E) / F	1.06	1.06	1.07	1.09
G) Total lysine requirement estimated from F, g/d	5.1	9.2	14.2	16.7
Total lysine requirement recommended by NRC (1988), g/d	5.3	9.0	14.3	18.7

A)Mean body weight for each stage of live weight. B)Expected body weight gain given by NRC.C) = B × 0.145 × 1000(Assuming that 14.5 % of body weight gain is protein accretion). D) = $36 \times A^{0.75} \div 1000$ (36 mg/kg $W^{0.75}$/d) . E) = 122 × C ÷ 1000 (122 mg／g body protein accretion). F) = 17.3 × B (Digestible lysine requirement of 17.3 g/kg body weight gain). G) = F ÷ 0.85 (Assuming that the lysine digestibility is 85%).

relationship between the amount of protein accretion and amino acid requirements. However, in growing pigs, accretion efficiency or the amount required for protein accretion have not so far been fully clarified with regard to lysine which is the most important amino acid and, therefore, this method has not yet been established as a practical method for expressing amino acid requirements. According to the values of Fuller *et al.*, the amounts of digestible lysine required for maintenance are 0.16, 0.27, 0.52 and 0.96 g/d respectively for the body weight of 5 to 10, 10 to 20, 20 to 50 and 50 to 110 kg, and the proportions of the maintenance requirement to digestible lysine requirements (g/d) calculated by the present method are very small and 3.7, 3.5, 4.3 and 6.8% respectively. It is considered that 93.2 to 96.5% of digestible lysine are used for the protein accretion.

Fig. 8-2 shows the ideal amino acid patterns (Fuller *et al.*, 1989) for maintenance and for protein accretion calculated as proportions of essential amino acids to lysine (100). The most characteristic feature is that the proportions of threonine and sulfur amino acid for maintenance are higher than those for protein accretion. Chung and Baker (1992) described maintenance as comprising 1) urinary excretion of unmodified amino acids (AA); 2) obligatory use of AA as precursors for other essential body metabolites (*e.g.*, creatine, taurine, glutathione, catecholamines, carnitine); 3) obligatory oxidation of AA; 4) AA lost from gastrointestinal epithelia (desquamated mucosal cells, mucus digestive enzymes); and 5) AA lost from integuments and epidermal

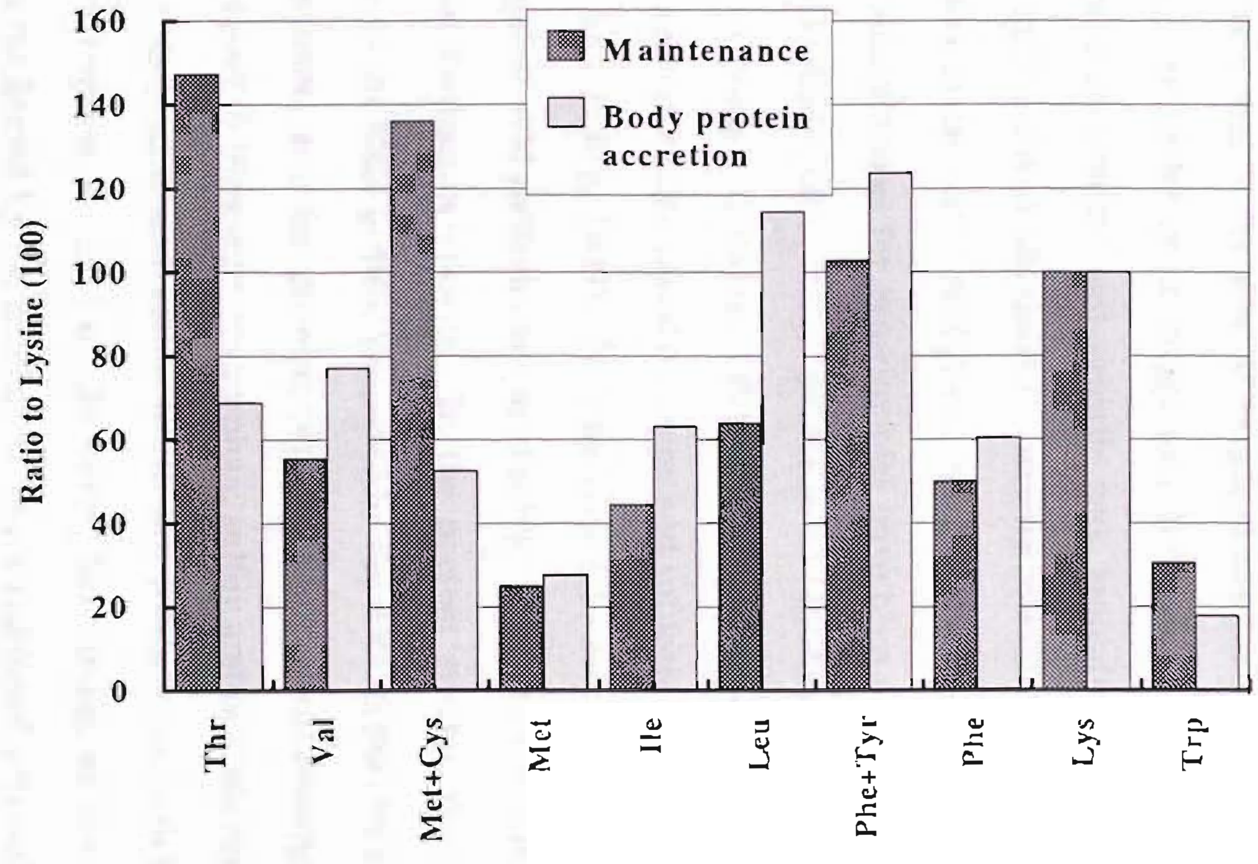

Fig. 8–2. Ideal amino acid pattern of growing pigs for maintenance and for body protein accretion (Fuller *et al.*, 1989).

structures. The results shown in Fig. 8–2 reflect that sulfur amino acid is abundant for items 2, 3 and 5 and threonine is abundant for items 3 and 4. An ideal amino acid pattern is expressed as the ratio of each amino acid that is the sum of required amounts for maintenance and growth. As Fuller *et al.* suggested, difference of amino acid pattern between maintenance and growth may indicate that a certain ideal amino acid pattern obtained is, strictly speaking, applicable only to particular body weight and particular growth rate of an animal. Ideal amino acid patterns for growing pigs have been obtained by estimation from literature (ARC, 1981), quotation of requirements of other animal species (Chung and Baker, 1992) or nitrogen retention obtained when pigs were housed in a metabolic cage and subjected to restricted feeding (Wang and Fuller, 1989). It is necessary to ascertain whether or not these amino acid patterns are applicable to the growth achieved under practical feeding conditions. In the present studies, the amino acid pattern in the ideal protein proposed by the ARC (1981) was revealed to be reasonable also for growing pigs under practical feeding conditions with respect to threonine, tryptophan, sulfur amino acids (methionine + cystine), isoleucine and valine which are liable to be deficient next to lysine (Chapters V and VI). However, the validity of the amino acid pattern confirmed by the present studies is restricted pigs weighing 20 to 40 kg and it is necessary to ascertain the validity for finishing pigs because, for instance, the proportion of the maintenance requirements for threonine and sulfur amino acid are expected to increase with the increase of body weight.

The expression method of amino acid requirements shown by the present studies is applied to practical feed formulation according to the following procedures. First, the expected body weight gain (g/d) is multiplied by 17.3 (g/d BWG) to obtain the requirement for digestible lysine (g/d). Then, the digestibility of lysine in diets is divided by the obtained digestible lysine requirement (g/d) to obtain total lysine requirement (g/d). On the other hand, the energy requirement (kcal/d) is calculated from the body weight, expected body weight gain and environmental temperature referring to the Japan Feeding Standard, which is divided by energy content (kcal/g) of the feed used to obtain feed supply (g/d). The total lysine requirement (%) can be calculated by dividing total lysine requirement (g/d) by feed supply (g/d) and multiply the resulting value by 100. The requirement for each of the other essential amino acids can be obtained by multiplying the total lysine requirement (%) by the ratio of the amino acid concerned to lysine shown in the ideal protein of the ARC (1981). In this manner, whether or not the amino acid requirements are satisfied can be ascertained by comparing the amino acid requirements obtained with amino acid composition of the formulated diet.

To make amino acid requirements obtained by either method of the present studies or a factorial method effective references in practically formulating diets, it is essential that the rate of body weight gain or that of protein accretion be understood in each of the cases. The amino acid requirements may be correctly expressed by excluding the influence of various affecting factors. However, understanding of the ability of growth of pigs is required to apply such knowledge to the formulation of diets. Further, in actually supplying the formulated diet to pigs, what is most important is that pigs ingest the expected amount of diet (nutrient) and show expected growth. In the future, there will be an increased importance to realize the responses of pigs to nutrient not only as independent areas of 1) feed intake (*ad libitum* intake), 2) digestion, absorption and metabolism, 3) ability of growth and 4) adaptation to environment but also from the view point of clarifying their mutual relationships.

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