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Determining the lean growth curve for growing-finishing pigs from the lysine requirements estimated by using plasma urea nitrogen as a rapid response criterion

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Determining the lean growth curve for growing-finishing pigs from the lysine requirements estimated by using plasma urea nitrogen as a rapid response criterion

by

Ruilan Wei

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Animal Nutrition

Major Professor: Dean R. Zimmerman

Iowa State University

Ames, Iowa

2001
This is to certify that the doctoral dissertation of

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TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION 1
Introduction 1
Dissertation Organization 3
Literature Cited 3

CHAPTER 2. LITERATURE REVIEW 4
From Dietary Protein to Body Protein 4
Estimation of Amino Acid Requirements for Growing-Finishing Pigs 10
PUN as a Response Criterion in Estimating Lysine Requirement 16
Protein Accretion Curve over the Growing-Finishing Period 26
Literature Cited 32

CHAPTER 3. LYSINE REQUIREMENTS OF PIC BARROWS DETERMINED BY USING PLASMA UREA NITROGEN AS A RAPID RESPONSE CRITERION 44
Abstract 44
Introduction 45
Materials and Methods 46
Results and Discussion 49
Implications 54
Literature Cited 55

CHAPTER 4. LYSINE REQUIREMENTS OF PIC BARROWS UNDER GROUP PENNED SITUATION 64
Abstract 64
Introduction 65
Materials and Methods 65
Results and Discussion 68
Implications 70
Literature Cited 71

CHAPTER 5. APPLICATION OF A REPEATED LATIN SQUARE DESIGN TO INCREASE THE PRECISION FOR ESTIMATING THE LYSINE REQUIREMENTS OF PIGS BY USING PLASMA UREA NITROGEN AS A RAPID RESPONSE CRITERION 77
Abstract 77
Introduction 78
Materials and Methods 79
Results and Discussion 82
Implications 87
Literature Cited 88
Determining the lean growth curve for growing-finishing pigs from the lysine requirements estimated by using plasma urea nitrogen as a rapid response criterion

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Three experiments (Exp. A, B and C) were conducted to determine the lysine requirements of PIC (327 × C22) barrows over the growing-finishing period, using plasma urea nitrogen (PUN) as a rapid response criterion. In Exp. A, either randomized complete block designs or completely randomized designs were used. Pretreatment PUN was used as a covariate. Results indicated that the true ileal digestible lysine requirements were 16.5 ± 0.2, 18.0 ± 0.5, 20.2 ± 1.5, 16.7 ± 0.8, and 14.4 ± 7.2 g/d/pig at 33, 52, 72, 93, and 113 kg body weight, respectively. The precision of the estimation decreased when pigs reached the finishing period. To increase the precision, three trials in Exp. C were conducted. Repeated 5 x 5 Latin square designs were used and feed intakes were limited to 90% of the ad libitum levels. The estimated true ileal digestible lysine requirements were 19.9 ± 0.57, 17.0 ± 0.47, and 18.1 ± 0.64 g/d/pig at the body weight ranges of 59 to 78, 78 to 95, and 96 to 115 kg, respectively. The last requirement might be overestimated because of a rapid feed consumption during the trial period. Compared with Exp. A, the precision of the lysine requirement estimation increased in Exp. C.

From the lysine requirements obtained in Exp. A and C, the lean growth rates of individually penned PIC barrows were calculated to be 334, 372, 404, 414, 340, 327, and 276
g/d at 33, 52, 69, 72, 87, 93, and 113 kg body weight, respectively. Fitting the data to a polynomial least square regression, a lean growth curve was derived: 

\[ Y = (-0.11606 + 0.04895X - 0.0005855X^2 + 0.000001963X^3) \times 347, \]

where \( Y \) was the lean growth rate in grams/day, \( X \) was the BW in kilograms.

Exp. B determined the lysine requirements of PIC barrows under group penned situation. The results showed that each barrow required 15.8 ± 1.7 and 18.9 ± 1.4 g true ileal digestible lysine/d at 32 and 50 kg body weight, respectively. PUN responses did not allow valid estimates of the lysine requirements for group penned finishing pigs.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

The NRC (1998) has incorporated in its growth model a lean growth curve to calculate the instantaneous lysine requirement of pigs over the growing-finishing period. It also allows the user to provide a unique curve determined with the user's pigs under farm-specific environments. The shape of the default curve is fixed, it simply moves up or down according to the overall lean growth rate input by the user (NRC, 1998). In the face of research evidence that the shape of a lean growth curve differs in response to genetics, sex and environments (Whittemore et al., 1988; Schinckel and de Lange, 1996; Van Lunen and Cole, 1998), a curve determined with the user's pigs would allow more accurate estimates of the nutrient requirements than the default curve provided by NRC (1998).

The classical method to determine a lean growth curve involves serial slaughter with carcass composition analysis (Whittemore et al., 1988; Van Lunen and Cole, 1998). This accurate method, however, is too expensive to be routinely conducted on an adequate number of pigs. An alternative method is the use of serial live weight and ultrasonic measurements. Compared to the serial slaughter method, the ultrasonic measurements are less accurate, therefore, a multivariate double sampling procedure has to be employed (Schinckel, 1994). For many smaller pork producers, a simpler and more economic procedure to determine a lean growth curve is needed (Schinckel et al., 1996).

The NRC (1998) estimates the lysine requirement for growing-finishing pigs via a factorial approach; it assumes that the daily maintenance requirement for true ileal digestible lysine is 36 mg/kg BW\(^{0.75}\), and that each gram of lean growth requires 47 mg true ileal digestible lysine. On the other hand, by using the same assumptions, the lean growth rate can
be calculated from an empirically determined lysine requirement. From a series of lean
growth rates over the growing-finishing period, a lean growth curve can be established.
Therefore, an easy and simple method to determine a lean growth curve can be developed if
there is an easy, inexpensive and accurate method to estimate the lysine requirement.

Plasma urea nitrogen (PUN) has been used as a rapid response criterion to determine
the lysine requirements for swine (Kaji and Furuya, 1987; Coma et al., 1995; Coma et al.,
1996). The rapid response of PUN allows repeated measurements for lysine requirements
over a range of body weights. PUN is easy to measure, the experimental procedure is simple,
and the cost for the experiment is low. The lysine requirement determined from PUN
response was consistent with the requirement from nitrogen balance (Coma et al., 1995).
Therefore, the objective of this research was to develop and demonstrate a technique to
determine the lean growth curve from the lysine requirements determined by using PUN as a
rapid response criterion. The experiment reported in Chapter 3 estimated the lysine
requirements of individually penned PIC (327 × C22) barrows at approximately 30, 50, 70,
90, and 110 kg body weights. The experiment reported in Chapter 4 was conducted to
estimate the lysine requirements of group penned PIC barrows over the growing-finishing
period. The experiment reported in Chapter 5 re-evaluated the lysine requirements of
individually penned barrows at approximately 70, 90 and 110 kg body weights. From the
lysine requirements determined in the experiments reported in Chapters 3 and 5, a lean
growth curve for the individually penned pigs over the growing-finishing period was
developed.
Dissertation Organization

This dissertation contains three papers that have been prepared in the style appropriate for submission to the *Journal of Animal Science*. The papers are preceded by a general introduction and literature review and followed by a general summary.

Literature Cited


CHAPTER 2. LITERATURE REVIEW

From Dietary Protein to Body Protein

*From dietary protein to the free amino acid pool(s)*

Dietary proteins are digested in the digestive tract to oligopeptides and amino acids, and then absorbed by the small intestinal mucosa, where the oligopeptides are hydrolyzed to amino acids. It was first assumed that all dietary amino acids absorbed by the small intestinal mucosa entered the portal circulation intact and were 100% available to the extraintestinal tissues. However, studies over the last two decades, reviewed by Wu (1998), indicate that there is an extensive metabolism of dietary amino acids in the small intestine. Small intestine tissue uses glutamine, glutamate and aspartate, instead of glucose, as its major fuels. Most of dietary glutamine, and almost all glutamate and aspartate are catabolized by the small intestinal mucosa, and CO₂ accounts for 56 to 64% of their metabolized carbons. Dietary amino acids are essential precursors of glutathione, nitric oxide, polyamines and nucleotides, which are obligatory for maintaining intestinal mucosal mass and integrity (Wu, 1998).

It is indicated that the net protein synthesis in the gut increases after a meal, and the rate of protein synthesis depends on the quality of dietary protein. Deutz et al. (1998) demonstrated that, gut protein synthesis in pigs tended to be lower with soy infusion than with casein infusion. In the postabsorptive state, proteins accumulated this way are hydrolyzed and provide free amino acids to other tissues (Waterlow, 1995).

Research by Stoll et al. with milk-fed piglets (1998b) revealed that there was an extensive catabolism of dietary essential amino acids in the small intestine. They found that 35% of lysine, 32% of leucine, 35% of phenylalanine, and 61% of threonine were consumed by the small intestine in their first pass, of those, only about 20% were recovered in mucosal
proteins, and the rest must have been catabolized. Stoll et al. (1998b) pointed out that it was not clear whether the catabolism of the essential amino acids represented a functional requirement of the intestine or if it was driven simply by the local availability of those substrates to the mucosal enterocytes. Nevertheless, the extensive catabolism of dietary essential amino acids by the small intestine decreases the efficiency of using absorbed amino acids for protein synthesis.

Amino acids that have escaped from metabolism in the small intestine enter the liver via the portal vein. While catabolism dominates the amino acid metabolism in the small intestine, hepatic protein synthesis accounts for the majority of the metabolism of the portal amino acids taken up by the liver (Stoll et al., 1998a). In piglets of fed state, hepatic protein synthesis utilized from 48 (threonine) to 90% (lysine) of the hepatic uptake of amino acids. Combining the data of metabolism of absorbed amino acids in the small intestine (Stoll et al., 1998b) and the liver (Stoll et al., 1998a), Stoll et al. (1998a) reported that the post-hepatic availability were 58, 24, 53, and 52% for the absorbed dietary lysine, threonine, leucine, and phenylalanine, respectively.

*Protein synthesis in the body tissues*

The absorbed amino acids, as well as those from body tissue protein degradation, form a pool in the plasma and smaller pools in various tissues. The amino acids in the pools are preferentially utilized for protein synthesis (Benevenga et al., 1993). Gut, liver and muscle are the major organs for protein synthesis, while muscle responds marginally (after gut and liver) with net protein synthesis after feeding (Waterlow, 1995). However, because of its large mass, protein accretion in muscle represents the greatest pool of deposited amino acids in growing-finishing pigs.
The amino acid composition of each cellular protein is specified by its mRNA, and the amount of mRNA determines the rate of protein synthesis. Elevation of the concentrations of free amino acids in the pools, up to a certain threshold level, stimulates the secretion of insulin, growth hormone, and glucocorticoids. The increase in the hormones initiates the transcription of mRNA that then increases protein synthesis. When this threshold level has been reached, however, any further increase has little or no effect on the rate of protein synthesis. The concentrations of free amino acids in the free amino acid pools depend on protein intake, therefore, the responses of protein synthesis versus dietary protein intake can be described as a linear increase and a plateau form. The minimum dietary protein intake corresponding to the protein synthesis plateau is believed to be the protein requirement (Hunt and Groff, 1990).

In addition to dietary protein intake, the quality of dietary protein also has a large impact on protein synthesis. Different dietary proteins initiate different amino acid metabolism in the body because of their different amino acid composition. Deutz et al (1998) infused soy and casein proteins in pigs and found that protein synthesis in the gut and the liver was lower after soy infusion than after casein infusion.

Amino acid catabolism

Animals can not store amino acids in free form. Any amount in excess of their requirements will be oxidized. Amino acid oxidation is the primary mechanism for regulating amino acid concentrations in the free pools (Hunt and Groff, 1990). Amino acid catabolism decreases dietary protein utilization and should be kept as low as possible. In general, amino acid oxidation has the following properties:
1. The oxidation relates inversely to protein synthesis. Gahl et al. (1998) reported that injecting rats with rbST increased protein syntheses in hind leg muscle and liver by 35 and 29%, respectively. At the same time, lysine oxidation decreased by 44%.

2. The oxidation of an amino acid is minimum when the dietary concentration of that amino acid ranges from deficient to the requirement. Beyond the requirement, the oxidation increases sharply and linearly with increased dietary concentration. The relationship can be described as a two-slope, broken-line response, and the dietary amino acid concentration corresponding to the breaking point is the requirement. Under this principle, Kim et al. (1983) estimated the phenylalanine requirement of piglets to be approximately 7 g/kg diet. Lysine requirements of rats (Brookes et al., 1972) and pigs (Chavez and Bayley, 1976) have been determined using the same principle.

3. Essential amino acid deficiency increases amino acid oxidation. The amino acid composition of each body protein is specified by its mRNA. When a diet is deficient in one essential amino acid, the relative excesses of other amino acids are necessarily oxidized. The oxidation decreases with the addition of the limiting amino acid until the requirement for that amino acid is met (Fuller and Garlick, 1994). Therefore, the adequacy of the limiting amino acid dictates the oxidation of other amino acids. Kim et al. (1983) demonstrated that the oxidation of phenylalanine was elevated when dietary histidine was deficient. The oxidation decreased by increasing dietary histidine concentration and reached a minimum when the histidine requirement was met. Harney et al. (1976) also reported that cystine supplementation to a low methionine diet resulted in decreases in oxidation of methionine, valine, and tyrosine and a significant increase in muscle protein synthesis.
4. Inevitable amino acid catabolism

Although in amino acid deficiency the first limiting amino acid is highly conserved for protein synthesis, the oxidation of that amino acid is still going on, which is referred to as obligatory oxidative loss (Fuller and Garlick, 1994). This inevitable catabolism occurs, even though non-protein energy supply is adequate, and dietary amino acids are well balanced and below their requirements (Heger and Frydrych, 1989). The data of Kim et al. (1983), Brookes et al. (1972), and Chavez and Bayley (1976) on amino acid oxidation illustrated oxidation of the amino acids tested, even when they were deficient. Fuller (1980) reported that a proportion of the absorbed first limiting amino acid was degraded. The oxidation of dietary amino acids in the small intestine and liver in their first pass might contribute in part to the inevitable catabolism. Heger and Frydrych (1989) reviewed the inevitable catabolism as an "inevitable consequence" of the operation of mechanisms controlling the degradation of amino acids in the body and an "unavoidable tax which the animal must pay for the ability to respond quickly to quantitative and qualitative changes in the protein supply".

Efficiency of using absorbed amino acid for protein accretion

Inevitable catabolism of absorbed amino acids decreases the efficiency of the first limiting amino acid for protein synthesis (Heger and Frydrych, 1985). There are controversial opinions about the question whether the inevitable catabolism increases with the increase of amino acid intake or not. The data of Heger and Frydrych (1985) and Batterham et al. (1990) indicated that the efficiency of the limiting amino acid decreased as the amino acid intake increased. However, Möhn et al. (2000) pointed out that the efficiencies estimated by Heger and Frydrych (1985) and Batterham et al. (1990) might be confounded by the variation of amino acid requirements among the experiment animals. To avoid that kind of confounding,
Möhn et al. (2000) prescreened pigs first, then used the pigs with similar protein accretion potentials to evaluate the efficiency of absorbed lysine for protein synthesis at dietary lysine concentrations supporting 70 and 90% of the pigs' maximum protein deposition, respectively. They found that the efficiency of using available lysine over and above maintenance was 69%, and the marginal efficiency for protein accretion was 75%. The efficiency was not affected by body weight, energy intake and dietary lysine concentration.

Bikker et al. (1995) also indicated that the efficiency of lysine for nitrogen retention was not affected by body weight. In reviewing the literature data on lysine efficiency for protein synthesis, Susenbeth (1995) drew the conclusion that when lysine was the only limiting factor for protein synthesis, 1 g of lysine intake would lead to 7.5 g of protein accretion. Therefore, 1 g of protein deposition needed 0.13 g (1 ÷ 7.5) lysine intake. Assuming a constant lysine concentration of 7.2% in the protein retained, the efficiency of using lysine for protein synthesis was calculated to be 0.55 (0.072 ÷ 0.13). The efficiency was independent of lysine intake, body weight, breed, sex, and energy intake. Therefore, lysine requirement for a specific protein accretion could be calculated by multiplying the protein accretion rate with 0.13. The NRC (1998), also based on literature data, assumes that for each gram of protein accretion, 0.12 g true ileal digestible lysine is required. Because lysine concentration in the body protein is usually within the range of from 6.5 to 7.5%, the coefficient reflects a marginal efficiency of 54 to 62% of using true ileal digestible lysine for protein accretion.
Estimation of Amino Acid Requirements for Growing-Finishing Pigs

Factorial approaches

The requirement for maintenance. The body proteins are in a dynamic state, being degraded and re-synthesized. The amino acids from the degradation, however, are not 100% re-utilized for protein synthesis. Therefore, there is a continuous loss of endogenous amino acid nitrogen via the urine. Amino acids of body origin are also lost via the digestive tract mucus, enzymes, shed mucosal cells, and from the shedding of skin and hair (Moughan, 1991). The need for dietary amino acids to replace the obligatory amino acid losses, plus the need for the amino acids converted into specialized metabolites (Hahn and Baker, 1995), are referred to as maintenance requirement, the amount of which is related to the metabolic body weight \((W^{0.75})\). Baker et al. (1966a, b, c) directly estimated maintenance requirement of pigs. Fuller et al. (1989) regressed protein accretion rate on daily amino acid intake and calculated the amount of each amino acid needed to maintain nitrogen equilibrium. NRC (1998) took the average of the requirements for each amino acid determined by Baker et al. (1966a, b, c) and Fuller et al. (1989) and expressed the requirement as the percentage of the lysine requirement. The derived amino acid pattern is the ideal pattern for maintenance. Maintenance requirement represents less than 10% of total amino acid requirements in the growing pigs (Fuller et al., 1989) and rats (Benevenga et al., 1994).

The requirement for protein accretion. Wang and Fuller (1989) determined an optimum amino acid pattern for maximum nitrogen retention in the growing pigs. However, because maintenance requirement was not estimated, the application of the pattern was restricted to the pigs with that particular protein accretion rate. To estimate amino acid requirements for maintenance and for protein accretion simultaneously, Fuller et al. (1989)
did regression of daily protein accretion on daily amino acid intake for each essential amino acid and calculated the amount needed to increase 1 g of body protein accretion. NRC (1998) derived its ideal ratios for protein accretion by using the ideal ratios among the essential amino acids determined by Fuller et al. (1989) and making appropriate adjustments in accordance with recent empirically determined amino acid requirements.

The existence of the ideal patterns for both maintenance and protein accretion reduces the task of estimation of requirements for all essential amino acid requirements to the estimation for one essential amino acid, which is lysine in most of the cases, because lysine is the first limiting amino acid in most swine diets.

Models to estimate lysine requirement for growing-finishing pigs. Moughan (1989) developed a computer model to estimate lysine requirement for 50 kg pigs with different protein accretion rates. It partitions the lysine requirement for maintenance into three parts and estimates each part separately: (1) lysine loss from body protein turnover is estimated from protein deposition rate; (2) integumental lysine loss is estimated from metabolic body weight; and (3) gut lysine loss is considered as a function of food dry matter intake. The amount of lysine needed for protein synthesis is estimated based on two assumptions: (1) the efficiency of the absorbed lysine for protein accretion is a curvilinear function of body protein deposition rate, and (2) the upper limit of body protein retention is determined by genetics. Simulated results from the model agree closely with published empirical estimates of lysine requirements (Moughan, 1989).

The NRC (1998) has incorporated in its growth model a protein accretion curve to calculate lysine requirements for pigs over the growing-finishing period. The model requires the user to input the body weight and mean lean growth rate of their pigs. Then, maintenance
requirement is calculated based on the body weight (36 mg true ileal digestible lysine/kg BW^{0.75}/d). The model determines lysine requirement for protein accretion in four steps: (1) dividing the mean lean growth rate by 2.55 to get the mean protein accretion rate; (2) using the protein accretion curve to calculate the protein accretion rate at the specific body weight, expressed as a proportion of the mean protein accretion rate; (3) multiplying the results from step 1 and step 2 to get the protein accretion rate; and (4) calculating the lysine requirement (g/d) by multiplying the protein accretion rate with 0.12. The user has the options of using the default protein accretion curve provided by the NRC (1998) or supplying a unique protein accretion curve determined with the user’s pigs at the farm-specific environment. Because protein accretion rate is influenced by numerous factors such as genetics, sex, and environment, a curve unique to the user’s pigs would allow more accurate estimation of lysine requirements for specific genetics and environment than the default curve. After the lysine requirement is determined, the requirements for the other amino acids are calculated from the ideal patterns of amino acids for maintenance and for protein accretion. The total requirements are the sum of requirements for maintenance and protein accretion.

**Empirical approaches**

The protocol of empirical approaches to determine lysine requirement includes (1) the administration of a range of dietary lysine concentrations; (2) measurement of response(s); and (3) estimation of the requirement by fitting the data into a statistical model.

*The range of lysine concentrations.* Dietary lysine concentration should range from deficient to overabundant, and a range from 50 to 150% of the projected requirement is recommended (Lewis, 1992). To get an objective estimate of the requirement, at least four dietary concentrations are needed to fit the dose-response into a descriptive response curve
(Baker, 1986). The normal route is to formulate a basal diet containing the lowest lysine concentration, and the other nutrients are at adequate supply. The dietary lysine additions are achieved by supplementation with crystalline L-lysine·HCl.

It is generally assumed that crystalline lysine is 100% bioavailable. However, it is shown that free amino acids are generally absorbed more rapidly than those from intact protein sources (Rolls et al., 1972; Baker, 1984; Baker and Izquierdo, 1985), resulting in a decreased efficiency of using lysine for protein synthesis and an overestimation of lysine requirement (Batterham and Murison, 1981). Frequent feeding to ensure a constant flow of digesta for absorption will minimize this effect and increase the lysine availability from crystalline form. The experiments with swine (Batterham, 1974; Batterham and O’Neill, 1978) showed that the utilization of pure lysine in a once-per-day feeding regimen was about 50% of that in a frequent feeding system.

Selection of response variables. The response variables for estimating lysine requirement can be divided into two categories: production traits and metabolic responses; and estimates of the requirement may differ because of different response criteria used (Lewis, 1992). The advantages and disadvantages of each response criterion were discussed by Lewis (1992) and Fuller and Garlick (1994). This literature review will focus on the discussion of using plasma urea nitrogen (PUN) as a response criterion for estimating lysine requirement.

Estimation of lysine requirement from a statistical model. A broken-line model is commonly used to estimate nutrient requirement from dose-response data. The model is based on the assumption that the response increases or decreases linearly to additions of the limiting nutrient and reaches a plateau when the requirement is met. Using the least square
method, the broken-line regression fits dose-response data into two lines with different slopes. The breaking point is the point where the two lines intersect and the dietary lysine corresponding to the breaking point is the requirement (Robbins, 1986).

The main criticism of the broken-line model is that it does not reflect the real response curves. Fuller and Garthwaite (1993) measured nitrogen retention changes, on an individual pig basis, to increasing dietary amino acid supply. They found that curvilinear models described the individual nitrogen retention response significantly better than rectilinear models. Curnow (1973) and Fisher (1973) demonstrated that even if individual members did have an abrupt change and plateau response, a population of animals would exhibit a smooth curvilinear response. Robbins et al. (1979) stated that the population mean response to limiting nutrient supply could be more accurately described by a curvilinear model than a broken-line model.

The curvilinear response of a population has been described by Baker (1986). The response changes with a lesser slope at doses between 0 and 30% of the requirement. The slope of the change becomes maximal and constant when the limiting nutrient concentration is between 30 and 70% of the requirement. At doses from 70 to 100% of the maximum requirement, the response follows the law of diminishing returns: the animals requiring the least get their requirements, as a result, the slope begins to decrease. The slope continues to decrease with increment of doses as more and more animals meet their requirements, and it finally reaches an asymptote at and above 100% of the maximum requirement level (when the requirement is met for all animals in the population).

Motulsky and Ransnas (1987) discussed fitting data to non-linear regression from a practical and nonmathematical review. Several nonlinear models, such as an exponential
model (Robbins et al., 1979), “Reading Model” (Fisher et al., 1973), saturation kinetics models (Mercer et al., 1987) and modified kinetics model (Mercer et al., 1989) have been developed and applied in the estimation for nutrient requirements.

One problem encountered in the curvilinear model is that a requirement estimate is not included in the model. To get the estimate the user has to define “requirement” criterion (e.g., 90 or 95% of maximum response in the curvilinear fit). That kind of choice is arbitrary and different criteria result in different estimates. In contrast, broken-line regression fits broken-lines by the least square method, so it selects the breaking point objectively and the estimate of requirement is part of the output of the model. The estimate represents the requirement of the average animal in the population. Because of its objectivity, the broken-line regression is preferred (Baker, 1986).

Robbins et al. (1979) pointed out that, although a curvilinear model described response more accurately over a wide range of doses than a broken line model, any response approaching an asymptote could be fitted by a broken-line model over a suitable narrow range of doses. In experiments estimating lysine requirement, dietary lysine concentrations usually range from moderate deficient to a degree of over supply. Therefore, the use of the broken-line regression can be justified.

Coma et al. (1995a) fit PUN response of growing pigs versus dietary lysine concentrations into four models: one-slope broken-line, two-slope broken-line, quadratic, and quadratic with plateau, respectively. Using R^2 as the criterion in assessing the fitness of the models, they found that broken-line models fit their data better than the quadratic models, and the two-slope broken line model had a larger R^2 than the one-slope broken-line model.
Therefore, they recommended a two-slope, broken-line model in estimating lysine requirement when PUN was used as a response criterion.

**PUN as a Response Criterion in Estimating Lysine Requirement**

*Urinary urea excretion*

When a diet is deficient in one essential amino acid, the relative excesses of the other amino acids are necessarily deaminated. The deaminated N is used in the liver for synthesis of urea via the urea cycle, and urea is excreted through urine. Rose et al. (1950, 1951) observed a great increase in urinary urea excretion in humans as a result of deficiency of valine or methionine. Urinary urea excretion reached a minimum in pigs when dietary protein was well balanced for amino acids (Fuller et al, 1979).

Kiriyama and Iwao (1967) treated rats with graded dietary concentrations of threonine. They found that increasing threonine decreased urinary urea excretion linearly until the point where the requirement for threonine was met. Brown and Cline (1974) investigated the effect of dietary amino acid deficiency on urinary urea excretion and applied this concept to determine amino acid requirements of swine. They found that an addition of 0.5% L-lysine-HCl to a lysine-limiting diet lowered daily urinary urea nitrogen excretion from 3.96 to 2.88 g. In another experiment they showed that urea excretion decreased with the addition of 0.12, 0.24 and 0.36% of L-lysine and was minimized at the 0.24% supplementation level. Their data suggested that the lysine concentration that minimized urinary excretion was a close estimate of the lysine requirement.

Urine collection requires restraint of the animals. The inconvenience of total urine collection makes it more convenient to measure PUN, rather than urinary urea, as the criterion in assessing lysine requirement of pigs.
Plasma urea concentration or PUN

Protein utilization status can also be reflected in the change of plasma urea concentration. Eggum (1970, 1972) standardized the blood urea technique to ensure that protein quality was the decisive influence on blood urea concentration. He obtained a negative correlation (0.95) between blood urea concentration and biological value of protein in rats. He concluded that blood urea concentration was a rapid and efficient criterion for evaluating protein quality under well-defined feeding conditions. A negative relationship between serum urea nitrogen and protein quality was also found in humans by Taylor et al. (1974), who stated that protein quality estimates derived from serum urea nitrogen were comparable to the values obtained with nitrogen balance. Slagle and Zimmerman (1979) used PUN as a criterion in evaluating a yeast single cell protein in young pigs.

Braude et al. (1974) used changes in plasma amino acids and urea concentration to determine the appropriate lysine supplementation level for a diet consisting of cereals and groundnut meal for growing pigs. They concluded that plasma urea concentration might be of more value in assessing the amino acid requirements and efficiency of protein utilization than plasma amino acid concentrations.

Lewis and Speer (1973) employed plasma urea as one of the criteria to determine the lysine requirement of sows. They formulated a basal diet that met the requirements for all essential nutrients except lysine and used supplementation with L-lysine·HCl to produce five dietary lysine concentrations: 0.25, 0.35, 0.48, 0.66 and 0.91%. Plasma urea concentration decreased as the dietary lysine concentration increased from 0.25 up to 0.66% and then reached a plateau. By fitting the plasma urea response versus dietary lysine concentration to
two separate linear functions, the lysine requirement was estimated to be 0.53%. This value was very close to requirements estimated by the same authors with other criteria.

In assessing the optimum proportion between dietary lysine and tryptophan for growing pigs, Lewis et al. (1981) found that plasma urea reached a minimum at 0.20% of dietary tryptophan concentration when lysine was adequate, beyond that point, plasma urea increased or plateaued. PUN was used as one of the criteria in estimating lysine (Lewis et al., 1981) and methionine (Balogun and Fetuga, 1981) requirements of weanling pigs.

**Variation in PUN**

In contrast to urinary urea estimation, which usually requires a 24 hour (h) collection period, PUN is a measure of the concentration, at a specific time, of a dynamic catabolite. Whether the PUN concentration at a bleeding time is actually representative of urea nitrogen excretion for the whole 24 h period is a very important issue. It is indicated that PUN concentration changes over time after feeding (Kumta and Harper, 1961; Eggum, 1970, 1972). Therefore, even if a representative bleeding time is determined for a group of animals, the difference in eating patterns between individuals still has an influence on PUN variation.

Urea is synthesized in the liver, released into the blood and excreted via urine. Urinary urea excretion requires considerable amounts of water. In the situations where water intake is not limited, each animal usually drinks the amount that is sufficient to eliminate waste salts and urea via the urine (Brooks and Carpenter, 1990). Therefore, ad libitum water intake has little effect on the total urinary urea excretion in a 24 h period. However, it does have an impact on the PUN concentration. Limiting water intake increased PUN in cattle (Utley et al., 1970) and swine (Cai and Zimmerman, 1995). This negative relationship between water intake and PUN concentration implies that PUN concentrations may be confounded by
variation in ad libitum water intake among the experiment animals, especially in the period before bleeding.

In summary, when PUN is used as the response criterion for the estimation of lysine requirement, the random factors (such as eating pattern and water intake) which have little effect on daily urinary urea production, do exert their influences on PUN concentration. Therefore, PUN concentrations would exhibit greater variation than daily urinary urea excretion.

Brown and Cline (1974) measured PUN responses to lysine supplementation along with urinary urea excretion. They observed a linear decrease both in PUN and urinary urea excretion as a result of lysine supplementation, however, PUN did not reach a plateau while urinary urea excretion did. The authors suggested that the discrepancy might be because the blood sample was obtained at a fixed time after feeding, whereas the urine collection was made over a 24 h period. They also speculated that the time of bleeding, which was 5.5 h after feeding, might not be representative of PUN average over a 24 h period. An inspection on their data revealed that PUN variation of one treatment group was unusually high, whereas urea excretion variation of the same group was quite close to other groups. Woerman and Speer (1976) reported that the coefficient of variation (CV) of PUN in pigs was two times as much as the CV of urinary nitrogen excretion. Wilkinson et al. (1982) failed to identify a dietary lysine requirement because of considerable variation in PUN between individual sows. In another of their experiments, they obtained a lysine requirement estimate from PUN response, but the estimate was not consistent with the estimates made by other criteria. They stated that an abnormally high PUN value in one treatment group distorted the statistical analysis and resulted in the imprecise estimation.
Data of PUN measurements among 48 pigs over a 35 day period, by Coma et al. (1995c), can be used as another example to demonstrate the large variation of PUN. At day 1 of the experiment, the mean PUN value was 7.7 ± 1.9, in mg/dl, with the minimum of 4.5 and the maximum of 12.4; At day 20, the value was 9.8 ± 2.7 with a minimum of 5.0 and a maximum of 17.4; At day 35, 10.1 ± 2.8 with a minimum of 5.1 and a maximum of 18.8. The calculated CV from their data were 25, 28 and 28%, respectively, and the maximum concentrations were approximately three times as large as the minimum values. Because of the large variation, a tightly controlled experiment and appropriate strategies to reduce PUN variation are necessary when PUN is used as a response criterion to estimate lysine requirement.

Factors contributing to PUN variation

Dietary factors. There are several dietary factors, in addition to lysine adequacy, influencing PUN concentration. Tightly controlling these factors in an experiment is necessary.

1. Dietary protein and energy concentrations. Eggum (1970, 1972) showed a positive correlation (0.95) between dietary protein concentration and blood urea concentration in rats. In pigs, plasma urea concentration increased with the increase of dietary protein concentration (Chen et al., 1999) and the increase in plasma urea was rapid when the ideal protein concentration was above the requirement (Yen et al., 1986a, b). Therefore, in experiments where PUN is used as a response criterion, it is necessary to equalize the total nitrogen level among all treatment diets (Eggum, 1970, 1972). In estimating lysine requirement, L-glutamic acid supplementation can be used to make treatment diets isonitrogenous.
It is known that N-acetylglutamate, a metabolite synthesized from glutamate and acetyl-CoA, regulates urea synthesis by activating allosterically carbamoyl phosphate synthetase I, which catalyzes the first committed step of the urea cycle. The rate of urea production in the liver is positively correlated with the N-acetylglutamate concentration (Veot and Voet, 1995). Because glutamate is the substrate of N-acetylglutamate, it was speculated that supplementations with different glutamic acid concentrations would cause different urea cycle activities among the treatment groups. However, because almost all of dietary glutamate is catabolized in the small intestine mucosa in the first pass (Wu, 1998), addition of glutamic acid should not interfere with the amino acid metabolism. Hayase et al. (1980) indicated that the concentrations of free amino acids in the liver were the main factors regulating the rate of urea synthesis when changing dietary protein from high quality to low quality. Research by Kumta and Harper (1961) showed that addition of 1% of glutamic acid to the diet did not raise plasma urea concentrations in rats.

Energy deficiency increases the oxidation of amino acid for ATP production and, thereby, increases PUN. Adequate and equalized energy level among treatment groups is very important.

2. Amino acid balance. Amino acid imbalance decreases dietary protein utilization and increases PUN concentration. Wang and Fuller (1989) found improvements in nitrogen retention when certain amounts of tryptophan, lysine, phenylalanine plus tyrosine were removed from the dietary amino acid mixture in the casein protein pattern. The result implied that the imbalance caused by excess of essential amino acids suppressed nitrogen retention. Kumta and Harper (1961) observed that blood urea concentration of rats was greatly elevated by supplementation with a mixture of methionine and phenylalanine, or arginine and
threonine. The rise in blood urea could be prevented by restoration of the amino acid balance with the addition of leucine and isoleucine, or valine and histidine, respectively. Kumta and Harper (1961) speculated that amino acid imbalance increased blood urea concentration via increasing the need for the first limiting amino acid or developing a new deficiency. In one of the experiments by Lewis et al. (1977), further addition of tryptophan beyond the requirement increased PUN. In estimating methionine requirements of weanling pigs, Balogun and Fetuga (1981) found that plasma urea decreased with methionine supplementation and was minimized at 0.55% dietary methionine, after that point, plasma urea increased in such a way that the slope of increase was much sharper than the slope of decrease. The imbalances among amino acids caused by excess methionine might contribute to PUN increase.

3. Electrolyte balance. Nitrogen excretion plays a central role in maintaining acid-base balance of the body (Patience, 1990). When there are excess anions to be excreted in urine, ammonia synthesis in the kidney increases. Strong acids, such as chloride, are excreted as ammonium salts (Duling, 1983). Under practical circumstances, ammoniagenesis is synchronized quantitatively with ureagenesis such that N excretion remains constant (Welbourne et al., 1986). Therefore, dietary electrolyte balance influences urea synthesis and PUN concentration. Cai and Zimmerman (1995) demonstrated that an increase in dietary cation (Na) increased urinary urea excretion. Slage and Zimmerman (1979) suggested that excess anion might be one of the reasons contributing to the low PUN in pigs fed a yeast single-cell protein. Honeyfield et al. (1985) reported that increasing dietary concentration of chloride decreased plasma urea concentration in growing pigs. Because commercial lysine is in the form of L-lysine-HCl, lysine additions change dietary chloride concentrations among
the treatments. When PUN was used as a criterion to determine the lysine requirement for pigs, the result was confounded by different chloride concentrations among the treatments. The confounding effect disappeared when treatment diets were made identical in electrolyte balance (Coma et al., 1995a). An identical electrolyte balance among the treatments can be achieved by partially replacing NaCl with Na₂CO₃, the amount of NaCl to be replaced depends on the amount of chloride added in the form of L-lysine-HCl (Coma et al., 1995a).

Bleeding time. Kumta and Harper (1961) measured plasma urea concentrations of rats at 1, 3, 6 and 8 h after feeding. The rats were fed once a day and trained to consume equal amount of either the basal diet or the diet containing additional methionine and phenylalanine in a 2 h period. They found that the greatest difference between the two treatment groups occurred 3 h after feeding. Eggum (1970) measured plasma urea changes of one pig at hourly interval after feeding, and suggested that 4 to 5 h after feeding would be the most suitable bleeding time.

Cai et al. (1994) compared the PUN changes over a 24 h period between pigs with free access to feed and pigs fed twice daily. A peak of PUN concentration at 4 h after feeding was observed among the pigs fed twice daily, whereas PUN of the pigs with free access to feed exhibited only a slight increase at that time. The authors explained that the slight increase of PUN in pigs with free access to feed might be the result of the influence of the meal-fed pig's feeding behaviors, because all pigs were housed in the same room. Based on the result, they concluded that for pigs with free access to feed, blood sampling at any time reflected average PUN concentrations for the whole 24 h period. For meal-fed pigs, bleeding 4 h after feeding was recommended.
Inherent factors.

1. Feed intake. At the same protein concentration, increasing feed intake elevates PUN because protein intake is increased. Coma et al. (1995c) found a positive correlation (0.50) between ADFI and PUN. Limiting feed intake reduced PUN (Coma, et al. 1995b).

2. Body weight. PUN tends to increase with body weight, probably because of an increase in voluntary feed intake (Coma, et al., 1995c). Chen et al (1999) also showed that plasma urea concentration in barrows increased over the body weight range of from 50 to 110 kg.

3. Lean growth. PUN can indicate the status of protein utilization in the body. Because muscle represents the largest pool of protein deposition, a negative relationship between PUN and lean growth rate would be expected. Gourley and Zimmerman (1993) found that the correlation of PUN and lean percentage were -0.86 and -0.80 in two strains of pigs, respectively. Whang et al. (1994a, b) demonstrated that PUN values were well correlated with rate of lean gain and feed efficiency in PIC pigs. Hahn and Baker (1994) also reported that rate of lean gain was negatively correlated with PUN in PIC pigs from 50 to 95 kg body weight. A strong negative relationship between PUN and lean tissue growth rate was reported by Coma et al. (1995c).

Correction for individual PUN variation not related to amino acid adequacy

The influence of dietary protein concentration, electrolyte balance, and bleeding time on PUN could be reduced to a minimum in a tightly controlled experiment. The body weight influence could be reduced by strict animal selection and blocking. Researchers can limit feed intake to get a more uniform feed intake among the experiment animals to reduce PUN variation caused by different feed intakes among animals. However, some inherent factors
(e.g., lean growth rate and individual eating and drinking pattern) cannot be manipulated by researchers. PUN variation resulting from this kind of factors should be corrected before the effect of lysine adequacy is investigated.

To find appropriate variable(s) to correct individual PUN variation not related to dietary lysine adequacy, Coma et al. (1995c) performed multiple regression of PUN concentration in the dietary treatment period (PUN1) to the pretreatment PUN (PUN0), daily carcass fat deposition, urine specific gravity, average daily feed intake, daily carcass lean gain, and serum creatinine concentration. Even though significant correlation was found between PUN1 and the first four variables, PUN1 was most closely related to PUN0, which explained more than 70% of the difference in PUN1. Adding all other variables only improved $R^2$ a little. They concluded that PUN0 could be used as a covariate to efficiently correct PUN1 in assessing lysine requirement of pigs.

**PUN as a rapid response criterion in estimating lysine requirement**

The change of amino acid metabolism to dietary supplies is a rapid response. Brown and Cline (1974) observed a maximum difference in urinary urea excretion between treatments 2 d after the initiation of dietary treatments. Kaji and Furuya (1987) reported that a new equilibrium of PUN was reached within 1 to 2 d after changing dietary crude protein concentrations. Coma et al. (1995a, 1996) found that a 3 d feeding time was required to re-equilibrate PUN concentrations after a change in the dietary lysine concentration for both growing pigs and sows.

Kaji and Furuya (1987) measured the PUN responses 2 d after the graded additions of L-lysine to a rice-soybean meal basal diet with pigs weighing about 25 kg. A linear decrease in PUN was observed until the dietary lysine concentration reached 0.9%. Their results
suggested that PUN could be used efficiently as a rapid response criterion for the
determination of amino acid requirements.

Coma et al. (1995a) investigated the use of PUN as a rapid response in determination
of amino acid requirements. After a series of studies, a protocol was established, which
included (1) equalizing dietary treatments on total nitrogen and electrolyte balance; (2) using
PUN\textsubscript{0} as a covariate to correct PUN\textsubscript{1}; (3) 5 d experiment period, of which the first 3 d was
used for PUN to reach a new equilibrium; and (4) using a two-slope, broken-line model to
estimate lysine requirement. The estimate derived this way was consistent with the result
from a nitrogen balance trial. The procedure has been used extensively for estimating lysine
requirements of growing and finishing pigs (Coma et al., 1995a, b, c) and sows (Coma et al.,
1996; Sparks, 1998).

**Protein Accretion Curve over the Growing-Finishing Period**

*Protein accretion rate versus body weight*

It is generally recognized that growth of animals from conception to maturity occurs
in a sigmoidal shape: self accelerating during the early phase until the inflexion, followed by
self inhibiting of the final phase approaching maturity. The growth curve might be described
by a Gompertz curve: $W = A \times \exp(-\exp(-B(t-t^*))$, where $W$ is the live weight in kilograms at
given time $t$ (usually in days of age), $A$ is the live weight at maturity, $B$ is the growth
coefficient, and $t^*$ is time corresponding to the inflexion of growth (Whittemore, 1994,
1998).

In swine nutrition, the protein accretion rate versus body weight allows more accurate
estimates for amino acid requirements to maximize lean growth than does body weight gain
alone (Friesen et al., 1996). To partition weight gain into protein accretion, an allometric
equation $Pr = a \times X^b$ can be used, where $Pr$ is protein mass, $X$ is body weight, $a$ is the scale constant, and $b$ is the growth coefficient. Protein accretion rate at a given body weight can be derived by using the following function: $d(Pr)/dt = dw/dt \times d(Pr)/dw$, where $d(Pr)/dt$ is the protein accretion rate at the body weight of $w$, $dw/dt$ is the live weight gain rate and $d(Pr)/dw$ is the rate at which body weight is partitioned into protein (Whittemore, 1988; Schinckel, 1994; Van Lunen and Cole, 1998b).

Protein accretion rate is influenced by numerous factors such as nutrition, environment, health, gender and genotype. Under situations in which the optimal levels of nutrients are provided to the pigs with a high health status in a suitable environment, protein accretion rate is determined by a pig’s genetic merit as well as sex and can be considered as potential protein accretion rate (Wittemore et al., 1988; Van Lunen and Cole, 1998b).

Potential protein accretion curves have been developed for different genotypes and sexes (Wittemore et al., 1988; Palmer et al., 1993; Friesen et al., 1996; Van Lunen and Cole, 1998b). From literature data, Carr et al. (1977) derived an equation to describe the relationship between maximum nitrogen retention and body weight. Generally speaking, protein accretion rate over the growing-finishing period occurs in a quadratic form: increasing from 20 up to about 60 (Palmer et al., 1993) or 70 kg (Wittemore et al., 1988; Van Lunen and Cole, 1998b), then decreasing. The major differences among genotypes are the overall protein accretion rate and the rate in which protein accretion declines after 90 kg live weight. In general, high protein accretion genotypes have and maintain higher protein accretion rates at heavier body weights than low or average protein accretion genotypes (Schinckel and de Lange, 1996). Within the same genotype, protein accretion rate is the highest in males, lowest in castrated males and intermediate for females (Campbell and King,
Factors influencing protein accretion

Under commercial conditions, other factors in addition to genetics and sex influence protein accretion rate. For growing-finishing pigs, the important factors are energy intake, effective ambient temperature, pen density and health status (Schinckel, 1994; Schinckel and de Lange, 1996). Each factor may have different impacts on the rate of body protein deposition at different growth stages.

Energy intake. Pigs with high protein accretion rate require high amino acid intakes to express their genetic potential (Stahly et al., 1988, 1991, 1994a). At the conditions of adequate protein supply, the response of protein accretion to energy intake is of the linear-plateau form (Dunkin et al., 1986; Van Lunen and Cole, 1998a). There is research evidence showing high growth potential in young pigs (Whittemore, 1986). Möhn and de Lange (1998) indicated that the maximum protein deposition over the growing-finishing period could be achieved at 25 kg body weight. To achieve the high growth potential, a high energy intake, between 30 and 32 MJ DE per day, is needed (Edwards and Campbell, 1998). A typical corn-soybean meal diet is about 14 MJ (3400 Kcal) DE per kg (NRC, 1998). Because of their low gut capacity (1.8 to 2.0 kg/d feed intake), growing pigs (below 50 kg) usually are unable to consume sufficient energy to achieve their protein accretion potential (Whittemore and Fawcett, 1976; Campbell and Taverner, 1986). As a result, protein accretion is linearly related to energy intake up to ad libitum level in pigs below 50 kg body weight (Campbell and King, 1982; Campbell and Dunkin, 1983; Campbell et al., 1985a; Whittemore, 1986; Close, 1994). Recently Smith et al. (1999b) reported that increasing the dietary energy
density (3.31, 3.44, and 3.57 Mcal ME/kg) and lysine:calorie ratio (2.75, 3.10, 3.45, and 3.80 g/Mcal ME) improved average daily gain and feed efficiency of the growing pigs.

The increase in protein accretion caused by per unit increase of energy intake is called a slope. The slope is steep in younger animals and becomes flatter as pigs grow (Black and Griffiths, 1975; Campbell and Taverner, 1988). Any factor that reduces feed intake or utilization of dietary energy for protein synthesis will have a greater restriction on protein deposition during the growing period than the finishing period.

As pigs grow, feed intake increases in such a way that after 50 kg body weight, energy requirement for maximum protein accretion tends to be below the appetite level, therefore, voluntary energy intake does not limit protein deposition (Campbell et al., 1984, 1985b; Dunkin et al., 1986). Campbell et al. (1985b) reported that in pigs between 48 to 90 kg body weight the protein accretion rate reached a plateau at about 80% of ad libitum energy intake. At this stage of growth, the maximum protein accretion rate is determined by the pig’s intrinsic factors, such as genetic merits and sex (Campbell and King, 1982; Campbell et al., 1985b; Dunkin et al., 1986). Coma et al. (1995b) showed that, in finishing pigs, some decrease in protein deposition caused by decreased feed intake can be overcome by increasing dietary amino acid concentrations.

*Effective ambient temperature.* When the effective ambient temperature falls below the lower critical temperature, pigs increase feed intake to produce more heat for maintaining body temperature (Holmes and Close, 1977; Verstegen and Close, 1994). As a consequence, feed energy for protein deposition is diminished. Verstegen et al. (1978) calculated, using literature data, that feed intake increased by 21 g per 1°C coldness from 20 to 5°C. Noblet et al. (1985) reported that heat production increased and protein retention decreased when the
environmental temperature decreased from 23 to 13°C. Verstegen et al. (1982) found that growing and finishing pigs needed about 25 and 39 g/d of feed to compensate for each degree centigrade coldness, respectively, and that pigs tended to get fatter under cold environment.

Pigs respond to a hot environment by reducing their feed intake. To overcome the effect of reduced feed intake on protein accretion, increasing the concentrations of lysine and other nutrients is necessary (Schenck et al., 1992a, b). Lopez et al. (1993a, b) investigated the effects of dietary lysine (0.60 versus 1.00%), source of amino acid fortification (intact protein versus synthetic amino acids formulated on an ideal protein basis) and environmental temperature (thermoneutral: 20°C versus hot, diurnal: 27.7 to 35.5°C) on carcass characteristics and protein accretion. They found that increasing dietary lysine concentration in the form of intact protein improved carcass leanness and growth performance of finishing gilts under hot stress; however, no improvement was observed by increasing synthetic lysine and other amino acids on an ideal protein basis.

*Pen density.* Restricting space allowance decreases daily feed intake and daily gains in pigs. Heitman et al. (1961) indicated that pigs with restricted space tended to grow more slowly and less efficiently. Lindvall (1981) reported that increasing number of pigs per pen had a detrimental influence on daily gain for nursery pigs. Jensen et al. (1973) observed decreases in both voluntary feed intake and gain as space allowance decreased for growing-finishing pigs. The reduction in growth rate could not be overcome by increasing lysine and/or energy concentration of the diet (Brumm, 1992, 1994; Brumm and Miller, 1993; Edmonds, 1998).

*Health status.* Diseases or immunogens decrease feed intake and protein accretion (Stahly, 1996). Hale and Stewart (1979) and Hale et al. (1981, 1985) reported reduced
nitrogen retention in pigs infected with worms *Trichuris suis*, *Ascaris suum* or *Oesophagostomum spp*. Schinckel (1994) proposed separate linear-plateau responses of protein accretion versus nutrient intake for high and low health status pigs. The main difference of the two curves is that high health status pigs have a higher protein accretion plateau and need a higher nutrient intake to reach that plateau than low health status pigs. Research indicates that pigs with low immune system activation deposit muscle more rapidly and efficiently than those with activated immune systems, therefore, they have higher lysine requirements (Williams et al., 1993a, b, 1994; Stahly et al., 1994b; Williams et al., 1997).

**Protein accretion curves determined under commercial conditions**

Because of the effects of various factors on protein accretion, pigs raised under commercial environments usually operate below their potentials for protein accretion (Campbell, 1988; Schinckel and de Lange, 1996). Schinckel and de Lange (1996) compared protein accretion curves for a high lean growth pigs raised in optimal condition and three commercial environments representing above average, average and below average levels of health and management. It was noticed that even for high health status pigs in a three site production unit, the protein accretion curve fell much lower than the ideal curve, especially during the growing period. The protein accretion reached approximately 70% of the potential at 20 kg, 85% at 70 kg, and about 100% after 100 kg body weight. One interesting observation was that the three curves determined under three different commercial environments shared a similar shape, with an approximately 20% fall from above average to average and from average to below average.

Schinckel et al. (1996) has developed a method to determine a protein accretion curve from mean fat-free lean growth data. This method offers producers an easy and economical
approach to derive protein accretion curves of their own pigs under their farm-specific environments. Farm-specific protein accretion curves have been developed by several researchers (Smith et al., 1999a; De La LLata et al., 2000).

**Literature Cited**


CHAPTER 3. LYSINE REQUIREMENTS OF PIC BARROWS 
DETERMINED BY USING PLASMA UREA NITROGEN AS A RAPID 
RESPONSE CRITERION

A Paper Prepared for Submission to the Journal of Animal Science

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ABSTRACT

Five trials (Trials 1, 2, 3, 4, and 5) were conducted to estimate the true ileal digestible 
lysine requirements of PIC (327 × C22) barrows at approximately 30, 50, 70, 90, and 110 kg 
BW, respectively, using plasma urea nitrogen (PUN) as a rapid response criterion. Pigs were 
individually penned and had free access to feed and water in and between the trials. The 
dietary treatments were five true ileal digestible lysine concentrations, which were 0.732, 
0.807, 0.882, 0.957, 1.032 in Trial 1; 0.527, 0.602, 0.667, 0.752, 0.827% in Trial 2; 0.430, 
0.510, 0.590, 0.670, 0.750% in Trial 3; 0.354, 0.434, 0.514, 0.594, 0.674% in Trial 4; and 
0.313, 0.393, 0.473, 0.553, 0.633% in Trial 5. In each trial, corn, wheat, soybean meal, L-
threonine, and DL-methionine were used to formulate a basal diet containing the lowest lysine 
concentration, and the other essential AA were at or above their ideal ratios (NRC, 1998) to 
the highest lysine concentration. The remaining four lysine concentrations were achieved by 
supplementation with L-lysine-HCl. Twenty PIC barrows with an initial BW of 19.1 kg were 
used in Trials 1 and 2, each was conducted as a randomized complete block design with five 
treatments and four blocks. Trials 3, 4, and 5 used another group of 20 PIC barrows with an 
initial BW of 59.0 kg in completely randomized designs. Pigs were fed diets containing 
adequate lysine and other nutrients before and after each trial. Trials 1, 2, 3, 4, and 5 started 
when pigs reached approximately 30, 50, 70, 90, and 110 kg, each consisted of a 5-d dietary
treatment period. On the last 2 d of the period, pigs were bled and PUN concentrations were
determined. Pretreatment PUN was obtained before the initiation of dietary treatments and
was used as a covariate to correct PUN variation not related to dietary lysine adequacy.
Variance analyses indicated that pretreatment PUN was highly correlated with PUN (P ≤
0.0003), and that the covariate correction reduced the experimental errors by approximately
57%. The true ileal digestible lysine requirements of PIC (327 × C22) barrows at 33, 52, 72,
93, 113 kg BW were 0.85 ± 0.01, 0.76 ± 0.02, 0.68 ± 0.05, 0.59 ± 0.03, and 0.42 ± 0.21%,
respectively. Expressed as daily intake, the requirements were 16.5 ± 0.2, 18.0 ± 0.5, 20.2 ±
1.5, 16.7 ± 0.8, and 14.4 ± 7.2 g/pig. The precision for the lysine estimation decreased when
pigs reached the finishing period. Further investigation is needed to increase the precision.
Key words: Lysine Requirement, Plasma Urea Nitrogen (PUN), Growing-Finishing Pigs

Introduction

When pigs are fed diets with different lysine concentrations from deficient to
excessive, plasma urea nitrogen (PUN) concentrations are expected to decrease until the
lysine requirement is met. This response has been used to titrate the lysine requirements of
pigs. PUN is a rapid response: it reaches a new equilibrium within 2 d (Kaji and Furuya,
1987) or 3 d (Coma et al., 1995a; 1996) after changing dietary protein or lysine
concentrations. Coma et al. (1995a, c) investigated the use of PUN as a rapid response
criterion in determining the lysine requirements for swine and established a PUN technique.
The lysine requirement determined by the PUN technique was consistent with the estimate
from a nitrogen balance trial (Coma et al., 1995a). In this experiment (Exp. A), five trials
were conducted to determine the lysine requirements of PIC (327 × C22) barrows at
approximately 30, 50, 70, 90, and 110 kg BW, using the PUN technique developed by Coma et al. (1995a).

Materials and Methods

Dietary treatments

In each trial, the dietary treatments were five equally spaced true ileal digestible lysine concentrations (Diets 1, 2, 3, 4, and 5), while the lysine concentration of Diet 3 was projected to be near the requirement level predicted by the NRC (1998) with an average lean growth rate of 350 g/d. Corn, wheat, soybean meal, L-threonine, and DL-methionine were used to formulate a basal diet containing the lowest lysine concentration, and the other essential AA were at or above their ideal ratios (NRC, 1998) to the highest lysine concentration. Wheat was used because of its high valine: lysine ratio compared to corn (NRC, 1998). The basal diet was sampled and ground through a 1-mm screen for total amino acid analysis. From the analyzed lysine concentration, the true ileal digestible lysine concentration was calculated by using the equation provided by NRC (1998). Lysine additions were achieved by supplementation with L-lysine-HCl, and the utilization of free lysine was assumed to be 100% (Batterham, 1974; Batterham and O’Neill, 1978; Batterham and Murison, 1981). The five true ileal digestible lysine concentrations were 0.732, 0.807, 0.882, 0.957, 1.032% in Trial 1; 0.527, 0.602, 0.667, 0.752, 0.827% in Trial 2; 0.430, 0.510, 0.590, 0.670, 0.750% in Trial 3; 0.354, 0.434, 0.514, 0.594, 0.674% in Trial 4; and 0.313, 0.393, 0.473, 0.553, 0.633% in Trial 5. The compositions of the basal diets are listed in Table 1.

In each trial, the five treatment diets were made isonitrogenous by supplementation with L-glutamic acid. Sodium carbonate was used to make the diets identical in electrolyte
balance (Na + K -Cl) thus preventing a dietary acid-base difference from affecting PUN (Cai and Zimmerman, 1995).

**Animals and experimental designs**

Pigs were individually penned in a room with pens that were 0.60 x 2.2 m in dimensions, had steel slatted flooring, and each contained a stainless steel self-feeder and a nipple drinker. The room was cleaned every 4 d and it was mechanically ventilated. During the adjustment and inter-trial periods, pigs were fed diets containing adequate lysine and other nutrients. The animals had free access to feed and water over the whole experiment period.

**Trial 1.** Twenty PIC (327 x C22) barrows with an initial BW of 19.1 kg were placed in the room 2 wk before the start of Trial 1. The pigs were grouped on litter and initial BW to form four blocks, and the five pigs in each block were located in contiguous pens.

Trial 1 started when pigs reached approximately 30 kg BW. The trial consisted of a 7-d period. On d 1 and d 2 pigs were bled between 0730 and 0800 via an orbital sinus, and about 10 ml blood was taken from each pig. Immediately after bleeding on d 2, pigs were weighed and fed the treatment diets. The blood samples from d 1 and d 2 were used for pretreatment PUN determination. After the initiation of dietary treatments, pigs were allowed a 3-d period for PUN to re-equilibrate. Then on d 6 and d 7, pigs were bled again for PUN measurement. After the bleeding on d 7, pigs were weighed, and the dietary treatments ended. ADFI over the dietary treatment period was recorded for each pig.

**Trial 2.** The dietary treatments began when the pigs reached approximately 50 kg BW. The blocks remained the same way as in Trial 1, but a new randomization of treatments was made for each block. The procedures were the same as in Trial 1.
Trials 3, 4, and 5. A new set of 20 PIC (327 x C22) barrows with an initial BW of 59.0 kg were repeatedly used in Trial 3, 4, and 5. Because of uniform BW among the pigs and lack of litter control, completely randomized designs were used. After a 1-wk period of adjustment to the pens, pigs reached approximately 70 kg, therefore, Trial 3 started. Trial 4 began when pigs weighed approximately 90 kg. Between Trials 4 and 5, four pigs were replaced to maintain uniform BW among the animals. After a 10-d adaptation period, Trial 5 was conducted, when pigs weighed approximately 110 kg.

PUN analysis

Plasma was harvested from blood sample by centrifugation and stored at -20°C until analyzed. Each sample was analyzed for PUN concentration by colorimetrically measuring the product formed in the direct reaction of urea and diacetyl monoxime, as described by Marsh et al. (1965).

Statistical analysis

PUN concentrations obtained on d 1 and d 2 were averaged for each pig and the average was used as pretreatment PUN. Similarly, PUN concentrations obtained on d 6 and d 7 were averaged for each pig and the average was considered as treatment PUN. The resulting data were analyzed by using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Pretreatment PUN was used as a covariate to correct PUN variation not related to lysine adequacy. Trials 1 and 2 were analyzed as randomized complete block designs; the class statement includes block and treatment, and the model statement includes pretreatment PUN, block, and treatment. In Trials 3, 4, and 5, the data were analyzed as completely randomized designs with treatment in the class statement and pretreatment PUN and treatment in the
model statement. Type I sum square from SAS output was used to test the significance of covariate analysis and treatment effect on PUN.

Dietary lysine requirement (%) was estimated by fitting the corrected PUN means versus dietary lysine concentrations to a two-slope, broken-line model by using NLIN procedures of SAS (Robbins, 1986). The lysine requirement (grams/day) was calculated as requirement (%) × ADFI.

Results and Discussion

The lysine requirements over the growing-finishing period

Trial 1. The initial and final BW were 31.0 ± 2.2 and 35.5 ± 2.8 kg. The room temperature over the trial period ranged between 20 and 23°C. ADFI was 1.94 ± 0.12 kg/pig, and no difference was found among the treatment groups (P = 0.57). Variance analysis indicated that pretreatment PUN was highly correlated with PUN (P = 0.0001), and the covariate correction reduced the experimental error from 3.38 to 1.78. The response of the corrected PUN means versus dietary lysine concentrations is shown in Figure 1, as well as in Table 2. As lysine increased, PUN decreased and then approached a plateau. Fitting the response to a two-slope, broken-line model, the estimated true ileal digestible lysine requirement was 0.85 ± 0.01%. The mean square error (MSE) for the broken-line model was 0.01. Expressed as daily intake, the requirement was 16.5 ± 0.2 g/pig.

Trial 2. Trial 2 started at 49.8 ± 5.2 kg BW and ended at 54.5 ± 5.2 kg. During the trial period, the room temperature fluctuated between 16 and 29°C. ADFI was 2.37 ± 0.13 kg/pig, and no difference was found among the treatment groups (P = 0.54). Pretreatment PUN was highly correlated with PUN (P = 0.0001) and the covariate correction reduced the experimental error from 3.51 to 1.82. In response to the increase of dietary lysine
concentration, PUN decreased and was minimized at the requirement level, which was estimated to be $0.76 \pm 0.02\%$ of true ileal digestible lysine (Figure 2 and Table 2). The MSE for the fitted broken-line model was 0.07. The daily requirement was $18.0 \pm 0.5$ g/pig.

The upper room temperature during Trial 2 was 6°C higher than that of the period between Trials 1 and 2 (16 to 23 versus 16 to 29°C). The reason for the sudden increase was that when Trial 2 started, a furnace malfunctioned. When the cause of the high temperature was identified and solved, the trial had been completed. Because pigs had been housed at a relatively constant temperature range, the sudden increase of 6°C was a heat stress, and pigs responded to the heat stress by reducing feed intakes (Stahly et al., 1979; Ferguson, 1999).

The ADFI during Trial 2 was 2.37 kg/pig, lower than that of the period between Trials 1 and 2 (2.53 kg/pig). The reduced feed intake resulted in a reduction in energy intake. Whether the reduced energy intake limited the pigs' lean growth rates or not was critical for the validation of the lysine requirement estimated from this trial. Several studies have demonstrated that for pigs above 50 kg BW, the energy requirement for the maximum lean growth is below the appetite level (Campbell and King, 1982; Campbell et al., 1985; Dunkin et al., 1986). Edwards and Campbell (1991), by reviewing the literature on the relationship between energy intake and maximum lean growth, pointed out that growing pigs required a energy intake of between 7170 to 7648 kcal DE/d to achieve their high lean growth potentials. Pigs in this trial had an average BW of 52 kg and an energy intake of 8347 kcal DE/d. Based on the above research evidence, it seemed that the reduced energy intake in this trial should not have limited the pigs' lean growth rates. From the PUN response, it could be seen that PUN responded to lysine increment up to the fourth lysine concentration (Figure 2). The lysine requirement was estimated to be 0.76%, approximately 92% of the highest lysine
concentration among the five treatments. The requirement was higher than the value predicted by NRC (1998) with an average lean growth rate of 350 g/d. The result was consistent with those from Campbell et al. (1984, 1985) and Coma et al. (1995b), that pigs on a low feeding level required a higher protein or lysine concentration than pigs with ad libitum feed intakes. Coma et al. (1995b) illustrated that, when the energy intake was not limiting lean growth rate, the lysine requirement, expressed as daily intake, was relatively constant and not affected by a reduced feed intake. Therefore, the lysine requirement (grams/day) from this trial was considered valid.

Trial 3. Trial 3 started at 69 ± 3.6 and ended at 74.9 ± 2.6 kg BW. The room temperature ranged between 18 and 26°C. ADFI was 2.97 ± 0.28 kg/pig, and no difference was found among the treatment groups (P = 0.95). Covariate analysis of pretreatment PUN was highly significant (P = 0.0005) and the experimental error was reduced from 3.38 to 1.59 by the covariate correction. The corrected PUN response to lysine increment is shown in Figure 3 and Table 2. Broken-line analysis indicated that pigs required 0.68 ± 0.05% true ileal digestible lysine. The MSE for the fitted model was 1.67. The daily intake requirement was 20.2 ± 1.5 g/pig.

Trial 4. The BW ranged from 90.5 ± 3.2 to 95.1 ± 3.6 kg during the trial period. The room temperature ranged between 19 and 25°C. ADFI was 2.83 ± 0.46 kg/pig, with no difference among the treatment groups (P = 0.70). However, in this trial, ADFI varied widely among the animals. To compare ADFI variation among the trials, the coefficients of variation (CV, %) were computed for each trial. The results were 6, 5, 9, and 16% for Trials 1, 2, 3 and 4, respectively, indicating that ADFI variation in Trial 4 increased greatly, when compared to the previous trials.
Covariate analysis indicated that both pretreatment PUN and ADFI were highly correlated with PUN (P = 0.0003 and 0.01, respectively). Pretreatment correction reduced the experimental error from 5.42 to 2.33, and ADFI correction reduced it further to 1.61. The highly significant correlation between ADFI and PUN might have been caused by the large ADFI variation in this trial. The corrected PUN versus lysine is shown in Figure 4, as well as in Table 2. The broken-line analysis indicated that the true ileal digestible lysine requirement was 0.59 ± 0.03%. The MSE for the model was 0.13. Expressed as daily intake, the requirement was 16.5 ± 0.8 g/pig.

Trial 5. The pigs weighted from 110 ± 3.6 to 115 ± 3.9 kg. The room temperature varied between 21 and 27°C. ADFI was 3.43 ± 0.31 kg/pig, and a significant difference was found among the five treatment groups (3.27, 3.47, 2.85, 3.56, 3.65 kg, P = 0.02). The reason for the difference was not clear. The correlation between pretreatment PUN and PUN was highly significant (P = 0.0001), and the covariate correction reduced the experiment error from 4.23 to 0.98. The corrected PUN means versus lysine concentrations are presented in Figure 5 and Table 2. As lysine increased, PUN decreased and exhibited a great variation when the dietary lysine concentration reached and exceeded the requirement level. It seemed that the response fit a three-slope, rather than a two-slope, broken-line model. The poor fitness of the model influenced the precision for the lysine requirement estimation: the true ileal digestible lysine requirement was 0.42 ± 0.21% and the MSE for the broken-line model was 3.9. The daily requirement was 14.4 ± 7.2 g/pig. The large MSE and standard error indicated that this estimate should be treated with caution.
Precision of the lysine requirement estimation

Coma et al. (1995c) found that pretreatment PUN was closely correlated with treatment PUN ($R^2 = 0.73$) and could be used as a covariate to reduce PUN variation not related to lysine adequacy. Results from the current experiment showed that pretreatment PUN was highly correlated with PUN ($P \leq 0.0003$), and that the covariate correction reduced the experimental errors by approximately 57%. Covariate correction adjusted each treatment PUN mean to the value that it would have been expected to have if all treatments had the same pretreatment PUN mean. In this way the covariate correction removed the effect of variations in pretreatment PUN means between treatments. The adjusted means were used to complete the broken-line analysis, therefore, the use of pretreatment PUN as a covariate increased the precision of lysine requirement estimation.

When PUN is used as a response criterion to estimate the lysine requirement, the variation in PUN response to lysine increment among experimental animals also influences the precision of lysine requirement estimation. Variation in PUN response between animals was reported by Wilkinson et al. (1982), who measured PUN response to lysine increment on an individual sow basis. Because of the large variation, they failed to identify a requirement. In another of their experiments, the large variation in PUN response distorted the statistical analysis by having an abnormally high value for one treatment group. As a result, the requirement from PUN response was not consistent with estimates by other criteria.

The results of the experiment reported here indicated that the precision of lysine requirement estimation decreased when pigs reached the finishing period, especially at 110 kg BW. Because the effect of pretreatment PUN correction did not decrease as BW increased, the decrease of the precision must have been caused by an increased variation in
PUN response among the pigs. The large variation in PUN response in heavy pigs might be caused by large variations in lysine requirement, BW and ADFI among the animals. Further investigation is needed to increase the precision of estimating the lysine requirements for finishing pigs.

It was noticed that as pigs grew, variation in ADFI increased, and that the effect of ADFI on PUN was not consistent among the trials. In Trial 4, ADFI was significantly correlated to PUN. The correlation was not detected in Trial 5, however, a significant difference in ADFI among treatment groups was found. Because the energy requirement for maximum lean growth is below the appetite level in finishing pigs, it might be desirable to equalize feed intake among the pigs in future studies to reduce the effect of ADFI on PUN and lysine requirement estimation.

Implications

The true ileal digestible lysine requirements of individually penned PIC (327 × C22) barrows at 33, 52, 72, 93, 113 kg BW were 0.85 ± 0.01, 0.76 ± 0.02, 0.68 ± 0.05, 0.59 ± 0.03, and 0.42 ± 0.21%, respectively. Expressed as daily intake, the requirements were 16.5 ± 0.2, 18.0 ± 0.5, 20.2 ± 1.5, 16.7 ± 0.8, and 14.4 ± 7.2 g/pig, respectively. Daily lysine requirement of PIC barrows increased with BW to 72 kg and then decreased.

Pretreatment PUN correction reduced the experimental errors by approximately 57% and increased the precision for estimating the lysine requirements. The precision decreased when pigs reached finishing period. The decrease may be caused by large variation in PUN response to lysine increment among the animals. How to increase the precision for estimating the lysine requirements of finishing pigs needs further investigation. To reduce the effect of ADFI variation on PUN, an equalized feed regime may be used in future studies.
Literature Cited


Table 1. Composition of basal diets\textsuperscript{a}, as-fed basis

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
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<tbody>
<tr>
<td>Corn</td>
<td>49.54</td>
<td>56.93</td>
<td>41.09</td>
<td>30.59</td>
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<td>Wheat</td>
<td>25.00</td>
<td>25.00</td>
<td>46.16</td>
<td>60.00</td>
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<td>Soybean meal</td>
<td>20.51</td>
<td>13.34</td>
<td>9.12</td>
<td>6.01</td>
<td>2.69</td>
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<tr>
<td>Fat</td>
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<td>2.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
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<td>Calcium carbonate</td>
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<td>0.79</td>
<td>0.83</td>
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<tr>
<td>Dicalcium phosphate</td>
<td>0.78</td>
<td>0.65</td>
<td>0.46</td>
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<tr>
<td>Sodium chloride\textsuperscript{b}</td>
<td>0.26</td>
<td>0.25</td>
<td>0.25</td>
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<tr>
<td>L-Glutamic acid\textsuperscript{c}</td>
<td>0.60</td>
<td>0.60</td>
<td>0.64</td>
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<td>0.64</td>
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<tr>
<td>L-Threonine</td>
<td>0.12</td>
<td>0.11</td>
<td>0.12</td>
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<td>DL-Methionine</td>
<td>0.07</td>
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<td>Vitamin premix\textsuperscript{d}</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
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<tr>
<td>Trace mineral premix\textsuperscript{e}</td>
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<tr>
<td>Tylan\textsuperscript{f}</td>
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<td>0.05</td>
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</table>

Calculated analysis

<table>
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<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>17.5</td>
<td>14.7</td>
<td>13.8</td>
<td>13.0</td>
<td>11.5</td>
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<tr>
<td>DE, kcal/kg</td>
<td>3529</td>
<td>3523</td>
<td>3468</td>
<td>3459</td>
<td>3462</td>
</tr>
<tr>
<td>True ileal digestible lysine\textsuperscript{g}, %</td>
<td>0.732</td>
<td>0.527</td>
<td>0.430</td>
<td>0.354</td>
<td>0.313</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Graded amounts of L-lysine-HCl were added to the basal diets to obtain five dietary true ileal digestible lysine concentrations for each trial.

\textsuperscript{b}Partially replaced by sodium carbonate to maintain an identical electrolyte balance (Na + K –Cl) among the treatments.

\textsuperscript{c}Partially or totally removed to make the five treatment diets isonitrogenous.

\textsuperscript{d}Contributed per kilogram of diet: 4,400 IU of vitamin A; 1,100 IU of vitamin D\textsubscript{3}; 22 IU of vitamin E; 6.6 g of riboflavin; 18 mg of d-pantothenic acid; 33 mg of niacin; 10.0 \mu g of vitamin B\textsubscript{12}.

\textsuperscript{e}Contributed in part per million of diet: Zn, 75.0; Fe, 87.5; Mn, 30; Cu, 8.8; I, 1.0.

\textsuperscript{f}Contributed per kilogram of diet: 44 mg tylosin.

\textsuperscript{g}Calculated from the analyzed lysine concentration by using the equation in NRC (1998). The amino acid analysis was performed in a commercial laboratory (Experiment Station Chemical Laboratories, Columbia, MO 65211).
Table 2. The response of plasma urea nitrogen (PUN) to dietary lysine concentrations

<table>
<thead>
<tr>
<th>Trial</th>
<th>Item</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>SEMa</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lysine, %</td>
<td>0.732</td>
<td>0.807</td>
<td>0.882</td>
<td>0.957</td>
<td>1.032</td>
<td></td>
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<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>11.77</td>
<td>9.98</td>
<td>8.90</td>
<td>8.92</td>
<td>8.67</td>
<td>0.67</td>
<td>0.040</td>
</tr>
<tr>
<td>2</td>
<td>Lysine, %</td>
<td>0.527</td>
<td>0.602</td>
<td>0.677</td>
<td>0.752</td>
<td>0.827</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>10.37</td>
<td>9.96</td>
<td>8.71</td>
<td>8.30</td>
<td>8.56</td>
<td>0.53</td>
<td>0.110</td>
</tr>
<tr>
<td>3</td>
<td>Lysine, %</td>
<td>0.430</td>
<td>0.510</td>
<td>0.590</td>
<td>0.670</td>
<td>0.750</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>11.17</td>
<td>9.52</td>
<td>9.25</td>
<td>7.50</td>
<td>7.32</td>
<td>0.63</td>
<td>0.005</td>
</tr>
<tr>
<td>4</td>
<td>Lysine, %</td>
<td>0.354</td>
<td>0.434</td>
<td>0.514</td>
<td>0.594</td>
<td>0.674</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>11.33</td>
<td>9.94</td>
<td>9.74</td>
<td>8.49</td>
<td>8.90</td>
<td>0.64</td>
<td>0.070</td>
</tr>
<tr>
<td>5</td>
<td>Lysine, %</td>
<td>0.313</td>
<td>0.393</td>
<td>0.473</td>
<td>0.553</td>
<td>0.633</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>12.06</td>
<td>10.80</td>
<td>9.58</td>
<td>10.86</td>
<td>8.99</td>
<td>0.50</td>
<td>0.005</td>
</tr>
</tbody>
</table>

aTrue ileal digestible lysine concentration.
bLeast square mean of plasma urea nitrogen (PUN) corrected by using pretreatment PUN as a covariate.

Standard error of the mean (n = 4; n = 3 for Diet 3 in Trials 2, 3, and 4).
P-value of obtaining such differences in PUN if dietary lysine has no effect on PUN concentration.
Figure 1. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine concentrations in PIC barrows weighing from 31.0 to 35.5 kg (Trial 1). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was $0.85 \pm 0.01\%$ true ileal digestible lysine.
Figure 2. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine concentrations in PIC barrows weighing from 49.8 to 54.5 kg (Trial 2). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was 0.76 ± 0.02% true ileal digestible lysine.
Figure 3. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine concentrations in PIC barrows weighing from 69.0 to 74.9 kg (Trial 3). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was 0.68 ± 0.05% true ileal digestible lysine.
Figure 4. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine concentrations in PIC barrows weighing from 90.5 to 95.1 kg (Trial 4). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was 0.59 ± 0.03% true ileal digestible lysine.
Figure 5. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine concentrations in PIC barrows weighing from 110.0 to 115.0 kg (Trial 5). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was 0.42 ± 0.21% true ileal digestible lysine.
CHAPTER 4. LYSINE REQUIREMENTS OF PIC BARROWS UNDER GROUP PENNED SITUATION

A Paper Prepared for Submission to the Journal of Animal Science

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**ABSTRACT**

Five trials (Trials 1, 2, 3, 4 and 5) were conducted to estimate the lysine requirements of PIC (327 x C22) barrows at approximately 30, 50, 70, 90, and 110 kg BW, respectively, using plasma urea nitrogen (PUN) as a rapid response criterion. One hundred barrows with an initial BW of 24.4 kg were used in the five trials, each was conducted as a randomized complete block design with four blocks and five treatments. Pigs were group penned and had free access to feed and water. The treatments were five true ileal digestible lysine concentrations, which were 0.723, 0.798, 0.873, 0.948, 1.023% in Trial 1, 0.577, 0.657, 0.737, 0.817, 0.897% in Trial 2, 0.541, 0.621, 0.701, 0.781, 0.861% in Trial 3, 0.404, 0.484, 0.564, 0.644, 0.724% in Trial 4, and 0.304, 0.384, 0.464, 0.544, 0.624% in Trial 5. In each trial, corn, wheat, soybean meal, L-theronine and DL-methionine were used to formulate a basal diet containing the lowest lysine concentration, and other essential AA were at or above their optimal ratios (NRC, 1998) to the highest lysine concentration. Lysine additions were achieved by supplementation with L-lysine-HCl. Trials 1, 2, 3, 4, and 5 started when pigs reached approximately 30, 50, 70, 90, and 110 kg BW, respectively. Each trial consisted of a 5-d dietary treatment period. On the last 2 d of the period, two pigs from each pen were selected and bled for PUN measurements. Pretreatment PUN was obtained before the initiation of dietary treatments and was used as a covariate to correct PUN variation not
related to lysine adequacy. The lysine requirement was estimated by fitting the corrected PUN means versus dietary lysine concentrations to a two-slope, broken-line model. The results indicated that each PIC barrow required 15.8 ± 1.7 and 18.9 ± 1.4 g true ileal digestible lysine per day at 32 and 50 kg BW, respectively. However, PUN response did not give reasonable estimates for the lysine requirements at approximately 70, 90, and 110 kg BW. Further studies are needed for estimating the lysine requirements of finishing pigs.

Key words: Lysine Requirement, Plasma Urea Nitrogen (PUN), Group Penned Pigs

Introduction

Lysine requirement is determined by protein accretion rate, which is influenced by genetics, sex, and environment factors, such as effective ambient temperature, pen density, and health status (Schinckel, 1994; Schinckel and de Lange, 1996). Therefore, pigs raised in individually penned and group penned situations may have different lysine requirements. In this experiment (Exp. B), five trials were conducted to determine the lysine requirements of group penned PIC (327 x C22) barrows at approximately 30, 50, 70, 90, and 110 kg BW, using a plasma urea nitrogen (PUN) technique, developed by Coma et al. (1995a).

Materials and Methods

Dietary treatments

In each trial, the dietary treatments were five equally spaced true ileal digestible lysine concentrations (Diets 1, 2, 3, 4, and 5), while the lysine concentration in Diet 3 was projected to be near the requirement predicted by the NRC (1998) for pigs with an average lean growth rate of 350 g/d. Corn, wheat, soybean meal, L-theronine and DL-methionine were used to formulate a basal diet containing the lowest lysine concentration, and the other essential AA were at or above their ideal ratios (NRC, 1998) to the highest lysine
concentration. Wheat was used because of its high valine: lysine ratio compared to corn (NRC, 1998). The basal diet was sampled and ground through a 1-mm screen for total amino acid analysis. From the analyzed lysine concentration, the true ileal digestible lysine concentration was calculated by using equation provided by NRC (1998). Lysine additions were achieved by supplementation with L-lysine-HCl, and the utilization of free lysine was assumed to be 100%. The five true ileal digestible lysine concentrations were 0.723, 0.798, 0.873, 0.948, 1.023% in Trial 1, 0.577, 0.657, 0.737, 0.817, 0.897% in Trial 2, 0.541, 0.621, 0.701, 0.781, 0.861% in Trial 3, 0.404, 0.484, 0.564, 0.644, 0.724% in Trial 4, and 0.304, 0.384, 0.464, 0.544, 0.624% in Trial 5. The compositions of the basal diets are listed in Table 1.

The five treatment diets were made isonitrogenous by supplementation with L-glutamic acid. Sodium carbonate was used to make the diets identical in electrolyte balance (Na + K –Cl) thus preventing a dietary acid-base difference from affecting PUN (Cai and Zimmerman, 1995).

**Animals and experimental design**

One hundred PIC barrows with an initial BW of 24.4 kg were used. The pigs were group penned, and each pen contained five pigs. The pens were 1.8 x 2.6 m in dimensions, had partially slatted concrete floor, and each contained a two-compartment stainless steel self-feeder and a nipple drinker. The animals had free access to feed and water. During the adjustment and inter-trial period, they were fed diets containing adequate lysine and other nutrients. The room was mechanically ventilated and high and low temperatures were recorded daily.
Trial 1. The animals were placed in the experimental room 1 wk before the trial started. The animals were blocked based on litter and initial BW to form four blocks, each block containing five contiguous pens.

Trial 1 started when BW averaged approximately 30 kg. The trial consisted of a 7-d period. On d 1 between 0730 and 0830, pigs were weighted, then from each pen two pigs with the BW nearest to the average BW of that pen were selected and bled via an orbital sinus. About 10 ml blood was taken from each pig. The selected pigs were bled at the same time on d 2 and then the dietary treatments were initiated. The blood samples from d 1 and d 2 were used for pretreatment PUN determination. After the initiation of dietary treatments, pigs were allowed a 3 d period for PUN to re-equilibrate. Then on d 6 and d 7, the selected pigs were bled again for PUN measurement. After the bleeding on d 7, all pigs were weighed, and the treatments ended. ADFI over the dietary treatment period was recorded on a pen basis.

Trials 2, 3, 4, and 5 began when the pigs reached approximately 50, 70, 90, and 110 kg, respectively. Pen groups and the blocks remained the same as in Trial 1, but a new randomization of treatments was made for each block in each trial. The procedures were exactly the same as in Trial 1.

When pigs reached 110 kg, they were measured by ultrasound at the 10th rib for fat depth and loin muscle area to estimate the average carcass lean growth rate over the growing-finishing period.

PUN analysis

Plasma was harvested from blood samples by centrifugation and stored at -20°C until analyzed. The PUN concentration was determined by colorimetrically measuring the product
formed in the direct reaction of urea and diacetyl monoxime, as described by Marsh et al. (1965).

**Statistical analysis**

PUN concentrations obtained on d 1 and d 2 were averaged for each pig and the average was used as pretreatment PUN. Similarly, PUN average on d 6 and d 7 was considered as PUN response to lysine. The resulting data were analyzed by using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Pretreatment PUN was used as a covariate to correct PUN variation not related to lysine adequacy on an individual pig basis and each pen was considered as an experimental unit after that. The data were analyzed as a randomized complete block design; the class statement includes block and treatment, and the model statement includes pretreatment PUN, block, and treatment. Type I sum square from SAS output was used to test the significance of covariate analysis and treatment effect on PUN.

Dietary lysine requirement (%) was estimated by fitting the corrected PUN means versus dietary lysine concentrations to a two-slope, broken-line model by using NLIN procedures of SAS (Robbins, 1986). The lysine requirement (grams/day) was calculated as requirement (%) \times ADFI.

**Results and Discussion**

**The lysine requirements of PIC barrows**

**Trial 1.** The trial started at 29.3 ± 3.5 and ended at 33.7 ± 4.0 kg BW. During the trial period, the room temperature ranged between 17 and 22°C. ADFI of pigs was 1.7 kg/pig, and no difference was found among the treatment groups (P = 0.37). Variance analysis indicated that pretreatment PUN was highly correlated with PUN (P = 0.0001). The response of the corrected PUN means versus dietary lysine concentrations is shown in Figure 1 and Table 2.
Fitting the response to a two-slope, broken-line model, the lysine requirement was estimated to be $0.93 \pm 0.10\%$ true ileal digestible lysine. Expressed as daily intake, the requirement was $15.8 \pm 1.7$ g/pig.

**Trial 2.** The initial and final BW were $47.0 \pm 5.4$ and $52.0 \pm 6.0$ kg, respectively. During the trial period, the room temperature fluctuated between 16 and 21°C. ADFI was 2.3 kg/pig, and no difference was found among the treatment groups ($P = 0.62$). Variance analysis showed that the correlation between pretreatment and treatment PUN was not significant ($P = 0.15$). This result was not consistent with the result of Coma et al. (1995b), who demonstrated that pretreatment PUN was closely related to treatment PUN. Because a highly significant correlation was found in our previous experiment (Exp.A), as well as in Trial 1 in this experiment, pretreatment PUN was still used as a covariate in this trial. The corrected PUN means versus dietary lysine concentrations are illustrated in Figure 2 and Table 2. Results from the broken-line analysis indicated that pigs required $0.82 \pm 0.06\%$ true ileal digestible lysine. Expressed as daily intake, the requirement was $18.9 \pm 1.4$ g/pig.

**Trials 3, 4 and 5.** The PUN responses to dietary lysine concentrations in Trials 3, 4 and 4 are shown in Table 2. Fitting the responses to broken-line models failed to obtain lysine requirement estimates for pigs at 70, 90 and 110 kg.

Based on the real-time ultrasound measurements on 10th rib fat depth and 10th rib loin eye area, the average fat-free lean growth of PIC (327 × C22) barrows over the growing-finishing period was calculated (NRC, 1998) to be $359$ g/d/pig. The dressing percentage was assumed as 74%.

Compared with Exp. A, the precision of lysine requirement estimation in Exp. B was lower (Table 3). The reason might be because only two pigs from each pen were used for
PUN measurements. In Exp. B, each trial was originally designed as a randomized complete block design, in which the pigs were blocked on litter and initial BW. Because of the large number of pigs (100), it was decided that from each pen, two pigs with the BW nearest to the average BW of that pen were used for PUN measurement. Among the selected pigs in the same block, some were littermates and some were not, therefore, the covariance structure between pigs within each block became complicated, and the covariance structure differed from block to block. The complicated covariance structure increased the variation of PUN response to dietary lysine concentration, thereby decreased the precision for the lysine requirement estimation. However, if pigs had been selected by litter instead of BW, the large variation in BW among pigs would have increased PUN variation also. Variance analyses indicated pen average BW was different among the blocks (P = 0.0001 and 0.06 in Trials 1 and 2, respectively). The best way to increase the precision for estimating lysine requirements was to measure the response on all animals.

As reported in Exp. A, the precision for estimating the lysine requirement decreased when pigs reached approximately 70 kg. In the current experiment, we failed to obtain lysine requirement estimates for finishing pigs. One possible reason might be that the variation of PUN response to lysine concentrations increases as BW increases. Therefore, when PUN is used as a response criterion to estimate the lysine requirements for finishing pigs, either the number of replications should be increased, or other experimental designs should be investigated to decrease the variation.

**Implications**

Group penned PIC (327 x C22) barrows required 0.93 ± 0.10 and 0.82 ± 0.06% true ileal digestible lysine at 32 and 50 kg BW, respectively. Expressed in grams/day/pig, the
requirements were 15.8 ± 1.7 and 18.9 ± 1.4, respectively. PUN technique could be used to estimate the lysine requirements for growing pigs under group penned situation. However, the number of pigs from each pen used for PUN measurements might have an impact on the precision of the estimation. Further studies are needed to estimate the lysine requirements for group penned finishing pigs.

Literature Cited


Table 1. Composition of basal diets\textsuperscript{a}, as-fed basis

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Corn</td>
<td>49.54</td>
<td>56.89</td>
<td>59.01</td>
<td>50.78</td>
<td>44.45</td>
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<tr>
<td>Wheat</td>
<td>25.00</td>
<td>25.00</td>
<td>25.00</td>
<td>38.62</td>
<td>49.35</td>
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<td>Soybean meal</td>
<td>20.51</td>
<td>13.34</td>
<td>12.28</td>
<td>7.15</td>
<td>2.69</td>
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<td>Fat</td>
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<td>2.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
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<td>Calcium carbonate</td>
<td>0.82</td>
<td>0.79</td>
<td>0.77</td>
<td>0.78</td>
<td>0.80</td>
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<td>Dicalcium phosphate</td>
<td>0.78</td>
<td>0.65</td>
<td>0.52</td>
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<td>0.30</td>
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<tr>
<td>Sodium chloride\textsuperscript{b}</td>
<td>0.26</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
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<tr>
<td>L-Glutamic acid\textsuperscript{c}</td>
<td>0.60</td>
<td>0.64</td>
<td>0.64</td>
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<td>0.64</td>
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<tr>
<td>L-Threonine</td>
<td>0.12</td>
<td>0.11</td>
<td>0.15</td>
<td>0.12</td>
<td>0.22</td>
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<tr>
<td>DL-Methionine</td>
<td>0.07</td>
<td>0.03</td>
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<tr>
<td>Vitamin premix\textsuperscript{d}</td>
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<td>0.20</td>
<td>0.20</td>
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<td>Trace mineral premix\textsuperscript{e}</td>
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<td>0.05</td>
<td>0.05</td>
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<td>0.05</td>
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<tr>
<td>Tylan\textsuperscript{r}</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
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</table>

Calculated analysis

<table>
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<tr>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>17.5</td>
<td>15.5</td>
<td>14.5</td>
<td>12.8</td>
<td>11.5</td>
</tr>
<tr>
<td>DE, kcal/kg</td>
<td>3529</td>
<td>3527</td>
<td>3490</td>
<td>3475</td>
<td>3462</td>
</tr>
<tr>
<td>True ileal digestible lysine\textsuperscript{g}, %</td>
<td>0.723</td>
<td>0.577</td>
<td>0.541</td>
<td>0.404</td>
<td>0.304</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Graded amounts of L-lysine-HCl were added to the basal diets to obtain five dietary true ileal digestible lysine concentrations for each trial.

\textsuperscript{b}Partially replaced by sodium carbonate to maintain an identical electrolyte balance (Na + K –Cl) among the treatments.

\textsuperscript{c}Partially or totally removed to make the five treatment diets isonitrogenous.

\textsuperscript{d}Contributed per kilogram of diet: 4,400 IU of vitamin A; 1,100 IU of vitamin D\textsubscript{3}; 22 IU of vitamin E; 6.6 g of riboflavin; 18 mg of d-pantothenic acid; 33 mg of niacin; 10.0 \mu g of vitamin B\textsubscript{12}.

\textsuperscript{e}Contributed in part per million of diet: Zn, 75.0; Fe, 87.5; Mn, 30; Cu, 8.8; I, 1.0.

\textsuperscript{f}Contributed per kilogram of diet: 44 mg tylosin.

\textsuperscript{g}Calculated from the analyzed lysine concentration by using the equation in NRC (1998). The amino acid analysis was performed in a commercial laboratory (Experiment Station Chemical Laboratories, Columbia, MO 65211).
Table 2. The response of plasma urea nitrogen (PUN) to dietary lysine concentrations

<table>
<thead>
<tr>
<th>Trial</th>
<th>Item</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lysine(^a), %</td>
<td>0.732</td>
<td>0.798</td>
<td>0.873</td>
<td>0.948</td>
<td>1.032</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>PUN(^b), mg/dl</td>
<td>11.14</td>
<td>10.92</td>
<td>9.53</td>
<td>9.06</td>
<td>8.92</td>
<td>1.45</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>Lysine, %</td>
<td>0.557</td>
<td>0.657</td>
<td>0.737</td>
<td>0.817</td>
<td>0.897</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>11.59</td>
<td>11.65</td>
<td>10.44</td>
<td>8.25</td>
<td>9.09</td>
<td>1.60</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>Lysine, %</td>
<td>0.541</td>
<td>0.621</td>
<td>0.701</td>
<td>0.781</td>
<td>0.861</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>10.88</td>
<td>10.41</td>
<td>10.71</td>
<td>8.99</td>
<td>8.34</td>
<td>1.71</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>Lysine, %</td>
<td>0.404</td>
<td>0.484</td>
<td>0.564</td>
<td>0.644</td>
<td>0.724</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>12.41</td>
<td>10.23</td>
<td>11.72</td>
<td>10.50</td>
<td>10.18</td>
<td>1.62</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>Lysine, %</td>
<td>0.304</td>
<td>0.384</td>
<td>0.564</td>
<td>0.544</td>
<td>0.624</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN, mg/dl</td>
<td>12.99</td>
<td>12.81</td>
<td>11.42</td>
<td>11.31</td>
<td>10.53</td>
<td>1.07</td>
<td>0.16</td>
</tr>
</tbody>
</table>

\(^a\)True ileal digestible lysine concentration.

\(^b\)Least square mean of plasma urea nitrogen (PUN) corrected by using pretreatment PUN as a covariate.

\(^c\)Standard error of the mean (n = 4).

\(^d\)Probability of obtaining such differences in PUN if dietary lysine has no effect on PUN concentration.
Table 3. Comparison of the lysine requirement estimates between Exp. A and Exp. B

<table>
<thead>
<tr>
<th>Item</th>
<th>Exp.B</th>
<th>Exp.A</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW*, kg</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>ADFI*, kg</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Lysine requirement, %</td>
<td>0.93 ± 0.10</td>
<td>0.85 ± 0.01</td>
</tr>
<tr>
<td>Lysine requirement*, g/d</td>
<td>15.8 ± 1.7</td>
<td>16.2 ± 0.2</td>
</tr>
<tr>
<td>BW*, kg</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>ADFI*, kg</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Lysine requirement, %</td>
<td>0.82 ± 0.06</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>Lysine requirement*, g/d</td>
<td>18.9 ± 1.4</td>
<td>18.2 ± 0.5</td>
</tr>
</tbody>
</table>

*Expressed as per pig basis.
Figure 1. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine increment in PIC barrows weighing from 29.3 to 33.7 kg (Trial 1). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was 0.93 ± 0.10% true ileal digestible lysine.
Figure 2. Use of a two-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine increment in PIC barrows weighing from 47.0 to 52.0 kg (Trial 2). The points represented least square PUN means corrected by using pretreatment PUN as a covariate, and the lines were fitted broken lines. The estimated lysine requirement was $0.82 \pm 0.06\%$ true ileal digestible lysine.
CHAPTER 5. APPLICATION OF A REPEATED LATIN SQUARE DESIGN TO INCREASE THE PRECISION FOR ESTIMATING THE LYSINE REQUIREMENTS OF PIGS BY USING PLASMA UREA NITROGEN AS A RAPID RESPONSE CRITERION

A Paper Prepared for Submission to the Journal of Animal Science

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ABSTRACT

Twenty PIC (327 x C22) barrows with an initial BW of 45.2 kg were used to estimate the true ileal digestible lysine requirements at three BW ranges: 60 to 80, 80 to 100, and 100 to 120 kg (Trials 1, 2 and 3, respectively) by using plasma urea nitrogen (PUN) as a rapid response criterion. The trials were designed as four 5 x 5 Latin squares. The dietary treatments were five true ileal digestible lysine concentrations: 0.50, 0.58, 0.66, 0.74, 0.82% in Trial 1, 0.35, 0.43, 0.51, 0.59, 0.67% in Trial 2, and 0.33, 0.41, 0.49, 0.57, 0.65% in Trial 3. Each trial lasted for 20 d and was divided into five 4-d periods. The five dietary treatments were allotted randomly within each square with pigs as rows and periods as columns. Pigs were individually penned and each was restricted to 2.6, 2.8, and 3.0 kg treatment diets per day during Trials 1, 2, and 3, respectively. Between 0730 and 0800 of the last day of each period, pigs were bled and plasma urea nitrogen (PUN) concentrations were determined. A one-slope broken-line model was used to estimate the lysine requirements. The results indicated each barrow required 19.9 ± 0.57, 17.0 ± 0.47, and 18.1 ± 0.64 g true ileal digestible lysine/d at BW ranges of 59 to 78, 78 to 95, and 96 to 115 kg, respectively. Because of the rapid feed consumption in Trial 3, the last requirement might be
overestimated. In summary, repeated Latin square design increased the precision of estimating lysine requirements, compared to a completely randomized design.

Key words: Lysine Requirement, Plasma Urea Nitrogen (PUN), Latin Square, Finishing Pigs

Introduction

When pigs are fed a series of diets with different lysine concentrations from deficient to excessive, plasma urea nitrogen (PUN) concentration decreases until the lysine requirement is met (Brown and Cline, 1974; Kaji and Furuya, 1987; Coma et al, 1995a). By fitting the response to a broken-line model the lysine requirement can be estimated. Coma et al. (1995c) discovered that pretreatment PUN was closely related to treatment PUN and could be used as a covariate to correct PUN variation not related to lysine adequacy. Results from our previous experiment (Exp. A) indicated that pretreatment PUN correction reduced the experimental errors by approximately 57%. The correction adjusted each treatment PUN mean to the value that it would have been expected to have if all treatments had the same pretreatment PUN mean, and thus, removed the effect of variation in pretreatment means between treatments. The adjusted means were used to complete the broken-line analysis for lysine requirement estimation. Therefore, pretreatment PUN correction increased the precision of lysine requirement estimation.

When PUN is used as a response criterion to estimate the lysine requirement, variation in PUN response to lysine increments also influences the precision of lysine requirement estimation. Wilkinson et al. (1982) reported a considerable variation in PUN response to lysine concentrations among sows. Because of the variation, they failed to obtain valid lysine requirement estimates in two experiments. In our previous experiments (Exp. A and B), it was found that after pigs reached approximately 70 kg BW, in individually penned
situation the precision of the lysine requirement estimation decreased, and for group penned pigs, PUN response did not permit any valid estimation of requirements. It is speculated that the decrease might be caused by an increase in the variation in PUN response to lysine additions.

Because PUN re-equilibrates within 3 d after a change in lysine concentration (Kaji and Furuya, 1987; Coma et al., 1995a; 1996), it is possible to measure PUN responses to lysine additions within each individual pig. Measuring and examining PUN response within each pig would control PUN variations both related to and not related to lysine adequacy. Each animal receives all treatments in a sequence, thus the number of experimental units is increased. The control of PUN variation and increase of the experimental units would increase the precision of the lysine requirement estimation. In this experiment (Exp. C), three trials were conducted to estimate the true ileal digestible lysine requirements of PIC (327 × C22) barrows over the BW ranges of 60 to 80, 80 to 100, and 100 to 120 kg, by measuring PUN responses to lysine additions within each pig. A repeated 5 × 5 Latin square design was used for each trial.

From Exp. A it was noticed that as pigs grew, variation in ADFI increased, and that the effect of ADFI on PUN was not consistent. Therefore, limiting feed intake to 90% of the ad libitum level (NRC, 1998) was used to reduce the effect of ADFI on the lysine requirement estimation.

**Materials and Methods**

*Dietary treatments*

In each trial, the treatments were five equally spaced true ileal digestible lysine concentrations, of which the third concentration was projected to be near the lysine
requirement predicted by the NRC (1998) for pigs with an average lean growth rate of 350 g/d. Corn, soybean meal, DL-methionine, L-isoleucine, L-threonine, L-tryptophan, and L-valine were used to formulate a basal diet containing the lowest lysine concentration, with the other essential AA being at or above their ideal ratios (NRC, 1998) to the highest lysine concentration. Lysine additions were achieved by supplementation with crystalline L-lysine-HCL, and the utilization of free lysine was assumed to be 100%. The basal diet was sampled and ground through a 1-mm screen for total amino acid analysis. The true ileal digestible lysine concentration was calculated from the analyzed lysine concentration by using the equation given by NRC (1998). The composition of the basal diet in each trial is listed in Table 1. The five true ileal digestible lysine concentrations were 0.50%, 0.58%, 0.66%, 0.74%, and 0.82% in Trial 1, 0.35%, 0.43%, 0.51%, 0.59%, and 0.67% in Trial 2, and 0.33%, 0.41%, 0.49%, 0.57%, and 0.65% in Trial 3, respectively.

L-glutamic acid was used to make the five treatment diets isonitrogenous. Sodium carbonate was used to make the diets identical in electrolyte balance (Na + K – Cl) thus preventing a dietary acid-base difference from affecting PUN (Cai and Zimmerman, 1995).

Animals and experimental design

Twenty PIC (327 x C22) with an initial BW of 47.2 ± 3.7 kg were used in the three trials. Four extra pigs with BW similar to the experimental animals were housed in the same room for replacement purposes. The pigs were individually penned. The pens were 0.6 x 2.2 m in dimensions, had steel slatted flooring, and each contained a stainless steel self-feeder and a nipple drinker. The room was cleaned every 4 d, mechanically ventilated and the room temperature ranged between 17 to 24°C.
The trials were conducted as repeated 5 × 5 Latin square designs. Each trial consisted of four squares, and each square contained five pigs weighed approximately 60 kg in Trial 1, 80 kg in Trial 2, and 100 kg in Trial 3. Each square lasted for 20 d and was divided into five 4-d periods. The five dietary treatments were allotted randomly within each square with pigs as rows and periods as columns.

Pigs were limited in feed intake to approximately 90% of the ad libitum amounts predicted by NRC (1998): 2.6, 2.8, and 3.0 kg/d/pig in Trials 1, 2 and 3, respectively. Pigs were fed once per day, feed waste was collected and feed intake recorded daily. On the last day of each 4-d period between 0730 and 0800, the pigs were bled via an orbital sinus and approximately 10 ml blood was taken from each pig. Body weight at the beginning and the end of the trials were recorded. At the end of Trial 3, pigs were measured by ultrasound at the 10th rib for fat depth and loin muscle area to estimate the average carcass lean growth rate over the growing-finishing period.

Plasma was harvested from blood samples by centrifugation and stored at -20°C until analyzed. The PUN concentration was determined by colorimetrically measuring the product formed in the direct reaction of urea and diacetyl monoxime, as described by Marsh et al. (1965).

Statistical analysis

The data were analyzed using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) to estimate the variance components associated with pigs and residual. The model statement included square, period and lysine concentration. Pig was considered as the random subject and a compound symmetry covariance structure (Neter et al., 1996) was used to describe the covariance structure between the observations within each pig.
To estimate the lysine requirement, PUN response versus lysine intake (grams/day) was fitted to a broken-line model with NLMIXED procedures of SAS. The random subject and the covariance structure were specified the same as above.

The lean growth curve of the pigs over the growing-finishing period was developed by performing a polynomial least square regression of lean growth rate on BW by using GLM procedures of SAS. The lean growth rates were derived from the lysine requirements estimated in Exp. A and C (individually penned pigs).

**Results and Discussion**

*Estimates of the lysine requirements*

*Trial 1.* The average BW at the beginning and the end of the trial were 59.1 ± 0.9 and 78 ± 1.6 kg, respectively. The average BW was 69 kg. The data from the pigs with daily feed intake less than 2.0 kg were removed, resulting in 65 observations. PUN response versus lysine intake along with the fitted broken lines are illustrated in Figure 1, where PUN observations from the same pig were represented by a particular symbol. As lysine intake increased, PUN decreased and then reached a plateau. By fitting the PUN response to a two-slope, broken-line regression, the requirement was estimated to be 20.2 ± 1.82 g true ileal digestible lysine/d/pig. When the response was fitted to a one-slope, broken-line model, however, the estimate was 19.9 ± 0.57 g/d/pig. The decrease of standard error for lysine requirement (from 1.82 to 0.57) indicated that a one-slope broken-line model fit the data better than a two-slope model (Motulsky and Ransnas, 1987). From Figure 1 it can be seen that linear decrease and plateau forms fit the PUN responses very well. Therefore, a one-slope, broken-line model was used to estimate lysine requirements in the three trials.
**Trial 2.** The BW ranged from $78 \pm 1.7$ to $95 \pm 2.3$ kg with an average BW of 87 kg during the trial period. One pig died and two pigs had FI less than 1 kg/d, therefore, a total of 85 observations were used for data analysis. PUN response with the fitted broken lines is illustrated in Figure 2. The daily true ileal digestible lysine requirement was estimated to be $17.0 \pm 0.47$ g/pig.

**Trail 3.** The initial and final BW were $96 \pm 1.7$ and $115 \pm 2.2$ kg, respectively. The average BW was 106 kg. A total of 90 valid PUN measurements were obtained in this trial. PUN response versus lysine intake is shown in Figure 3. The broken-line regression indicated that pigs at this stage of growth required $18.1 \pm 0.62$ g true ileal digestible lysine/d/pig. However, it was observed that in Trial 3, pigs consumed the allotted feed rapidly. Consequently, the obtained requirement might have been overestimated (Batterham, 1974; Batterham and O’Neill, 1978; Batterham and Murison, 1981).

Based on the real-time ultrasound measurements on 10th rib fat depth and 10th rib loin eye area, the average fat-free lean growth of PIC ($327 \times C22$) barrows over the growing-finishing period was calculated (NRC, 1998) to be 348 g/d. The dressing percentage was assumed as 74%.

**Precision of the estimation for lysine requirements**

Variance analyses indicated that measuring PUN responses to lysine additions within individual pig reduced the experimental error from 3.65 to 1.02 in Trial 1, from 2.18 to 0.68 in Trial 2, and from 1.84 to 0.90 in Trial 3. The experimental errors were reduced by approximately 64%. In Exp. A, it was found that the experimental errors were reduced by approximately 57% via pretreatment PUN correction. In comparison, measuring the response within each pig was more efficient because it controlled PUN variations both related and not
related to lysine adequacy, and only the latter part was adjusted by pretreatment PUN
correction. What was more important was that Latin square design increased the number of
experimental units compared with a completely randomized design. As a result, the precision
of the estimation for lysine requirements increased in Exp. C, compared to the precision in
Exp. A. The standard errors of the lysine requirement estimates at approximately 70, 90, and
110 kg BW were 0.57, 0.47, 0.62 in Exp. C, and the corresponding values in Exp. A were
1.5, 0.8, 7.1, respectively.

Period effect on PUN

To measure the responses of PUN versus dietary lysine concentrations on individual
pigs requires that each animal be fed all treatment diets in a sequence over a period of time.
Therefore, the period effect on PUN had to be considered. Coma et al. (1996) reported that in
lactating sows, PUN concentrations were not affected by the period (P > 0.50). Fuller and
Garthwaite found that in growing pigs, nitrogen retention rate (expressed as g/kg BW^{0.75}) did
not change significantly over a 60 d period. On the other hand, the graph of PUN in growing
pigs, reported by Coma et al. (1995a), indicated that PUN increased slightly over a 20-d
period. Other researches also showed that PUN increased as pigs grew (Coma et al., 1995c;
Chen et al., 1999). The increase might be because of an increase in feed intake over time. To
minimize the effect of feed intake on PUN, the pigs were limited to the same amount of feed
during each trial. In addition, even though there was a period effect on PUN, the effect would
be averaged because of the balanced structure of Latin square design. The variance analyses
showed no significant period effect in Trials 1 and 2 (P = 0.21). However, the effect was
significant in Trial 3 (P = 0.007). To examine the period effect in detail, a plot of PUN versus
period was made for Trail 3 and illustrated in Figure 4. From the graph, it can be seen that
PUN increased over the trial period, especially from period 3 to period 4, at that time the pigs would weigh approximately 110 kg. Because the feed intake was equalized over the trial, the increase in PUN might imply that the lysine requirement of the pigs decreased over the trial period. However, why there was a sharp increase in PUN from period 3 to period 4 was not clear. Even though the period effect on PUN was highly significant, the magnitude of the effect was very small compared to the treatment effect (5 versus 85 in F-value). Therefore, the period effect on PUN should not have influenced the lysine requirement from the broken-line analysis.

_Effects of limiting feed intake on protein synthesis and free lysine utilization_

In this experiment pigs were limited in feed intake to approximately 90% of the ad libitum levels predicted by NRC (1998), therefore, energy intake was limited. However, the reduced energy intake should not have a negative impact on lean growth of the pigs. It was indicated that the energy requirement for the maximum protein accretion of finishing pigs was below the appetite level (Campbell et al., 1984, Dunkin et al., 1986). Campbell et al. (1985) reported that the protein accretion rate of pigs between 48 to 90 kg BW reached a plateau at about 80% of ad libitum energy intake. Coma et al. (1995b) further illustrated that some decrease in protein deposition caused by decreased feed intake in finishing pigs can be overcome by increasing dietary amino acid concentrations.

Pigs in this experiment were fed once daily. Experiments with swine (Batterham, 1974; Batterham and O’Neill, 1978; Batterham and Murison, 1981) indicated that the utilization of free lysine in a once-per-day feeding regimen was only 50% of the utilization in pigs having continuous access to feed. Therefore, assuming 100% utilization of free lysine for pigs fed once per day may overestimate the lysine requirement. However, in their
experiments, the pigs that received once-per-day feeding had been trained to consume the assigned amount of feed in less than 1 h. In this way the difference of the absorption rates between free and intact AA was enlarged. As a consequence, the utilization of free lysine in pigs fed once-per-day may be underestimated in their experiments. In Trials 1 and 2 of this experiment, most pigs had access to feed for about 8 h after feeding and some pigs did not consumed the allotted feed in 24 h. Baker and Izquierdo (1985) found that feeding twice a day in chickens did not reduce utilization of lysine. Batterham (1984) reported a similar result in swine. There is evidence in the literature that animals may conserve lysine during periods of inadequate lysine ingestion and thus prevent a reduction in the efficiency of lysine utilization (Yamashita and Ashida, 1969; Chu and Hegsted, 1976). Therefore, the assumption of 100% utilization in this experiment might be valid. However, in Trial 3 the pigs consumed their feed more rapidly, most finished their allotted amount in less than 4 h. As a result, the lysine requirement at this stage might have been overestimated. In the future studies, feeding pigs twice a day would be recommended.

**Lysine requirements over the growing-finishing period**

Combining the results from Exp. A and C, there were seven valid lysine requirement estimates for individually penned PIC (327 × C22) barrows over the growing-finishing period (Table 2). The estimate of Trial 3 in Exp. C was not included because we didn’t know to what extent the requirement might have been overestimated. Each requirement (grams/day) minus the lysine requirement for maintenance (0.036 g/ BW^{0.75}) was the requirement for protein accretion. Applying the assumptions of NRC (1998) that 0.12 g true ileal digestible lysine is required for each gram of whole body protein accretion, and that the conversion of whole body protein to lean tissue is 2.55, the lean growth rates were calculated.
to be 334, 372, 404, 414, 340, 327, and 276 g/d at 33, 52, 69, 72, 87, 93, and 113 kg BW, respectively. The lean growth rates at 69 and 72, 87 and 93 kg BW were averaged to get the lean growth rates at approximately 70 and 90 kg BW. Using the estimates at approximately 30, 50, 70, 90, and 110 kg BW, the average lean growth rates over growing–finishing period was calculated to be 345 g/d. Combining it with the estimate from the ultrasound measurements (348 g/d), an average lean growth rate of 347 g/d was used.

Each lean growth rate was scaled as the proportion of the average lean growth rate of the seven estimates, and the resulting data were used to perform a polynomial least square regression on BW. A cubic response was derived, which was expressed as:

\[
Y = (-0.11606 + 0.04895X - 0.0005855X^2 + 0.000001963X^3) \times 347,
\]

where Y was the lean growth rate in gram/day, X was the BW in kilogram. The \(R^2\) was 0.89. The plot of the lean growth rates and the fitted curve are illustrated in Figure 4.

Implications

PIC (327 x C22) barrows required 19.9 ± 0.57 and 17.0 ± 0.47 g true ileal digestible lysine/d/pig at BW ranges of 59 to 78 and 78 to 95, respectively. Repeated Latin square allowed a relatively precise estimate for lysine requirement of swine when PUN was used as a rapid response criterion. PUN responses versus lysine concentration fit a one-slope broken-line model, for which the NLMIXED procedures of SAS were recommended. The lean growth curve of the barrows over the growing-finishing period could be expressed as

\[
Y = (-0.11606 + 0.04895X - 0.0005855X^2 + 0.000001963X^3) \times 347,
\]

where Y is the lean growth rate in grams/day, and X is BW in kilograms.
Literature Cited


Table 1. Composition of basal diets\textsuperscript{a}, as-fed basis

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>83.20</td>
<td>88.89</td>
<td>89.85</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>12.79</td>
<td>6.96</td>
<td>6.12</td>
</tr>
<tr>
<td>Fat</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.66</td>
<td>0.61</td>
<td>0.64</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.67</td>
<td>0.87</td>
<td>0.74</td>
</tr>
<tr>
<td>Sodium chloride\textsuperscript{b}</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Glutamic acid\textsuperscript{c}</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.15</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.09</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>0.05</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>L-Valine</td>
<td>0.06</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin premix\textsuperscript{d}</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Trace mineral premix\textsuperscript{e}</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Tylan\textsuperscript{r}</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
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</table>

Calculated analysis

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>14.0</td>
<td>11.7</td>
<td>11.4</td>
</tr>
<tr>
<td>DE, kcal/kg</td>
<td>3513</td>
<td>3500</td>
<td>3497</td>
</tr>
<tr>
<td>True ileal digestible lysine\textsuperscript{g}, %</td>
<td>0.50</td>
<td>0.35</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Graded amounts of L-lysine-HCl were added to the basal diet to obtain five dietary true ileal digestible lysine concentrations in each trial.

\textsuperscript{b} Partially replaced by sodium carbonate to maintain an identical electrolyte balance (Na + K - Cl) among the treatments.

\textsuperscript{c} Partially or totally removed to make the five treatment diets isonitrogenous.

\textsuperscript{d} Contributed per kilogram of diet: 6,600 IU of vitamin A; 1,650 IU of vitamin D\textsubscript{3}; 33 IU of vitamin E; 9.9 g of riboflavin; 26 mg of d-pantothenic acid; 50 mg of niacin; 33 \mu g of vitamin B\textsubscript{12}.

\textsuperscript{e} Contributed in part per million of diet: Zn, 75.0; Fe, 87.5; Mn, 30; Cu, 8.8; I, 1.0.

\textsuperscript{f} Contributed per kilogram of diet: 44 mg tylosin.

\textsuperscript{g} Calculated from the analyzed lysine concentration by using the equation in NRC (1998). The amino acid analysis was performed in a commercial laboratory (Experiment Station Chemical Laboratories, Columbia, MO 65211).
Table 2. Lean growth rates over the growing-finishing period

<table>
<thead>
<tr>
<th>BW(^a), kg</th>
<th>True ileal digestible lysine requirement(^b), g/d</th>
<th>Requirement for maintenance(^c), g/d</th>
<th>Requirement for protein accretion(^d), g/d</th>
<th>Lean growth rate(^e), g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>16.2 ± 0.2</td>
<td>0.5</td>
<td>15.7</td>
<td>334</td>
</tr>
<tr>
<td>52</td>
<td>18.2 ± 0.5</td>
<td>0.7</td>
<td>17.5</td>
<td>372</td>
</tr>
<tr>
<td>69</td>
<td>19.9 ± 0.5</td>
<td>0.9</td>
<td>19.0</td>
<td>404</td>
</tr>
<tr>
<td>72</td>
<td>20.4 ± 1.5</td>
<td>0.9</td>
<td>19.5</td>
<td>414</td>
</tr>
<tr>
<td>87</td>
<td>17.0 ± 0.5</td>
<td>1.0</td>
<td>16.0</td>
<td>340</td>
</tr>
<tr>
<td>93</td>
<td>16.5 ± 0.8</td>
<td>1.1</td>
<td>15.4</td>
<td>327</td>
</tr>
<tr>
<td>113</td>
<td>14.3 ± 7.1</td>
<td>1.3</td>
<td>13.0</td>
<td>276</td>
</tr>
</tbody>
</table>

\(^a\)Average BW over the trial period.
\(^b\)Estimate ± standard error.
\(^c\)Daily maintenance requirement for the true ileal digestible lysine was assumed to be 0.036 g/kg BW\(^{0.75}\).
\(^d\)Total requirement – requirement for maintenance.
\(^e\)(Lysine requirement for protein accretion ÷ 0.12) × 2.55.
Figure 1. Use of a one-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine intake in PIC barrows weighing from 59 to 78 kg (Trial 1). Each symbol represented the five PUN concentrations from the same pig, and the lines were fitted broken lines. The true ileal digestible lysine requirement was estimated to be 19.9 ± 0.57 g/d.
Figure 2. Use of a one-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine intake in PIC barrows weighing from 78 to 95 kg (Trial 2). Each symbol represented the five PUN concentrations from the same pig, and the lines were fitted broken lines. The true ileal digestible lysine requirement was estimated to be 17.0 ± 0.47 g/d.
Figure 3. Use of a one-slope, broken-line model to describe the response of plasma urea nitrogen (PUN) to dietary lysine intake in PIC barrows weighing from 96 to 115 kg (Trial 3). Each symbol represented the five PUN concentrations from the same pig, and the lines were fitted broken lines. The true ileal digestible lysine requirement was estimated to be 18.1 ± 0.64 g.
Figure 4. The effect of period on PUN in Trial 3. The BW corresponding to the sharp increase in PUN would be approximately 110 kg.
Figure 4. The plot of observed lean growth rates with the fitted lean growth curve for PIC barrows over the growing-finishing period. The curve was expressed as:

\[ Y = (-0.11606 + 0.04895X - 0.000585X^2 + 0.000001963X^3) \times 347, \]

where \( Y \) was the lean growth rate in grams/day, \( X \) was the BW in kilograms. The \( R^2 \) was 0.89. The average lean growth rate was 347 g/d.
CHAPTER 6. GENERAL SUMMARY

General Summary

Three experiments (Exp. A, B and C) were conducted to determine the lysine requirements of PIC (327 × C22) barrows over the growing-finishing period, using plasma urea nitrogen (PUN) as a rapid response criterion. From the results, the procedure of determining the lean growth curve for growing-finishing pigs from the lysine requirements was developed. In Exp. A, the lysine requirements of individually penned barrows at approximately 30, 50, 70, 90, and 110 kg body weights were determined by using a PUN technique developed by Coma et al. (1995a). Pretreatment PUN was used as a covariate to correct PUN variation not related to lysine adequacy. A two-slope, broken-line model was used to estimate the lysine requirement. The results indicated that the true ileal digestible lysine requirements of pigs at 33, 52, 72, 93, 113 kg body weights were 16.5 ± 0.2, 18.2 ± 0.5, 20.2 ± 1.5, 16.7 ± 0.8, and 14.4 ± 7.2 g/d/pig, respectively. The precision for estimating the lysine requirements decreased after pigs reached approximately 70 kg body weight.

Exp. B determined the lysine requirements of PIC barrows under group penned situation. Two pigs from each pen were selected for PUN measurements. The results indicated that each PIC barrow required 15.8 ± 1.7 and 18.9 ± 1.4 g/d true digestible lysine at 32 and 50 kg body weights, respectively. PUN responses to dietary lysine concentrations failed to give valid lysine requirements for pigs at approximately 70, 90 and 110 kg body weights.

The precision of the lysine requirement estimation in Exp. B was lower than that in Exp. A. The reason might be that from each pen of five pigs, only two pigs with the body weight nearest to the pen average were selected for PUN measurements. Exp. B was
originally designed as a randomized complete block, in which pigs were blocked on litter and initial body weight. However, among the selected pigs in the same block, some were littermates and some were not, therefore, the covariance structure between pigs within each block became complicated, and the covariance structure differed from block to block. The complicated covariance structure increased the variation of PUN response to dietary lysine concentration, thereby decreased the precision of the lysine requirement estimation. Increasing the number of animals used for PUN measurements might increase the precision of lysine requirement estimation for group penned pigs.

Results of Exp. A and B indicated that after pigs reached approximately 70 kg BW, in individually penned situation the precision of lysine requirement estimation decreased, and for group penned pigs. PUN response did not permit any valid estimation of requirements. The possible explanation might be that PUN variation increased as pigs grew. When PUN is used as a response criterion to estimate the lysine requirement, the variation in PUN can be divided arbitrarily into two parts, the variation not related to lysine adequacy and the variation related to lysine adequacy. The variation not related to lysine adequacy refers to PUN differences among animals when they were fed a diet with the same and adequate lysine concentration. This kind of variation can be partially corrected by using pretreatment PUN as a covariate. The variation related to lysine adequacy means that different animals have different PUN responses to the same series of lysine additions. Wilkinson et al. (1982) reported a considerable variation in PUN response to lysine concentrations among sows. Fuller and Garthwaite (1993) fit different models to describe the response of protein accretion to dietary lysine supply for 14 pigs. This kind of variation can not be corrected by pretreatment PUN correction. Results from Exp. A indicated that pretreatment PUN
correction reduced the experimental errors by 48, 49, 53%, 57%, and 77% for the pigs at 33, 52, 72, 93, 113 kg body weights, respectively. Because the effect of pretreatment PUN correction did not decrease as pigs grew, the decrease of the precision of lysine requirement estimation for pigs beyond 70 kg must have been caused by an increased variation in PUN response to dietary lysine concentrations. The increased variation in PUN response in heavy pigs might be caused by large variations in lysine requirement, body weight and ADFI among the animals.

Because PUN re-equilibrates within three days after a change in dietary lysine concentration (Kaji and Furuya, 1987; Coma et al, 1995a; 1996), it is possible to measure PUN responses to lysine additions within each individual pig. Measuring and examining PUN response within each pig would control PUN variations both related to and not related to lysine adequacy. Each animal receives all treatments in a sequence, thus the number of experimental units is increased. The control of PUN variations and increase of the experimental units would increase the precision of the lysine requirement estimation. Based on the above hypotheses, in Exp. C three trials were conducted to estimate the true ileal digestible lysine requirements of PIC barrows over body weight ranges of 60 to 80, 80 to 100, and 100 to 120 kg. A repeated 5 x 5 Latin square design was used and feed intake was limited to 90% of the ad libitum level during each trial.

Results of Exp. C showed that the true ileal digestible lysine requirements of PIC (327 x C22) barrows were 19.9 ± 0.57, 17.0 ± 0.47, and 18.1 ± 0.64 g/d/pig at the body weight ranges of 59 to 78, 78 to 95, and 96 to 115 kg, respectively. During the last trial period, pigs consumed feed very rapidly, as a consequence, the last requirement might have been overestimated. The standard errors of the requirements indicated that measuring PUN
responses to dietary lysine additions within each pig increased the precision of the estimation. The broken-line analysis in this experiment revealed that a one-slope, broken-line model fit the PUN response better than a two-slope, broken-line model.

From seven lysine requirements at different body weights estimated in Exp. A and C, the lean growth rates were calculated, and a least square regression was performed to get the lean growth curve for individually penned PIC (327 ×C22) barrows over the growing-finishing period. The curve was expressed as:

\[ Y = (-0.11606 + 0.04895X - 0.0005855X^2 + 0.000001963X^3) \times 347, \]

where \( Y \) was the lean growth rate in grams/day, and \( X \) was body weight in kilograms.

To determine the lean growth curve from the lysine requirements estimated by using PUN as a response criterion, at least five requirements at different body weights over the growing-finishing period are required. Either the PUN technique (Coma et al., 1995a) or a repeated Latin square design can be used to estimate the lysine requirements for growing pigs. For finishing pigs, a repeated Latin square design is recommended. An ad libitum feeding is recommended for growing pigs, while an equalized feed intake, twice a day feeding system might be used for finishing pigs. A one-slope, broken-line model is recommended to estimate the lysine requirement from PUN response. From the lysine requirements, the lean growth rates can be calculated by using the assumptions of NRC (1998) and these data can be used to derive the lean growth curve by using least square regression.
Literature Cited


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