Use of plasma urea nitrogen as a rapid response criterion to estimate the lysine requirements of growing and lactating pigs

Jaime Coma
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Use of plasma urea nitrogen as a rapid response criterion
to estimate the lysine requirements
of growing and lactating pigs

by

Jaime Coma

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Animal Nutrition

Approved:
Signature was redacted for privacy.

In Charge of Major Work
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For the Major Department
Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1995
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CHAPTER 1. INTRODUCTION

The growing pig requires dietary amino acids primarily for maintenance and for protein accretion. Because potential protein accretion, maintenance requirement, and voluntary feed intake change as the animal matures, the dietary amino acid requirements of a growing pig changes continuously with body weight. However, most of the response criteria used to assess amino acid requirements in domestic animals provide an estimate over a considerable weight range. Moreover, not all response criteria result in the same estimate of the amino acid requirement. Direct or indirect assessments of protein accretion (i.e., nitrogen retention) provide precise assessments of amino acid adequacy. Some response criteria that assess protein accretion allow short-term determinations of dietary amino acid requirements but specialized instruments are needed and costs are high. Plasma urea nitrogen (PUN) concentrations are inversely related to quality of the dietary protein and their measurement is rapid and simple. Therefore, PUN concentration has been extensively used in long-term treatment periods to provide an estimate of the lysine requirement for growing, gestating, and lactating swine over a weight or time interval. Because nitrogen metabolism has a rapid response to changes in dietary amino acid concentrations, PUN concentration was hypothesized to have potential value as a rapid response criterion in determination of amino acid requirements. The five experiments summarized in Chapter 3 were conducted to evaluate the potential use of PUN in short-term trials to assess the dietary lysine required to maximize nitrogen utilization by pigs of a specific body weight. The experiment reported in Chapter 2 was conducted to refine the response of PUN to dietary lysine from variation not related to amino acid adequacy and to investigate the relationship between PUN and protein accretion.
Lysine was chosen as the amino acid to investigate because it is the first-limiting amino acid for swine in all the cereal grains with the exception of corn. In corn, some research has indicated that lysine is first-limiting, other indicates tryptophan, whereas in still other research, lysine and tryptophan are co-limiting. Lysine is also first-limiting in most plant protein sources; exceptions are soybean and canola meals. Because of the deficiency of lysine in most dietary ingredients and the high concentration of lysine in muscle tissue deposited by the growing pig and in milk synthesized by the lactating sow, lysine is the most important amino acid in formulation of practical swine diets.

The performance of growing pigs is directly related to their daily feed intake. A number of physiological, environmental, and dietary factors influence feed intake (i.e., energy and amino acids intakes). Under conditions of protein adequacy, protein accretion is linearly related to energy intake from near maintenance to ad libitum intakes in young pigs. But in later stages of growth (body weights greater than 50 kg), protein deposition response to increasing energy intake reaches a plateau at energy intakes below the appetite level. Because the relationship between energy intake and protein accretion depends on the stage of growth, the effect of a reduction in feed intake (i.e., energy intake) on daily lysine required to maximize protein accretion was hypothesized to be different depending on the body weight of the animal. In the experiment reported in Chapter 3, PUN and average daily gain were used to assess the effect of a reduction in feed intake on the lysine requirement of pigs at two stages of growth: growing and finishing phases.

The lactating sow requires dietary amino acids for maintenance and for synthesis of milk protein. Current ARC (1981) and NRC (1988) recommendations are based on research in which sows nursed seven to eight pigs. In modern swine production, litters of nine and ten pigs are very common. The increase in litter size and, therefore, increase in demand of
milk production increases the dietary lysine required by the lactating sow. Plasma urea nitrogen concentration was hypothesized to have potential value to determine the lysine requirement of lactating sows. Because of the rapid response of PUN to changes in dietary amino acid concentrations, it is possible to assess the effect on PUN of different dietary lysine concentrations fed to the same sow over a lactation period. Therefore, the requirement could be determined using a small number of sows in an efficient statistical design. In Chapter 4, the dietary lysine intake required to maximize efficiency of dietary amino acid use for milk synthesis by adult sows nursing 10 pigs was estimated by using PUN as a rapid response criterion.

**Thesis Organization**

The thesis is divided into a Literature Review, four papers, and a General Summary. The papers were prepared in the style appropriate for submission to the *Journal of Animal Science*. The paper in Chapter 3 has been published in *J. Anim. Sci.* 1995. 73:472-481. The papers in Chapter 4 and 5 have been, whereas the one in Chapter 6 will be, submitted to the same journal. J. Coma is the senior author of the four papers.
CHAPTER 2. LITERATURE REVIEW

Lysine Requirement of the Growing Pig

Factorial approach

The growing pig requires dietary amino acids primarily for replacing obligatory losses (i.e., maintenance) and for protein (mostly muscle) accretion. The amino acid needs for maintenance have been estimated based on body weight (Baker et al., 1966; and Fuller et al., 1989) and body protein mass (Whittemore, 1983). Baker et al. (1966) and Fuller et al. (1989) estimated the requirement of digestible lysine for maintenance to be 25 and 38 mg/kg\textsuperscript{75}, respectively. Whittemore (1983) proposed that the maintenance requirement for protein is .004 of the body protein mass. Fuller et al. (1989) reported that 2.27 g of digestible lysine are needed for each 100 g of maintenance protein.

The dietary lysine needs for protein deposition are directly related to the rate of lean (i.e., protein) deposition achieved by the animal. There is considerable variation in the reported estimates of the efficiency of digestible lysine utilization for protein deposition. Campbell and Tavener (1988) reported that 1 g of digestible lysine supported 5.0 and 7.5 g of body protein deposition in barrows and boars, respectively, of an unimproved strain, whereas 9.0 g were supported in boars of an improved strain. Fuller et al. (1989) determined that 68 mg of digestible lysine were required to deposit 1 g of body protein (i.e., 1 g of digestible lysine required to support 14.7 g of protein deposition). Ji et al. (1991) indicated that 1 g of digestible lysine supported 9.8 g of body protein accretion in ractopamine-treated pigs (40 mg/d) whereas it only supports 6.0 g in non-treated pigs. Batterham et al. (1990b) suggested similar values for boars and gilts (11.0 and 11.2 g of protein deposited per gram of digestible lysine). Chiba et al. (1991) reported a value of 6.1
in barrows and gilts. The average of the reported efficiencies is 8.9 g of body protein deposited for each gram of digestible lysine. This equates to approx. 112.5 mg of digestible lysine required to deposit 1 g of protein. Different lysine concentrations of body protein have been reported: 6.8% (Aumaitre and Duee, 1974), 6.0% (Moughan and Smith, 1987), 6.5% (Campbell et al., 1988b), 6.5% (Batterham et al., 1990b), 6.2% (Chung and Baker, 1992a), 7.1% (Kyriazakis et al., 1993), 6.2 and 7.3% (Hahn and Baker, 1995). Using an average value of 6.5% lysine in protein deposited, the average efficiency of lysine utilization would be 57.8%. Estimates of utilization of digestible lysine ranging from 39% (Boyd et al., 1991) to 86% (Batterham et al., 1990a) have been reported in different strains and sexes.

**Empirical studies**

In the current NRC (1988) publication, empirical studies evaluating the lysine needs of the growing pig were summarized to recommend dietary lysine requirements of pigs at different stages of the life-cycle. Recent literature not included in the NRC (1988) publication is presented in Table 1. Part of the data was summarized by Kerr (1993). Growth performance and/or carcass composition were used as response criteria. When rates of protein deposition were not reported, they were calculated from carcass composition data by using formulas summarized by Kerr (1993). If carcass composition data were not available, the protein deposition characteristics of the animals were classified as high, medium and low based on rate and efficiency of body weight gain.
Table 1. Summary of studies evaluating the dietary lysine requirement, as a percentage of the diet and as a daily intake, of the growing pig

<table>
<thead>
<tr>
<th>BW, kg</th>
<th>Initial</th>
<th>Final</th>
<th>Sex</th>
<th>PD^ g/d</th>
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<td>1.20</td>
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<td>Growth Rate</td>
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<td>Krick et al., 1990b</td>
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*B = boars, G = gilts, and C = castrated males.
°Whole empty body protein deposition.

**Effects of body weight, sex and strain**

The potential protein deposition in the growing pig has been extensively described as a function of body weight (Carr et al., 1977; Black et al., 1986; Whittemore et al., 1988). At a specific body weight, the rate of protein deposition is a function of a wide variety of environmental influences (temperature, humidity, herd health, social factors, dietary amino acid and energy intake, space allowance) but is primarily determined by the sex (Whittemore et al., 1988, Hansen and Lewis, 1993b) and genetic characteristics (Campbell and Taverner, 1988; Schinckel, 1994) of the animal. Therefore, the dietary amino acid needs for optimum protein deposition depends basically on the body weight (ARC, 1981; NRC, 1988), sex (Baker et al. 1967, Watkins et al., 1977; Batterham et al.,...
1985; Yen et al., 1986a,b; Cromwell et al., 1990, Hansen and Lewis, 1993a,b) and genetic characteristics (Christian et al., 1980, Campbell and Tavemer, 1988, Stahly et al., 1991) of the pig. However, current ARC (1981) and NRC (1988) recommendations of dietary amino acid requirements do not take into consideration sex or genetic characteristics and give only a value for all pigs over a specific weight range.

The effect of sex on the dietary amino acid needs of the growing pig is highly dependent on body weight. Boars, gilts and castrates have similar rates of protein deposition up to 30 kg body weight. However, between 30 and 100 kg, boars deposit more protein than gilts and gilts similar to, or slightly greater than, barrows (Whittemore et al., 1988; Kanis and Koops, 1990; Hansen and Lewis, 1993a,b). Moreover, between 30 and 100 kg, differences occur among sexes in feed intake patterns with barrows consuming more feed than boars, and boars more than gilts. NRC (1987) described the differences in feed intake among sexes as a quadratic function for pigs above 25 kg, with a maximum difference of approximately 9%. Kerr (1993) proposed a 5% difference between boars and gilts, and a 5% difference between gilts and barrows in voluntary feed intake. Consequently, when amino acid requirements are expressed as a percentage of the diet rather than as daily intake, the differences in feed intake among sexes enlarge the differences due to protein deposition.

Small differences in the digestible lysine requirements for optimum body weight gain have been reported in boars, gilts, and castrates up to 50 kg (Batterham et al., 1985; Yen et al., 1986a; Campbell et al. 1988a). Batterham et al. (1990b), however, reported a greater lysine requirement (20%) in boars than in gilts between 20 and 45 kg, associated with differences in rates of protein deposition observed at those weights. After 50 kg body weight, boars have been reported to require from 10 to 31% more lysine than gilts.
(Williams et al., 1984; Batterham et al., 1985; Yen et al., 1986b; Campbell et al., 1988a) and gilts to require from 8 to 39% more than barrows (Baker et al. 1967, Watkins et al., 1977; Christian et al., 1980; Yen et al., 1986b; Cromwell et al., 1990, 1993; Hahn and Baker, 1994) depending on the range of BW, animals' characteristics, experiment conditions, and response criteria used.

Considerable differences in potential rates of protein deposition exist among and within genetic strains of pigs (Christian and Moeller, 1992; Walter and Christian, 1994; Schinckel, 1994). Moreover, genetic strains differ in their patterns of voluntary feed intake (Bark et al., 1988). Generally, animals with greater lean deposition capabilities exhibit their peak of energy intake at a heavier weight than those with lower rates of lean deposition. Therefore, it has been extensively demonstrated (Kornegay et al., 1973, Henry et al., 1979, Christian et al., 1980; Campbell and Taverner, 1988; Stahly et al., 1988, 1991) that protein or lysine requirements differ among genetic strains with improved (i.e., leaner) animals having a greater and more efficient protein deposition at greater lysine concentrations than those pigs with lower capabilities of protein deposition.

**Effects of environmental factors**

Environmental factors influence feed intake and metabolic processes. Body protein accretion can be affected by decreased feed intake if daily intakes of dietary amino acids and/or energy become limiting (Black and Griffiths, 1975). Therefore, environmental factors have the potential to affect the amino acid requirements of the animal for protein deposition.

The thermal environment in which a pig is maintained influences its voluntary feed intake, and therefore, the rate, efficiency and composition of gain (Verstegen et al., 1978; Noblet and LeDividich, 1982; Close and Stanier, 1984). Pigs kept in a cool environment
(relative to thermoneutrality) consume more feed and are less efficient for growth because of their elevated energy demand to produce heat required for maintaining body temperature (Holmes and Close, 1977). On the other hand, pigs in a hot environment reduce their feed intake to reduce the amount of body heat produced via digestive and metabolic processes. As a result of the effects of thermal environment on feed intake, Schenck et al. (1992a,b) reported that weanling pigs (7 to 25 kg BW) raised in a hot environment (32°C) require greater dietary lysine concentrations than pigs raised in a cool environment (20°C) to optimize performance.

Social factors such as number of pigs per pen and floor space affect performance of growing pigs. Pig density has a greater effect on growth rate than does the number of pigs per pen when the space per pig is kept constant (Heitman et al., 1961). Restricting floor space allowance decreases daily feed intake and daily gains in pigs, and this reduction is more pronounced in the weanling pig than in the growing pig or finishing pig (LeDividich, 1979; Kornegay et al., 1980, 1981; Lindvall, 1981; Lindemann et al., 1987; NCR-89 Committee; 1993). Kornegay and Notter (1984) suggested that approximately 75% of the decrease in daily gain could be explained by reductions in feed intake. However, Kornegay et al. (1993) reported that the single addition of lysine to the diet is not effective in overcoming the reduction in performance of weanling pigs (7 to 20 kg) caused by the restricted floor space allowance. Similar results have been obtained with pigs from 20 to 110 kg (Brumm, 1992, 1994; Brumm and Kerr, 1993). Therefore, the reduced lysine intake does not seem to be the only factor responsible for the decreased growth in conditions of space restriction.

Health factors also have an effect on the amino acid requirements of animals. The interaction between nutrition and immune system has been extensively described in poultry
(Klasing et al., 1987, 1991; Klasing, 1988; Klasing and Barnes, 1988; Roura et al., 1991; Piquer et al., 1993a,b). When the immune system is stimulated by the presence of an antigen, it releases cytokines that, by altering metabolic processes in the body, reduce feed intake and skeletal muscle accretion, increase basal metabolism rate and body temperature, and modify carbohydrate, protein and lipid metabolism. The result is a reduced rate of body growth, less efficient utilization of feed, and reduced protein synthesis in animals with a high stimulation of the immune system. Klasing and Barnes (1988) reported that chicks challenged with a chronic immune response had a decreased lysine and methionine requirement because of the decreased need for protein accretion when compared with non-challenged chicks. In pigs, Hale and Stewart (1979) and Hale et al. (1981, 1985) reported reduced nitrogen digestibility and N retention in pigs that were experimentally infected with worms compared with non-infected pigs. The effects of the activation of the immune system on the lysine requirement of pigs from weaning to market weight have been described by Williams et al. (1993a,b, 1994) and Stahly et al. (1994b). Based on these studies, pigs with a low activation of the immune system have a greater capacity for muscle tissue accretion and, thus, a greater dietary amino acid need than pigs with a high immune system activation.

**Relationship between Energy Intake and Protein Deposition**

Protein growth can be affected by changes in either protein or energy intake (Black and Griffiths, 1975). Campbell et al. (1984, 1985a) demonstrated that energy and protein intake have separate and different effects on protein retention. The response curve of protein retention has two phases: an initial protein-dependent phase that is followed by an energy-dependent phase. Under conditions of protein deficiency, protein retention responds linearly
to change in protein intake, but is independent of energy intake. When protein intake is adequate, protein retention is a function of energy intake and independent of protein intake.

Different relationships have been described between protein retention and energy intake (ARC, 1981). Whittemore and Fawcett (1976) proposed that under conditions of protein adequacy, the relationship between energy intake and protein deposition in pigs has a linear-plateau form. Protein deposition responds linearly to increasing energy intake until a maximum deposition is attained at which the response plateaus. Maximal lean tissue growth (plateau) has been demonstrated for pigs above 50 kg BW and is determined by intrinsic factors (Campbell et al. 1985b; Dunkin et al. 1986). But below 50 kg, because of their limited ingestive capacity, pigs are unable to consume sufficient energy to achieve their genetic potential for muscle growth. Consequently, for young pigs, protein deposition increases linearly with increasing energy intake from near maintenance to ad libitum energy intake (Campbell et al. 1975, 1986; Campbell and Dunkin, 1983a,b; Whittemore, 1986).

Because of this relationship, during the early stages of development, pigs can be given high levels of energy intake to achieve rapid growth without excessive fat deposition or a reduction in feed conversion efficiency. On the other hand, any factor that reduces feed intake or the utilization of dietary energy will restrict protein deposition (Dunkin and Black, 1987). After 50 kg body weight, the potential for muscle growth tends to be below the appetite level and, thus, the voluntary intake of energy does not limit the maximum protein deposition rate (Campbell et al. 1985b; Dunkin et al. 1986). As energy intake increases above the requirement for maximum lean growth rate, it is utilized for fat deposition, resulting in a marked increase of the lipid:protein deposition ratio, backfat thickness and the feed:lean gain ratio (Fuller and Livingstone, 1978; Moughan et al., 1987).

The relationship between energy intake and protein growth is affected by BW, sex,
strain, and administration of growth hormone and repartitioning agents (Campbell, 1988). Research reported by Dunkin et al. (1986) and Dunkin and Black (1987) suggest that the slope of the linear portion of the energy intake-protein retention curve decreases with increasing BW and that demonstrates that, as the animal progresses towards maturity, the capacity for maximal protein deposition becomes more constrained by intrinsic factors rather than nutritional factors. Campbell et al. (1985b) and Campbell and Tavemer (1988) demonstrated that the slope of the linear-response phase of protein deposition and the maximal protein deposition were less for females than for males. In the same study of Campbell and Tavemer (1988), it was demonstrated that the slope of the linear relationship was greater for faster-growing animals than for slower-growing animals. Administration of growth hormone results in an increase of the slope relating energy intake and protein deposition (Campbell et al., 1987).

Lysine Requirement of the Lactating Sow

Factorial approach

The lactating sow requires dietary amino acids for maintenance and for synthesis of milk protein. As previously indicated, different studies have assessed the digestible lysine requirement for maintenance in swine (Baker et al., 1966; Whittemore, 1983; Fuller et al., 1989), with the value of 38 mg/kg\textsuperscript{75} reported by Fuller et al. (1989) being the one most extensively used.

The quantity of digestible lysine required for milk production is directly related to the amount of milk synthesized. Estimates of the efficiency of utilization of dietary protein for milk protein synthesis are 65% (Mullan et al., 1989) and 70% (Whittemore and Morgan, 1990). Data collected by Schoenherr et al. (1989), King et al. (1989), Toner et al. (1991),
Stahly et al. (1992) and King et al. (1993), using the deuterium oxide dilution method, indicate that litters can consume an average of .95 kg/d of milk for each piglet in the litter. Assuming a .35% lysine concentration in milk (Elliott et al., 1971; Speer, 1990) and a 65 to 70% efficiency of milk protein production from dietary protein, an estimate of the digestible lysine requirement required for milk production can be obtained from litter size, that is approximately 4.75 to 5.0 g of digestible lysine for each piglet.

Alternatively, in a literature review by Pettigrew (1993), a need of 26 g of total lysine to produce the amount of milk needed for each kilogram of litter growth was suggested. The part of the total needs that is provided by mobilization of body protein needs to be considered to calculate the amount of dietary lysine that is required. As estimated by ARC (1981), the overall efficiency of dietary protein deposited as tissue protein in the sow and then mobilized for milk protein synthesis is 56%, which results from a 70% efficiency of conversion of dietary to tissue protein and a 80% efficiency of tissue to milk protein synthesis.

**Empirical studies**

There is a considerable range in published recommendations for the lysine requirement of sows. Early research indicated a requirement of less than 20 g/d (Boomegaardt et al., 1972). The ARC (1981) and NRC (1988) recommend lysine intakes of 33 and 31.8 g/d, respectively, for optimum lactational performance. Recent reports (Johnston et al., 1991; Stahly et al., 1990; 1992; Laurin et al. 1993; Monegue et al., 1993; Knabe et al., 1993; King et al., 1993; Sauber et al. 1994a) have suggested much higher requirements than the earlier recommendations. Differences in milk production between sows, attributable to factors such as litter size, parity and stage of lactation, are mostly
responsible for the variation in the published lysine requirements of the lactating sow. The amount of body tissue reserves (i.e., fat and muscle) and the ability of the sow to mobilize these reserves to support milk production (Etienne et al., 1985) also complicate the assessment of the requirements in the lactating sow. Different response criteria, as well as different experimental designs, are other factors that may explain part of the considerable variation in the published estimates (ARC, 1981).

The ARC (1981) and NRC (1988) recommendations are based on research in which sows nursed litters of seven to eight pigs with milk yields of 5 to 7 kg/d. However, modern sows nursing >9 pigs are capable of producing much greater amounts of milk with mean daily yields of >10 kg being common for first-litter sows (King et al., 1989; Sauber et al.; 1994b). Milk yields are not available in all the studies in which lysine requirement has been studied. In most circumstances, the growth of the piglets has been used as an indicator of the milk production of the sow. But milk yield of the sows can be estimated by using a conversion ratio of milk into pig gain of 3.8 g/g as reported by Noblet et al. (1986). Stahly et al. (1990; 1992) indicated that litter weight gain increased and sow weight loss decreased linearly as daily lysine intake increased from approximately 20 to 47 g/d in 190-kg sows nursing 10 to 11 pigs. In the first study (Stahly et al., 1990), a milk yield of 7.9 kg/d was estimated from the litter growth data by using the ratio reported by Noblet et al. (1986), whereas the deuterium oxide technique was used to calculate the daily milk yield in the second study (Stahly et al., 1992). In the work conducted by Johnston et al. (1991), sows nursing 9.5 to 10 pigs with an average litter growth of 2.21 kg/d (estimated milk yield = 8.3 kg/d) required a daily lysine intake of 55.0 g to optimize litter weight gain, whereas greater intakes were required to minimize BW loss in sows. Laurin et al. (1993) indicated that primiparous sows nursing 11 pigs with an average growth of 2.13 kg/d (estimated milk
yield = 8.11 kg/d) required a daily lysine intake of 42.5 g for optimum litter weight gain with greater intakes up to 57 g/d being needed for minimum BW loss. Monegue et al. (1993) reported that 200-kg sows nursing more than 11 pigs required a minimum of 45 g/d of lysine to minimize sow BW loss and maximize litter weight and daily milk yield, which was determined by using the weigh-suckle-weigh procedure. Knabe et al. (1993) reported no effect of increasing the dietary lysine intake from 34 to 51 g on lactation BW loss in sows nursing 9 to 10 pigs, however increasing lysine from 34 to 43 g resulted in increasing litter weaning weights with no further improvement after 43 g/d. In the work of King et al. (1993), first-litter sows weighing 150 kg BW required 48.1 g/d of dietary lysine to maximize N balance during early and late lactation, whereas lactational performance measured by using the deuterium oxide technique seemed to reach maximum levels at lower dietary lysine concentrations. Sauber et al. (1994a) reported a linear increase in litter weight gain as daily lysine intake increased from 28 to 55 g/d in primiparous sows nursing 14 pigs with an average growth of 2.5 kg/d (estimated milk yield = 9.5 kg/d). A summary of these studies is presented in Table 2.

Amino Acid Balance

There is evidence that the protein level of the diet can affect the amino acid requirement of the animal (Boomgaardt and Baker, 1973; Baker et al., 1975). In addition to the level of the first-limiting amino acid, the balance between individual essential amino acids, as well as between the overall essential amino acids and protein, may affect both voluntary feed intake and efficiency of dietary energy utilization (Fuller et al., 1987; Noblet et al., 1987) and, consequently, production performance. For instance, feeding high-protein diets to growing pigs is known to limit voluntary feed intake and carcass fatness (Henry,
Table 2. Summary of studies evaluating the dietary lysine requirement, expressed as a daily intake, of the lactating sow

<table>
<thead>
<tr>
<th>Litter</th>
<th>Requirement g/d</th>
<th>Size</th>
<th>Growth kg/d</th>
<th>Milk yield kg/d</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stahly et al., 1990</td>
</tr>
<tr>
<td></td>
<td>&gt;45.0</td>
<td>10.5</td>
<td>2.09</td>
<td>7.9</td>
<td></td>
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<tr>
<td></td>
<td>55.0</td>
<td>9.5 to 10</td>
<td>2.18</td>
<td>8.3</td>
<td>Johnston et al., 1991</td>
</tr>
<tr>
<td></td>
<td>&gt;46.0</td>
<td>10.5</td>
<td>2.45</td>
<td>10.2</td>
<td>Stahly et al., 1992</td>
</tr>
<tr>
<td></td>
<td>42.5</td>
<td>11</td>
<td>2.13</td>
<td>8.1</td>
<td>Laurin et al., 1993</td>
</tr>
<tr>
<td></td>
<td>&gt;45.0</td>
<td>11 to 12</td>
<td>2.49</td>
<td>10.4</td>
<td>Monegue et al., 1993</td>
</tr>
<tr>
<td></td>
<td>43.0</td>
<td>9.5</td>
<td>1.80</td>
<td>6.9</td>
<td>Knabe et al., 1993</td>
</tr>
<tr>
<td></td>
<td>48.1</td>
<td>9</td>
<td>2.16</td>
<td>9.1</td>
<td>King et al., 1993</td>
</tr>
<tr>
<td></td>
<td>&gt;51.0</td>
<td>14</td>
<td>2.50</td>
<td>9.5</td>
<td>Sauber et al., 1994a</td>
</tr>
</tbody>
</table>

1985; Cole and Chadd, 1989).

When lysine is the first-limiting amino acid, additional protein in excess of the requirement has no specific effect on feed intake, whereas growth and feed efficiency are decreased as a result of the additional cost of catabolism of excess amino acids (Henry et al., 1992a). Noblet et al. (1987) reported that the same energy deposition in the growing pig required .8% more ME per one percentage unit increase in protein level, thus explaining an increase in feed/gain with increased protein level. The depression in feed intake that occurs when high-protein diets are fed to pigs has been explained by Henry et al. (1992b) as a dietary imbalance between tryptophan and the less-limiting essential amino acids (large neutral amino acids) resulting in a tryptophan deficiency that limits the production of the neuromediator serotonin in the brain.
Amino acid antagonisms may occur because structurally similar amino acids compete with each other in the absorption or transport processes (Harper et al. 1970). However, although a leucine-isoleucine-valine (D'Mello and Lewis, 1970) and a lysine-arginine (Austic and Calvert, 1981) antagonisms have been demonstrated in poultry, the importance of these antagonisms in swine seems minimal (Oestemer et al., 1973; Henry et al., 1976; Southern and Baker, 1982; Hagemeier et al., 1983; Anderson et al., 1984; Edmonds et al., 1987).

Therefore, there may be beneficial effects in improving the amino acid balance of pig diets through supplementation with limiting amino acids in crystalline form and concomitant reduction of excess protein. The "ideal protein" with a perfect balance of amino acids, both among the essential amino acid and between essential and nonessential amino acids has been defined for poultry (Dean and Scott, 1965; Baker et al., 1979) and swine (ARC, 1981; Wang and Fuller, 1989; Chung and Baker, 1991, 1992b). Fuller et al. (1989) estimated the optimum amino acid profiles for maintenance and tissue protein accretion, separately. Because the two profiles are different and the importance of maintenance increases as pigs advance towards market weight, a recent study (Hahn and Baker, 1995) demonstrated a different optimum amino acid profile for finishing than for growing pigs.

**Amino Acid Availability**

The amino acid requirements for pigs recommended by NRC (1988) are expressed as total amino acid concentrations. However, not all dietary amounts of amino acids are absorbed in a form suitable for utilization by the pig (Batterham, 1980; Austic, 1983; Sauer and Ozimek, 1986; Sauer et al., 1989). The diets that are used in most of the studies conducted to determine the amino acid requirements of pigs contain amino acids from both
intact ingredients and from crystalline sources. The digestibility of the amino acids in the intact ingredients is variable depending on characteristics of the diet, type of feedstuff, variation among samples of the same feedstuff, and the procedure used to measure amino acid digestibility (Sauer and Ozimek, 1986; Southern, 1991; Fan, 1994). For instance, Fan et al. (1994) reported that the apparent ileal digestibility of an amino acid depends on the content of the same amino acid in the diet. Because of the modifying action of the microflora in the large intestine on amino acids (Zebrowska, 1973; Tanksley and Knabe, 1984; Sauer and Ozimek, 1986), the ileal procedure is more exact than the fecal procedure to determine the digestibility of amino acids. Further refinement of the digestibility value is achieved in true ileal digestibility of amino acids, by correcting the apparent ileal digestibility for endogenous secretion of amino acids (De Lange et al., 1990). Use of true ileal digestibility values is especially important when expressing the threonine or tryptophan requirements (total or relative to lysine) because of their high content in the endogenous protein of ileal digesta (De Lange et al., 1989).

Although crystalline amino acids are assumed to be 100% available, the efficiency of crystalline lysine utilization is lower when pigs are fed once per day than when they are fed more frequently (Batterham, 1984; Cook et al., 1985; Partridge et al., 1985). The reason for the lower efficiency is presumed to be related to the rapid absorption of crystalline lysine relative to other amino acid derived from intact proteins. As reported by Lewis (1992), overestimation of the bioavailability of crystalline amino acids leads to overestimates of amino acid requirements.
Response Criteria to Estimate Amino Acid Requirements

Several different response criteria have been used in experiments to determine amino acid requirements. The value determined for the amino acid requirement of an animal in a given experiment may depend on the response that is evaluated, because not all response criteria result in the same estimate of the requirement.

Production traits

Because of the economical importance, production traits such as weight gain, feed efficiency, and body composition in growing animals, and milk yield and litter size in lactating animals are the ultimate criteria when assessing requirements for farm animals. Therefore, it is important that other response criteria are validated relative to these traits. Although they are usually easy to measure and do not require any expensive equipment, production traits have several disadvantages (Lewis, 1992). Measurements of production traits usually have high variation and, therefore, experiments need large number of animals or experimental units. The large number of animals and the cost of experimental diets can limit the feasibility of the amino acid requirement estimation in long-term experiments, particularly in the breeding and lactating sow. Different production traits yield different estimates of amino acid requirements. For instance, feed efficiency tends to yield higher estimates of requirement than does weight gain, and carcass leanness gives still higher requirements (NRC, 1988). In some instances, rate of weight gain does not provide a good measurement of amino acid adequacy because of variations in composition of gain with age and/or dietary balance (Hogberg and Zimmerman, 1979). In the growing animal, direct or indirect assessment of protein gain provides a more precise assessment of amino acid adequacy (Brown et al., 1951; Smith et al., 1967; Wyllie and Owen, 1978) than does body weight gain.
Nitrogen balance

The classical method of assessing protein or amino acid adequacy in human and animal nutrition is nitrogen (N) balance (Lewis, 1992). In a N balance experiment, the optimum dietary amino acid concentration is considered to be that which, for a given N intake, results in the greatest retention of N. The N retention is calculated as the difference between N intake and N excreted in feces and urine. Although the procedure is laborious and time consuming, it has the advantage, especially in humans, of being noninvasive. The original determinations of the amino acid requirements of men and women were on the basis of nitrogen balance (Irwin and Hegsted, 1971). The use of nitrogen balance in humans was reviewed by Manatt and Garcia (1992). Nitrogen balance has been extensively used for the determination of amino acid requirement in pigs (ARC, 1981, NRC, 1988; Fuller et al., 1989; Wang and Fuller, 1989). Nitrogen balance is directly related to protein deposition in the growing pig and to changes in weight of piglets and weight of adult sows (Lewis and Speer, 1974; 1975; Yen et al., 1986a,b). However, because there are always losses in N balance that are difficult to measure and errors associated with N intake, collection and analysis processes, balance methods tend to overestimate N retention when compared to carcass analysis (McLaughlan and Campbell, 1969).

In N balance experiments, N excreted via feces is fairly constant and most of the differences are caused by changes in urinary N excretion. Thus, changes in urinary N excretion under conditions of constant protein intake can be used as a reflection of total protein utilization to titrate amino acid requirements. Brown and Cline (1974), Fuller et al. (1979) and Grosbach et al. (1985) have used changes in urinary urea excretion to assess the response of pigs to supplements of amino acids.
Plasma amino acid concentrations

Because free amino acids in blood are the primary route of transportation of dietary protein to the tissues, the concentration of a free amino acid in blood is closely related to the level of the same amino acid in the diet (Morrison et al., 1961; Puchal et al., 1962; Zimmerman and Scott, 1965). In growing animals, the concentration of an essential amino acid in plasma remains low and relatively constant until the dietary requirement for that amino acid is met. This response has been used to estimate amino acid requirements of pigs (Mitchell et al., 1968; Lewis and Speer, 1973, 1974, 1975; Rosell and Zimmerman, 1984; Arentson et al., 1989). However, experiments using plasma amino acid concentrations as a response must be controlled very carefully in terms of diet formulation, time of bleeding, and processing of blood samples (Lewis, 1992).

Ratios of plasma amino acid concentrations before and after a meal have also been used to determine amino acid requirements of pigs (Meisinger and Speer, 1979; Leonard, 1982). A ratio of less than one indicates an amino acid deficiency, whereas a ratio greater than one indicates that the requirement is met.

Amino acid oxidation

When an amino acid is limiting, the major proportion is used for protein synthesis and only a minimal part is oxidized to carbon dioxide. However, as the dietary supply exceeds the animal needs for protein synthesis, greater amounts of amino acid are oxidized and, therefore, greater amounts of carbon dioxide are produced. Brookes et al. (1972) designed a method to determine the lysine requirement of rats. Rats were injected with [C\(^{14}\)] lysine and were fed graded levels of dietary lysine. The production of radioactive carbon dioxide was used as an index of the amount of lysine oxidized. The same direct oxidation
method was used by Chavez and Bayley (1976) to estimate the lysine requirement of the pig and they concluded that estimates of amino acid requirement derived from the oxidation method agree closely with estimates obtained from growth rate and plasma amino acid concentrations.

The direct oxidation method requires a labeled tracer for each essential amino acid. Alternatively, Kim et al. (1983a) developed an indirect oxidation method by using an indicator amino acid. The principle is that, as the supply of the first-limiting amino acid is increased, the oxidation of the other amino acids is decreased until the production of carbon dioxide reaches a plateau at a point at which the requirement for the limiting amino acid is met. The indirect oxidation method has been used to estimate the histidine requirement by using \([\text{C}^{14}]\) phenylalanine as an indicator (Kim et al., 1983a), and the lysine and threonine requirement by using \([\text{C}^{14}]\) histidine as an indicator (Kim et al., 1983b). Both approaches of the oxidation method, direct and indirect, allow short-term determinations of dietary amino acid requirements but specialized instruments are needed and costs are high.

**Whole body protein turnover**

Whole body protein turnover can be partitioned into protein synthesis and protein breakdown being possible to examine the effect of protein quality on the two different processes. Fuller et al. (1987) demonstrated that increases in protein deposition in response to lysine supplementation were caused by a decrease in protein breakdown rather than an increase in protein synthesis. However, there are two approximations with associated errors in this approach (Bier, 1989). The first one is that there is no single whole body protein, but instead there are innumerable individual body proteins turning over at various rates. The second problem is that there are 20 different amino acid subsystems and different amino
acids have different turnover rates from tissue pools. For instance, lysine is known to be retained in tissues, and therefore, it has a slower turnover than either methionine or threonine (Chu and Hegsted, 1976; Blemings et al., 1989). Moreover, the large effort involved with this approach limits its use as a practical method to determine amino acid requirements.

Isotope methods

Because of the high cost of isotopes and equipment, methods involving the use of isotopes to determine amino acid requirements have been practically limited to human nutrition. Young and Bier (1987) used a continuous, stable isotope infusion procedure to estimate the amino acid requirements of adult humans. Arentson et al. (1989) compared an isotope method with more traditional methods of estimating amino acid requirement of pigs. Leucine and phenylalanine fluxes were measured, as well as growth performance, plasma amino acids, plasma urea nitrogen, nitrogen retention and body composition to estimate the threonine requirement of young pigs. All traditional methods yielded similar estimates, whereas no differences in amino acid fluxes were detected among the different dietary threonine concentrations.

Plasma urea nitrogen concentrations

Plasma urea N (PUN) concentrations are affected by the quality and the quantity of the dietary protein (Eggum, 1970). Quality is inversely, whereas the quantity of dietary protein is directly correlated with PUN concentrations. Because the amount of the dietary first-limiting amino acid that a protein contains is one of the factors affecting protein quality, PUN concentration is a valid criterion to determine amino acid requirements under
conditions of constant protein intake. Early research demonstrated that, as dietary amino acid balance is improved by supplementation with the first-limiting amino acid, PUN concentrations are reduced because of the increasing use of non-limiting amino acids for protein synthesis (Rose et al., 1950; Kumta and Harper, 1961; Brown and Cline, 1974). The reduction in PUN concentrations reflects a decrease in amino acid catabolism, more efficient total N utilization and, thus, decreased urea synthesis in pigs fed a diet adequate in first-limiting amino acid concentrations. When the dietary requirement of the first-limiting amino acid is met, urea excretion is minimized, and PUN concentrations tend to reach a minimum followed by a plateau (Brown and Cline, 1974) or a low increase (Lewis et al., 1977a, 1977b, 1980). Because PUN concentrations reflect the protein metabolism of the animal, greater dietary amino acid intakes are needed to optimize PUN concentrations than to optimize ADG (Kovar et al., 1993). The main advantage of using PUN concentrations in determining protein and amino acid utilization is the rapid and simple measurement (Marsh et al., 1965). On the other hand, PUN concentrations can be affected by factors not related to protein utilization (Cai, 1992).

Plasma urea N concentration has been extensively used in long-term treatment periods to provide an estimate of the lysine requirement for growing (Brown and Cline, 1974; Lewis et al., 1977a; 1980; 1991; Yen et al., 1986a,b; Chiba et al., 1991; Nam and Aherne, 1994), gestating (Woerman and Speer, 1976; Sohail et al., 1978), and lactating (Lewis and Speer, 1973; Wilkinson et al., 1982) swine over a weight or time interval.
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CHAPTER 3. USE OF PLASMA UREA NITROGEN AS A RAPID RESPONSE CRITERION TO DETERMINE THE LYSINE REQUIREMENT OF PIGS\textsuperscript{1,2,3}

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J. Coma\textsuperscript{4}, D. Carrion, and D. R. Zimmerman\textsuperscript{5}

**Abstract**

Five experiments were conducted to evaluate the use of plasma urea N (PUN) concentrations as a rapid response criterion to determine amino acid requirements. A preliminary experiment (Exp. 1) indicated that a 3-d feeding time is required to re-equilibrate PUN concentrations after a change in the dietary concentration of lysine. In Exp. 2, 3, and 4, PUN was used to estimate the lysine requirement of growing pigs at different specific BW. Thirty individually-penned crossbred pigs weighing 32 and 44 kg in Exp. 2 and 3, respectively, were assigned to five dietary treatments (.60, .70, .80, .90, and 1.00% lysine) for 5 d. The PUN decreased quadratically (P < .05) to increasing dietary lysine. A two-slope, broken-line regression model estimated the requirement at .85% in Exp. 2 and at .76% in Exp. 3. In Exp. 4, 60 crossbred pigs (30 barrows and 30 gilts) weighing 70 kg were assigned to five dietary lysine concentrations: .50, .60, .70, .80, and .90% for 4 d.

\textsuperscript{1}Reprinted with permission from J. Anim. Sci. 1995. 73:472-481.
\textsuperscript{2}Journal paper no. J-15806 of the Iowa Agriculture and Home Economics Experiment Station, Ames; Project 2919.
\textsuperscript{3}The authors wish to express appreciation to Biokyowa, Chesterfield, MO for providing some amino acids used in this project.
\textsuperscript{4}Supported by a Fulbright/Ministry of Education and Science (Spain) grant.
\textsuperscript{5}To whom correspondence should be addressed.
Increasing lysine caused PUN to decrease quadratically \( (P < .01) \). The estimated requirements were different \( (P < .05) \) between sexes: .69% for barrows and .75% for gilts.

In Exp. 5, the validity of using PUN as a rapid response criterion was verified by comparing the estimated lysine requirement based on PUN with the requirement determined in a 7-d N balance. Twenty crossbred barrows averaging 19 kg were used. Dietary lysine concentrations were .60, .75, .90, 1.05, and 1.20%. A quadratic response was observed in PUN \( (P < .05) \) and N retention (NR) \( (P < .01) \) with increasing lysine. The estimated lysine concentrations that maximized rates of NR and minimized PUN (1.03 vs. 1.05) were not different \( (P > .10) \). Therefore, PUN concentrations can be used in short-term trials to accurately estimate the dietary lysine required to maximize total N utilization in pigs at a specific BW. In addition, the two-slope broken-line regression model had the highest \( R^2 \) and lowest MSE when compared with three other models as means for estimating lysine requirement from PUN concentrations.

**Key words:** Pigs, Lysine, Plasma Urea Nitrogen, Linear Models

**Introduction**

The potential protein retention in the growing pig has been extensively described as a function of BW (Carr et al., 1977; Black et al., 1986; Whittemore et al., 1988). Thus, the intake of amino acids required to achieve this potential growth changes as the animal matures (Carr, 1977). Voluntary feed intake in the growing pig is also closely related to BW (ARC, 1981; NRC, 1987). Therefore, theoretically, the dietary amino acid requirements of a growing pig are continuously changing with BW. However, most of the response criteria used to assess amino acid requirements provide an estimate over a considerable weight range (NRC, 1988).
Plasma urea N (PUN) concentrations have been used in long-term treatment periods to provide an estimate of the lysine requirement for growing (Brown and Cline, 1974; Lewis et al., 1977a; Lewis et al., 1980), gestating (Woerman and Speer, 1976), and lactating (Lewis and Speer, 1973) swine. Nitrogen metabolism has been reported to have a rapid response to changes in dietary amino acid concentrations (Brown and Cline, 1974; Fuller et al., 1979). However, the use of PUN concentrations as a rapid response criterion in determination of amino acid requirements has never been investigated. Thus, five experiments were conducted to evaluate the potential use of PUN concentrations in short-term trials to assess the lysine requirement of swine at specific BW. In addition, four regression models were compared as means for estimating lysine requirement from PUN concentrations.

Materials and Methods

Lysine requirement of pigs as assessed by PUN

The objective of Exp. 1 was to investigate the feeding time required to obtain a re-equilibration of PUN concentrations after a modification in the dietary concentration of lysine. Experiments 2, 3, and 4 were conducted to investigate the potential use of PUN concentrations to estimate the lysine requirement of growing pigs at different specific BW. In Exp. 5, the validity of using PUN in short-term trials to estimate the lysine requirement of pigs at a specific BW was verified by comparing the estimated lysine requirement based on PUN with the requirement determined by N balance.

Animals and Treatments. In all experiments, (Yorkshire x Landrace)x(Hampshire x Duroc) pigs with a lean growth potential of .30 to .35 kg/d were used. Lean growth potential was previously calculated using the NPPC (1983) formula. In Exp. 1, 12 pigs
initially averaging 17.1 ± .4 kg BW were randomly allotted to six blocks based on litter and initial BW. Sex was ignored in the allotment procedure. Pigs were individually penned in 1.2-m x 1.2-m raised deck pens with woven-wire floors in a room kept at 24°C. After a 6-d adaptation period during which a .75% lysine diet (NRC requirement) was fed, pigs were randomly assigned to two dietary treatments (.60 and .90% lysine diets). After a 10-d treatment period, all pigs were again fed the .75% lysine diet for 5 d. Pigs were allowed ad libitum access to feed and water.

In Exp. 2, 30 individually-penned pigs (15 barrows and 15 gilts) with an average BW of 25.2 ± .4 kg were allotted from outcome groups based on litter, initial BW and sex to six blocks. Animals were housed in 1.8-m x 2.0-m concrete-floored, partially slotted pens. Room temperature was maintained at 22°C. After an 8-d adaptation period during which a .85% lysine diet was fed, five dietary treatments (.60, .70, .80, .90, and 1.00% lysine) were randomly assigned to pigs within blocks and fed for 5 d. Pigs were allowed ad libitum access to feed and water.

In Exp. 3, 30 pigs (15 barrows and 15 gilts) with an initial BW of 35.7 ± .7 kg were used. All procedures were the same as in Exp. 2, except the composition of the diets.

In Exp. 4, 60 pigs (30 barrows and 30 gilts) initially averaging 61.3 ± .6 kg BW were blocked by weight and randomly allotted to 15 pens with two barrows and two gilts each. Pigs were allocated .90 m² of floor space in concrete-floored, partly-slotted pens. Room temperature was maintained at 16°C. After a 10-d adaptation period during which a .78% lysine diet was fed, five dietary treatments (.50, .60, .70, .80, and .90% lysine) were randomly assigned to pens within blocks. The diets were fed for 5 d. Pigs were provided ad libitum access to feed and water.

In Exp. 5, 20 barrows with an average BW of 18.7 ± .4 kg were allotted on a litter
basis to four blocks. Pigs were housed in stainless steel metabolic crates in a room kept at 24°C. During a 7-d adjustment period to the new environment, a .95% lysine diet was fed. After the adjustment period, pigs were randomly assigned to one of five experimental diets (.60, .75, .90, 1.05, and 1.20% lysine) for 11 d; a 4-d adaptation period to the dietary treatments and a 7-d collection period. The diets were given to the animals at a rate of 115 g/kg of the expected BW at the middle of the treatment period and it was supplied in two equal portions at 12-h intervals. Refused feed was measured daily. Animals were provided ad libitum access to water. Total feces and urine were collected during the 7-d collection period.

Experimental Diets. Dietary treatments of Exp. 1 were obtained by crystalline lysine supplementation of a basal corn-soybean meal diet containing .60% lysine (Table 1). Crystalline methionine, threonine, and tryptophan were added in all diets to maintain constant, optimal ratios of these essential amino acids to the lysine content in the diet (Chung and Baker, 1992).

In Exp. 2, 3, 4, and 5, corn-soybean meal diets formulated to contain the lowest lysine concentration of the treatment sets (Table 1) were supplemented with crystalline lysine to produce the other four dietary treatments. Optimal ratios of essential amino acids to lysine (Chung and Baker, 1992) were calculated using the greatest lysine concentration of the treatment set. The resulting amino acid concentrations were maintained constant in all diets by supplementation with crystalline methionine, threonine, isoleucine, tryptophan, histidine, and valine. Therefore, the only difference among experimental diets was the dietary lysine concentration; all other essential amino acid concentrations were constant across treatments.

In Exp. 3, 4, and 5, diets were formulated to have identical electrolyte balance
(Na+K-Cl) by modifying the crystalline lysine, sodium chloride and sodium carbonate concentrations in the diet. In Exp. 5, diets were made isonitrogenous by replacing crystalline lysine with L-glutamic acid.

Minerals and vitamins were added to meet or exceed NRC (1988) standards. An antibacterial agent was included in the diets.

*Sample Collection and Analyses.* In Exp. 1, blood samples were obtained daily for 5 d before and 5 d after each change of diets. Blood was collected via orbital sinus puncture in heparinized tubes. In Exp. 2, 3, and 4, blood samples were collected daily for 3 d before feeding the experimental diets and for the last 3 d of the treatment period. Blood was collected by anterior vena cava puncture in heparinized tubes. In Exp. 5, blood samples were obtained daily for the last 3 d before feeding the experimental diets and for d 3, 4, and 5 of the collection period. Blood was collected via orbital sinus puncture in heparinized tubes.

In all experiments, plasma was separated by centrifugation and stored at -20°C until it was analyzed. The PUN concentrations were determined by colorimetrically measuring the product formed in the direct reaction of urea and diacetyl monoxime, as described by Marsh et al. (1965). In Exp. 5, N concentration of diets, urine, and feces were determined. Diet subsamples were ground through a 1-mm screen for chemical analysis. Urine was collected through glass wool into 4-L bottles containing 25 mL each of concentrated hydrochloric acid (HCl) and toluene as preservatives. A 10% aliquot was retained and stored at -10°C until it was analyzed for N. Feces were collected twice a day and preserved in 1 N HCl. At the end of the collection period, fecal collections were weighed and freeze-dried to determine DM content. Dried samples were allowed to air-equilibrate, ground similarly to diet samples, and analyzed for N content. Nitrogen content of diet, feces, and
urine samples was determined by the Kjeldahl procedure (AOAC, 1990).

Statistical Analyses. In all experiments, data were analyzed as randomized complete block designs. Treatments of Exp. 4 were arranged as a split-plot, with dietary lysine concentration in the whole-plot and sex in the subplot. All statistical analyses were performed using the appropriate GLM procedures of SAS (1988). In Exp. 2, 3, 4, and 5, initial PUN (pretreatment period) was used as a covariate (P < .01) to correct the final PUN (treatment period) for individual animal differences. A two-slope, broken-line regression model (Robbins, 1986) was used to estimate an inflection point for the lysine concentration-final PUN concentration response curve. In Exp. 5, the same model was used to calculate an inflection point for the ADG and N retention (NR) response curves to increasing lysine.

Regression models to estimate the requirement

The two-slope, broken-line regression model was compared with three other models as means for estimating lysine requirement from PUN concentrations: one of them was another linear model (one-slope, broken-line regression model), and two were nonlinear models (quadratic regression and quadratic model with plateau).

One- and Two-Slope Regression Line Models. The broken-line regression model described by Robbins (1986) was used. A one-slope, broken-line model consists of two parts: a straight line with an increasing or decreasing slope and a horizontal line. Their point of intersection is the breakpoint. The lines are fitted by the method of least squares. In the two-slope, broken-line model, both intersecting straight lines have non-zero slopes.

The general model of the one-slope, broken-line is as follows:

\[ Y = L + U (R - X_{LR}) \]

For the two-slope, broken-line, the model is as follows:
Y = L + U (R - X_{L,R}) + V (X_{GR} - R). In these equations, L is the ordinate and R the abscissa of the inflection in the curve. U is the slope of the line for X < R, and, in the two-slope equation, V is the slope of the line at X > R. Thus, by definition, (R - X_{L,R}) is zero for values of X greater than R, and (X_{GR} - R) is zero for values of X less than R. The dietary lysine concentration (R) at which the breakpoint is achieved is estimated as the lysine requirement.

**Quadratic Model.** A quadratic response is described by:

Y = b_0 + b_1X + b_1X^2. The concentration at which the response reached 90% of the minimum response was estimated as the requirement.

**Quadratic Model with Plateau.** The quadratic model with plateau is a segmented model with two theoretical hypothesis: Y = b_0 + b_1X + b_1X^2, if X < R, and Y = P, if X > R. That is, for values of X less than R, the equation relating Y and X is quadratic (a parabola), and for values of X greater than R, the equation is a horizontal line (P = plateau value). The dietary lysine requirement is estimated to be the lysine concentration (R) for which the response is the breakpoint.

The four models were fitted to the PUN data obtained in Exp. 3 (Table 2) by using the appropriate GLM and NLIN procedures of SAS (1988). Mean square error (MSE) and coefficient of determination (R^2) were used to assess the goodness of fit for the different models.

**Results and Discussion**

*Lysine requirement of pigs as assessed by PUN*

**Experiment 1.** The response of PUN concentrations to the changes in dietary concentration of lysine is shown in Figure 1. Plasma urea N responded to the dietary
treatments in less than 24 h (P < .01). A new equilibrium in PUN concentration was reached 2 to 3 d after changing the lysine concentration of the diet (P < .001). These data are in agreement with results reported by Kaji and Furuya (1987), wherein PUN concentrations were re-equilibrated within 2 d of changing crude protein levels of the diet. Moreover, Brown and Cline (1974) and Fuller et al. (1979) reported that rate of urea excretion reached a new equilibrium within 2 to 3 d of adding limiting amino acids to the diet. Because, under controlled conditions, renal filtration of urea is proportional to plasma concentrations (Gans and Mercer, 1984), the rapidity of re-equilibration of urinary urea and PUN concentrations after changes in the dietary concentrations of the first-limiting amino acid are expected to be similar. Therefore, it was concluded that the feeding time required to obtain a constant PUN concentration after a modification in the dietary concentration of lysine is 3 d.

**Experiment 2.** Initial and final average BW for the treatment period were 31.7 ± .6 and 35.7 ± .6 kg, respectively. Plasma urea N results are presented in Table 2. Increasing concentrations of lysine caused PUN to decrease quadratically (P < .01). There was no effect of sex on PUN concentrations (P > .05) as indicated in a comparison of sex blocks. Quality of dietary protein is inversely correlated with PUN concentrations (Eggum, 1970). Therefore, early research demonstrated that, as dietary amino acid balance is improved by supplementation with the first-limiting amino acid, PUN concentrations are reduced (Kumta and Harper, 1961; Brown and Cline, 1974). The reduction in PUN concentrations presumably reflects a more efficient total N utilization and, thus, decreased urea synthesis in pigs fed adequate lysine concentrations. Therefore, when the dietary requirement of the first-limiting amino acid is met, urea excretion is minimized, and PUN concentrations tend to reach a plateau (Brown and Cline, 1974).
Although a plateau in PUN concentrations was not obtained, a two-slope, broken-line regression model estimated an inflection point at .85% lysine (Figure 2). Absolute value of the slope below the inflection point was eight times greater than that above the inflection point. Therefore, a dietary lysine concentration of .85% was assumed to be the lysine requirement of the (YxLR)x(HxD) pigs weighing 32 to 36 kg BW. The NRC (1988) recommends a .75% lysine diet for 20- to 50-kg pigs. The BW of pigs used in this study was the average weight of the NRC interval. Therefore, results suggest that the lysine concentration required to maximize total N utilization of these pigs, as indicated by minimum PUN, was greater than NRC (1988) recommendations.

**Experiment 3.** Pigs averaged 44.1 ± .8 kg BW at the beginning of the treatment period and 48.7 ± .9 kg BW at the end. The results for PUN are reported in Table 2. Plasma urea N concentrations changed in a quadratic manner (P < .01) with increasing lysine concentration in the diet. The inflection point estimated by a two-slope, broken-line regression model was .76% (Figure 3). Sex did not have an effect on PUN concentrations (P > .05) as indicated in a comparison of sex blocks.

Dietary lysine concentrations greater than the requirement tended to result in an increase of PUN concentrations. A similar pattern has been observed by Lewis et al. in pigs (1977a, 1980) and rats (1977b). Feeding concentrations greater than the requirement results in an excess of amino acids that must be catabolized. Thus, the increased rate of urea synthesis is reflected in greater PUN concentrations; however, this trend was not observed in Exp. 2. This difference may have been because the diets were not balanced for electrolytes in Exp. 2; therefore, the chloride present in the crystalline form of lysine (lysine·HCl) tended to decrease the cation:anion ratio. Slagle and Zimmerman (1979) observed that pigs fed a diet with excess anion had extremely low PUN concentrations.
Similar results were reported by Honeyfield et al. (1985) when feeding increasing dietary concentrations of chloride. Furthermore, Cai et al. (1992) reported a tendency for greater PUN concentrations and significantly greater urea excretion in pigs fed excess cation, although total N excretion remained constant. The decreasing effect of anions on PUN concentrations is attributed to their acidifying properties. Welbourne et al. (1986) indicated that, in the maintenance of acid-base balance status in the body, synthesis of urea is quantitatively synchronized with ammonia production so that N excretion remains constant. Therefore, in Exp. 2, ammonia synthesis may have been enhanced to compensate for the acidification produced by the chloride in crystalline lysine, decreasing PUN concentrations, regardless of lysine effect.

As reported in the previous experiment, the lysine requirement estimated by using PUN was greater than NRC (1988) recommendations. Plasma urea N data indicated that the lysine requirement of the (YxLR)(HxD) pigs weighing 44 to 49 kg BW was .76% of the diet.

**Experiment 4.** Initial and final BW for the treatment period averaged 70.1 ± .7 and 73.8 ± .8 kg, respectively. Results for PUN are presented in Table 2. Increasing dietary lysine caused PUN to decrease quadratically (P < .01). The PUN response to dietary lysine concentrations was different (P < .05) between the sexes. A two-slope, broken-line regression model estimated an inflection point (requirement) at .69% lysine for barrows and at .75% lysine for gilts (Figure 4).

Differences in the protein or lysine requirement of barrows and gilts have been extensively demonstrated (Baker et al. 1967, Watkins et al., 1977; Cromwell et al., 1990). Gilts have a lower voluntary feed intake and gain body weight less rapidly than barrows. However, rate of lean tissue deposition in gilts is equal to, or slightly greater than, barrows
in the finishing phase of growth. Therefore, gilts require a greater lysine percentage in the
diet than barrows.

The NRC (1988) recommends a dietary lysine concentration of .60% for pigs
weighing 50 to 110 kg without sex distinction. Our data indicated a greater requirement for
both sexes in pigs weighing the average BW of the NRC interval. Thus, the reported results
confirm that lysine requirements estimated by PUN are greater than NRC recommendations.
In conclusion, the lysine requirement estimated by using PUN for these (YxLR)x(HxD) pigs
weighing 70 to 74 kg was .69% of the diet for barrows and .75% of the diet for gilts.

Experiment 5. Growth and N utilization results are presented in Table 3. The average
initial and final BW for the collection period were 25.5 ± .5 and 30.0 ± .7 kg,
respectively. Average daily gain during the 11-d treatment period improved quadratically
(P < .05) with increasing lysine content of the diet. A broken-line, regression model
estimated the optimum at 1.00% dietary lysine.

Increasing concentrations of dietary lysine caused PUN to decrease quadratically (P
< .05). Plasma urea N concentrations were estimated by a two-slope, broken-line
regression model to be minimized in pigs fed 1.05% dietary lysine (Figure 5).

Average daily gain was estimated to be optimized at a slightly lower dietary lysine
concentration than were PUN concentrations (1.00 vs 1.05%). Similar differences were
observed by Kovar et al. (1993) when a broken-line regression model was used to describe
the response of both criteria to graded levels of dietary threonine. It is well established that
maximum protein accretion requires a greater intake of amino acids than does maximal rate
of weight gain (NRC, 1988). Therefore, because PUN concentrations reflect the protein
metabolism of the animal, estimates of the requirement assessed by PUN tend to be higher
than when daily weight gain is used as a criterion.
Dietary lysine concentration did not (P > .10) alter the amount of N lost via feces. Urinary N excretion decreased in a quadratic manner (P < .05) with increasing percentage of dietary lysine. Therefore, NR increased quadratically (P < .01) as dietary lysine concentrations increased. Based on the broken-line analysis, the rate of NR was maximized at 1.03% dietary lysine (Figure 5).

Nitrogen balance has been extensively used for the determination of amino acid requirement in pigs (Fuller et al., 1979; Fuller et al., 1989; Wang and Fuller, 1989). In a N balance experiment, the optimum dietary amino acid concentration is considered to be that which, for a given N intake, results in the greatest retention of N. The maximum NR reached with the optimum concentration is a result of the absorbed total amino acids being used most efficiently for tissue growth. Increasing amino acid utilization decreases urea synthesis and, thus, decreases PUN concentrations. Therefore, theoretically, PUN concentrations are expected to be minimized and NR to be maximized in animals fed a dietary concentration of the first-limiting amino acid close to the requirement. Braude et al. (1974) reported maximum NR and minimum PUN concentrations at the same dietary lysine concentration when using both criteria to assess the requirement in growing pigs. In contrast, Balogun and Fetuga (1981) obtained a slightly greater estimate of the methionine requirement of weanling pigs by using PUN than by using NR. In the present study, the highest rates of NR and the lowest PUN concentration were obtained at the same dietary lysine concentration (1.05%). When data were fitted by a two-slope, broken-line regression model, the estimated lysine requirements using PUN concentrations and NR (1.05 vs 1.03%, respectively) were not (P > .1) different. The results indicate that PUN concentrations and NR result in the same estimate when both are used as rapid response criteria in lysine requirement determination. Therefore, PUN concentrations can be used in
short-term trials to accurately estimate the dietary lysine required to maximize total N utilization in pigs at a specific body weight.

**Regression models to estimate the requirement**

The PUN response of growing pigs to increasing concentrations of dietary lysine was fitted by the four models as shown in Table 4. The one-slope, broken-line model (Figure 6a) yielded a requirement of .743%. The requirement estimated by the two-slope model (Figure 6b) was .764%. The nonlinear models tended to estimate greater requirements: .787% by the quadratic model (Figure 6c), and .809% by the quadratic with plateau (Figure 6d). These estimates represent a variation of 9% from the highest to the lowest.

The linear models had lower MSE and higher R² than the nonlinear models. The two-slope model was more precise than the one-slope model (.0660 vs .0880, and .97 vs .94; MSE and R² values, respectively) because the slope of the second line was different (P < .05) from zero.

The broken-line model assumes (1) that a growing animal responds linearly to additions of a limiting indispensable nutrient until the exact requirement is met, after which (2) no further improvement is observed. There is evidence that nutrient-response relationships exhibit a saturation phenomenon, known as "law of diminishing returns" (Fisher et al., 1973; Morgan et al., 1975; Morris and Blackburn, 1982). Even if the individual members exhibit abrupt linear responses, Curnow (1973) demonstrated that a population would exhibit a smooth "saturation" response curve. Therefore, the broken-line model is clearly inadequate from a biological point of view. Motulsky and Ransnas (1987) pointed out that many types of biological data are best analyzed by nonlinear regressions. Robbins et al. (1979) demonstrated that the population mean response to limiting nutrient
supply can be more accurately described by a curvilinear model. Furthermore, Fuller and Garthwaite (1993) indicated that curvilinear models describe the individual NR responses to increasing dietary amino acid supply significantly better than do rectilinear models. However, Robbins et al. (1979) indicated that any response approaching an asymptote can be fitted by a linear model for a suitable narrow range of doses.

When describing the PUN concentration response to graded concentrations of dietary lysine, the four models fit the data adequately, and the differences in the closeness of fit were minimal, although linear models tended to fit the data better than the nonlinear models. These results seem to disagree with research discussed before; however, closeness of fit of the four models may vary among data sets.

The nonlinear models predicted a greater requirement than the broken-line regression models. As reported by Lewis (1992), estimates derived from broken-line models usually yield requirements that are less than the dose necessary to achieve maximal or minimal response. Baker (1986) indicated that the broken-line response selects a requirement value representing the average animal in the population. Thus, fitted broken lines, if properly constructed, should generally predict a requirement that is less than that predicted by fitted curvilinear procedures. The magnitude of the difference depends on the percentage of the nonlinear maximum/minimum response selected (e.g., 90 or 95%).

The problem with fitted curvilinear response lines is that breakpoints are not a component. Therefore, an arbitrary point has to be selected (e.g., 90% or 95% of the upper or lower asymptotic value), whereas a continuous broken line calculated by least squares objectively selects the breakpoint in the response line (Robbins, 1986). To avoid subjectivity of the estimate, a broken-line method is preferred.

The two-slope, broken-line seems to be a better model to describe PUN response to
dietary lysine than the one-slope regression model. Concentrations of PUN showed an increasing trend when concentrations greater than the requirement were fed. A similar response was reported by Lewis et al. (1977a,b, 1980). Thus, if both intersecting straight lines have non-zero slopes, PUN response is fitted more precisely.

It is concluded that PUN response to dietary lysine supply is precisely described by a two-slope, broken-line regression model. Therefore, this linear model can be used to estimate the lysine requirement of the average pig in the population from PUN data.

Implications

Amino acid requirements, expressed as percentage of diet, of growing pigs are continuously changing with BW. Nitrogen metabolism has a rapid response to changes in the dietary concentration of amino acids. This rapid response allows the use of plasma urea N concentrations in short-term trials to accurately estimate the dietary lysine required to maximize N utilization by pigs of a specific BW. Development and application of the technique will contribute to the identification of the amino acid requirement for a defined population of pigs under defined environmental and management conditions at specific stages of growth.

Literature Cited


Table 1. Composition (%) of basal diets*, as fed basis

<table>
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<tr>
<th>Ingredient</th>
<th>Item 1</th>
<th>Item 2 and 3</th>
<th>Item 4</th>
<th>Item 5</th>
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<td>83.57</td>
<td>82.44</td>
<td>86.46</td>
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<tr>
<td>Soybean meal</td>
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<tr>
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<td>.80</td>
<td>.84</td>
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<td>.25</td>
<td>.25</td>
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<tr>
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<td>.03</td>
<td>.03</td>
<td>.03</td>
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<td>1.00</td>
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<td>.00</td>
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<td>L-histidine</td>
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<td>L-valine</td>
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<td>.00</td>
<td>.00</td>
<td>.03</td>
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</table>

Calculated analysis:

| CP, %                      | 13.28   | 13.20        | 11.78  | 13.94  |
| ME, kcal/kg                | 3,280   | 3,279        | 3,298  | 3,322  |
| Ca, %                      | .70     | .70          | .60    | .65    |
| P, %                       | .60     | .60          | .50    | .55    |
| Lysine*                    | .60     | .60          | .50    | .60    |

*Graded concentrations of crystalline lysine were added to the basal diet to obtain the dietary treatments in each experiment.

Partly replaced by sodium carbonate when crystalline lysine was added, to maintain an identical electrolyte balance (Na+K-Cl) in Exp. 3, 4, and 5.

Contributed per kilogram of diet: 110 mg of chlortetracycline, 110 mg of sulfathiazole, and 55 mg of penicillin in Exp. 1, and 33 mg of bacitracin activity from bacitracin methylene disalicylate in Exp. 2, 3, 4, and 5.

Contributed per kilogram of diet: 4,400 IU of vitamin A; 1,100 IU of vitamin D₃; 22 IU of vitamin E; 6.6 g of riboflavin; 18.0 mg of d-panthotenic acid; 33 mg of niacin; 10.0 μg of vitamin B₁₂.

Contributed in parts per million of diet: Zn, 150; Fe, 175; Mn, 60; Cu, 17.5; I, 2.0.

*Contributed in parts per million of diet: Zn, 150; Fe, 175; Mn, 60; Cu, 17.5; I, 2.0.

*Analyzed values were: .58, .62, .59, .52, and .61% lysine in Exp. 1, 2, 3, 4, and 5, respectively. The values were determined by ion exchange chromatography following acid hydrolysis as described by Gehrke et al. (1985).
Table 2. Effects of dietary lysine on plasma urea N (PUN\textsuperscript{a}) concentrations (mg/dL) in growing pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>.50</th>
<th>.60</th>
<th>.70</th>
<th>.80</th>
<th>.90</th>
<th>1.00</th>
<th>CV</th>
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<tbody>
<tr>
<td>Exp. 2\textsuperscript{b}</td>
<td>8.50</td>
<td>7.21</td>
<td>5.11</td>
<td>4.23</td>
<td>3.99</td>
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<tr>
<td>Exp. 3\textsuperscript{c}</td>
<td>7.98</td>
<td>6.37</td>
<td>5.28</td>
<td>5.96</td>
<td>5.75</td>
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<tr>
<td>Exp. 4\textsuperscript{d}</td>
<td>Barrow: 9.91</td>
<td>8.54</td>
<td>7.12</td>
<td>7.87</td>
<td>8.21</td>
<td>9</td>
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<tr>
<td></td>
<td>Gilt:   11.58</td>
<td>8.73</td>
<td>7.34</td>
<td>6.75</td>
<td>8.11</td>
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\textsuperscript{a}Least squares means (n = 6, in Exp. 2 and 3; n = 3, in Exp. 4) of plasma urea N (PUN) corrected by using initial PUN as a covariate;

\textsuperscript{b}Initial and final average BW (n = 30) were 31.7 ± .6 and 35.7 ± .6 kg, respectively; quadratic effect of lysine (P < .05).

\textsuperscript{c}Initial and final average BW (n = 30) were 44.1 ± .8 and 48.7 ± .9 kg, respectively; quadratic effect of lysine (P < .05).

\textsuperscript{d}Initial and final average BW (n = 60) were 70.1 ± .7 and 73.8 ± .8 kg, respectively; quadratic effect of lysine (P < .01); sex x lysine interaction (P < .05).
Table 3. Effects of dietary lysine concentrations on growth performance and N utilization in growing pigs^a (Exp. 5)

<table>
<thead>
<tr>
<th>Item</th>
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<tr>
<td></td>
<td>.60</td>
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<tr>
<td>Growth, kg/d</td>
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<tr>
<td>ADG^b</td>
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<td>N utilization, g/d</td>
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<td>N losses</td>
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<td>Fecal</td>
<td>4.5</td>
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<tr>
<td>Urine^b</td>
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<td>N retained</td>
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<td>NR^c</td>
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<td>PUN^d, mg/dL</td>
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^aInitial and final average BW (n = 20) were 25.5 ± .5 and 30.0 ± .7 kg, respectively; quadratic effect of lysine (P < .05).
^bQuadratic effect of lysine (P < .05).
^cLeast squares means (n = 4) of N retention (NR) corrected by using initial body weight as a covariate; quadratic effect of lysine (P < .01).
^dLeast squares means (n = 4) of plasma urea N (PUN) corrected by using initial PUN as a covariate; quadratic effect of lysine (P < .05).
Table 4. Estimation of lysine requirement of growing pigs (Exp. 3) from plasma urea N concentrations by two linear (one- and two-slope, broken-line) and two nonlinear (quadratic and quadratic with plateau) regression models

<table>
<thead>
<tr>
<th>Model</th>
<th>Model parameters$^a$</th>
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<th>R²</th>
<th>MSE$^b$</th>
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<tr>
<td>One-slope broken-line</td>
<td>$L = 5.66$</td>
<td>.743</td>
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<td>$U = 16.10$</td>
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<tr>
<td></td>
<td>$R = .743$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-slope broken-line</td>
<td>$L = 5.34$</td>
<td>.764</td>
<td>.97</td>
<td>.0660</td>
</tr>
<tr>
<td></td>
<td>$U = 16.10$</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>$V = 2.35$</td>
<td></td>
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<tr>
<td></td>
<td>$R = .764$</td>
<td></td>
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<tr>
<td>Quadratic</td>
<td>$b_0 = 30.30$</td>
<td>.787$^c$</td>
<td>.90</td>
<td>.2190</td>
</tr>
<tr>
<td></td>
<td>$b_1 = -56.85$</td>
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<tr>
<td></td>
<td>$b_{11} = 32.50$</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Minimum = .875</td>
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<tr>
<td>Quadratic with plateau</td>
<td>$b_0 = 40.52$</td>
<td>.809</td>
<td>.94</td>
<td>.1253</td>
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<tr>
<td></td>
<td>$b_1 = -86.18$</td>
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<td>$b_{11} = 53.28$</td>
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</tr>
<tr>
<td></td>
<td>Plateau = 5.68</td>
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</tr>
<tr>
<td></td>
<td>R at plateau = .809</td>
<td></td>
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</tr>
</tbody>
</table>

$^a$L = ordinate (PUN concentration) of the inflection point; $U =$ slope below inflection point; $V =$ slope above inflection point; $R =$ abscissa (dietary lysine concentration) of the inflection point; in a quadratic equation: $b_0 =$ intercept, $b_1 =$ b-value for variable lysine (Lys), and $b_{11} =$ b-value for variable Lys$^2$.

$^b$Mean square error.

$^c$Estimated at 90%.
Figure 1. Mean plasma urea N (PUN) concentration (mg/dL) response to changes in the dietary concentration of lysine (Exp. 1). Space between arrows indicate the period of time during which diets containing .60 or .90% lysine were fed. There was an effect of lysine concentration (P < .01) on PUN concentrations for the entire treatment period. Standard error of PUN least squares means = .696.
Figure 2. Use of a two-slope, broken-line regression model to describe the response of plasma urea N (PUN) to dietary lysine concentration ($R^2 > .95$) in pigs weighing 32 to 36 kg (Exp. 2). Standard error of PUN least squares means = .323.
Figure 3. Use of a two-slope, broken-line regression model to describe the response of plasma urea N (PUN) to dietary lysine concentration ($R^2 > .96$) in pigs weighing 44 to 49 kg (Exp. 3). Standard error of PUN least squares means = .343.
Figure 4. Use of a two-slope, broken-line regression model to describe the response of plasma urea N (PUN) to dietary lysine concentration ($R^2 > .94$) in pigs weighing 70 to 74 kg (Exp. 4). Standard error of PUN least squares means = .458.
Figure 5. Use of a two-slope, broken-line regression model to describe the response of plasma urea N (PUN) and N retention (NR) to dietary lysine concentration ($R^2 > .94$) in pigs weighing 25 to 30 kg (Exp. 5). Standard errors of PUN and NR least squares means were .779 and .640, respectively.
Figure 6. Description of the mean plasma urea N (PUN) response to dietary lysine concentration in a growing pig (Exp. 3) by: (a) one-slope, broken-line, (b) two-slope, broken-line, (c) quadratic, and (d) quadratic with plateau regression models.
CHAPTER 4. VARIATION IN PLASMA UREA NITROGEN NOT RELATED TO AMINO ACID ADEQUACY: RELATIONSHIP WITH LEAN TISSUE GROWTH¹,²

A paper submitted to the *Journal of Animal Science*

J. Coma³, D. R. Zimmerman⁴ and D. Carrion

Abstract

The objectives of this study were (1) to investigate the relationship between plasma urea N concentrations (PUN) and lean tissue growth and (2) to compare the value of different variables, related to lean growth and renal function, to correct the dietary lysine-PUN response for variation not related to amino acid adequacy. Forty-eight gilts (64.8 kg BW) were individually penned (blocks based on initial BW) for 50 d: a 10-d adjustment, a 35-d pretreatment, and a 5-d treatment period. During the pretreatment period, ADFI, urine specific gravity (SG), serum creatinine (SC), PUN, and daily fat-free carcass lean (DFFCL), empty body protein (DEBP), total carcass fat (DCF), empty body lipid (DEBLI) depositions were measured. Partial correlation coefficients (ADFI effect removed) indicated a strong and inverse relationship between PUN and lean growth (DFFCL and DEEP) (r = -.88 and -.91, respectively, P < .01) and a positive relationship between PUN and fat depositions.

¹Journal paper no. J-16119 of the Iowa Agriculture and Home Economics Experiment Station, Ames; Project 2919.
²Funded by Iowa Pork Producers Association. The authors acknowledge the assistance of S. J. Moeller in the collection and analyses of ultrasound measurements.
³Supported by a Fulbright/Ministry of Education and Science (Spain) grant.
⁴To whom correspondence should be addressed.
deposition (DCF and DEBLI) (r = .66 and .54, respectively, P < .22). Treatments consisted of six dietary lysine concentrations (.475, .550, .625, .700, .775, and .850%). Initial and final BW's in the treatment period were 103.3 and 107.7 kg, respectively. Pretreatment PUN (PUN0) was the pretreatment variable with the greatest R² and the smallest MSE when used in the model describing the response of PUN (PUN1) to dietary lysine. The estimated lysine requirements from the PUN1 response corrected with either PUN0 or with the combination of PUN0, ADFI, DFFCL, DCF, SG, and SC were not (P > .05) different (.656 vs .678%, respectively). It is concluded that (1) PUN concentrations have a potential value as an indicator of the efficiency of lean tissue growth and (2) pretreatment PUN is a very useful variable to correct treatment PUN for variation not related to amino acid adequacy.

Key words: Pigs, Lysine, Plasma Urea Nitrogen, Lean growth

Introduction

Plasma urea N (PUN) concentration has been extensively used as a response criterion in lysine requirement determination of growing (Brown and Cline, 1974; Lewis et al., 1980; Chen et al., 1994), gestating (Woerman and Speer, 1976; Sohail et al., 1978), and lactating (Lewis and Speer, 1973; Wilkinson et al., 1982) swine. The value of PUN concentration as a rapid response criterion to accurately estimate the dietary lysine required by pigs of a specific BW to maximize N utilization has been demonstrated in a previous study (Coma et al., 1995).

However, PUN concentration also is affected by factors other than amino acid adequacy (Cai, 1992). Concentrations of urea (or urea N) in blood or plasma have been classically used as an indicator of renal function (Smith, 1951; Finco, 1989). Moreover, a
relationship between PUN and lean growth has been recently reported (Gourley and Zimmerman, 1993; Whang et al., 1994a,b; Hahn and Baker, 1994). Correction of PUN values for the variation not related to amino acid adequacy is needed to obtain accurate and precise estimates of lysine requirements of animals.

The objectives of this study were (1) to further investigate the relationship between PUN and lean tissue growth and (2) to compare the use of different variables (related to lean growth and renal function) as covariates when analyzing the response of PUN to dietary lysine.

Materials and Methods

General. Forty-eight individually-penned gilts (Yorkshire x Landrace) x (Hampshire x Duroc) initially averaging 64.8 ± .4 kg BW were allotted to eight blocks based on initial BW. Lean growth potential of this strain of pigs was previously calculated to be .30 to .35 kg/d using NPPC (1983) formulas. Animals were placed in .6-m x 2.4-m pens with steel-slatted flooring in a room kept at 18.0 ± 2.0 °C during 50 d: a 10-d period of adjustment to the new environment, a 35-d pretreatment period, and a 5-d treatment period. All pigs were fed the same diet (Table 1) during the adjustment and the pretreatment period. After the pretreatment period, pigs were randomly assigned within blocks to one of six dietary lysine treatments. Animals were provided ad libitum access to feed and water.

Lean and fat deposition of the pigs were measured during the pretreatment period. These measurements were related to PUN concentrations in the pretreatment period (initial PUN) to assess the relationship between PUN and the variation in rate of lean growth that existed within the strain of pigs (objective 1).

Renal function indicators were also measured in the pretreatment period. In the
treatment period, PUN was measured (final PUN) as a response criterion to the dietary lysine concentrations. Initial PUN, ADFI, rates of lean and fat deposition, and renal measurements in the pretreatment period were evaluated to explain the variation in final PUN not related to amino acid adequacy (objective 2).

**Measurements.** Pig weights and feed consumption were determined during the 35-d pretreatment period to determine ADG and ADFI and gain to feed ratio (G:F).

Real-time ultrasound (Aloka 500V, 12.5 cm 3.5-MHz linear array transducer, Corometrics Medical Systems, Wallingford, CT) was used to determine last rib backfat depth (BFL), and 10th rib longissimus muscle area (LEA) and backfat depth (BF) at d 1 and 35 of the pretreatment period. Ultrasound measurements were recorded on VHS videotape for later viewing and analysis. All measurements and analysis of images were done by the same person. Using formulas specific for each BW, provided by Dr. Allan Schinckel (personal communication), fat-free carcass lean (FFCL), empty body protein (EBP), total carcass fat (CF), and empty body lipid (EBLI) were estimated at d 1 and d 35 from ultrasound measurements and BW. Daily accretions of FFCL, EBP, CF, and EBLI from d 1 to 35 were calculated (DFFCL, DEBP, DCF, and DEBLI, respectively).

Blood samples were obtained between 0730 and 0900 on d 1, 20, 34, and 35 of the pretreatment period and on d 4 and 5 of the treatment period. Blood was collected via anterior vena cava puncture in heparinized tubes and also in nonheparinized tubes on d 35. Plasma and serum were separated by centrifugation and stored at -20°C until they were analyzed. The PUN concentrations were determined by colorimetrically measuring the product formed in the direct reaction of urea and diacetylmonoxime, as described by Marsh et al. (1965). Average of PUN concentrations in the pretreatment period: d 1 (PUN0A), d 20 (PUN0B), and average of d 34 and 35 (PUN0C) was called initial PUN (PUN0).
Average PUN concentration of d 4 and 5 of the treatment period was called final PUN (PUN1). Creatinine concentrations of serum (SC) from blood collected on d 35 were determined by using an automated version of the method described by Folin and Wu (1965).

Urine samples were collected on d 35 of the pretreatment period. Samples were collected upon spontaneous urination, starting at 0600 when lights were turned on and before pigs started eating or drinking, and it was completed at 0730. Urine specific gravity (SG) was measured by refractometry (T/C refractometer, AO Instrument Company, Buffalo, NY).

**Dietary Treatments.** Dietary treatments consisted of six lysine concentrations: .475, .550, .625, .700, .775, and .850%. A corn-soybean meal diet formulated to contain .475% lysine (Table 1) was supplemented with crystalline lysine to obtain the other five dietary lysine concentrations. Optimal ratios of essential amino acids to lysine (Chung and Baker, 1992) were calculated for the greatest lysine concentration of the treatment set (.850%). The resulting amino acid concentrations were maintained constant in all diets by supplementation with crystalline methionine, threonine, and tryptophan. Therefore, the only difference among experimental diets was the dietary lysine concentration; all other amino acid concentrations were constant across treatments. Diets were made isonitrogenous by replacing crystalline lysine with L-glutamic acid.

Diets were formulated to have identical electrolyte balance (Na+K-Cl) by modifying the crystalline lysine, sodium chloride and sodium carbonate concentrations in the diet. The reason for this modification was that PUN has been shown to be responsive to alterations of the dietary electrolyte balance (Cai and Zimmerman, 1991; Cai et al., 1992). Minerals and vitamins were added to meet NRC (1988) standards. An antibacterial agent was included in the diets.
Statistical Analyses. Simple correlation coefficients were calculated among different variables. Multivariate procedures of SAS (1988) were used to calculate partial correlation coefficients where ADFI effect was removed. Data of the response of PUN to dietary lysine were analyzed as a randomized complete block design by using the appropriate GLM procedures of SAS (1988). The experiment model was: \[ \text{PUN}_1 = (1- \text{ to 5-variable combination that served as a covariate}) + \text{block} + \text{lysine effect}. \]

The five variables used as covariates to correct PUN1 for variation not related to the lysine effect were the following measurements: PUN0, ADFI, DFFCL, DCF, SG, and SC. Empty body data were not used to avoid repetition of the information in carcass data. When assessing the goodness of the covariates, data were analyzed using maximum R² improvement procedures (SAS, 1988), with the five measurements serving as independent variables and PUN1 as the dependent variable. Two-slope, broken-line regression models (Robbins, 1986) were used to estimate inflection points (requirements) for the corrected response curves of PUN1 concentrations to increasing lysine.

Results and Discussion

Relationship of plasma urea nitrogen with lean growth

Body composition estimated from ultrasound measurements and BW (Table 2) were used to calculate DFFCL, DCF, EBP, and EBLI (Table 3). Results were in agreement with lean dissection data previously collected from the same strain of pigs (unpublished data). Plasma urea N concentrations tended to increase with BW, probably because of an increase in feed intake. Urine specific gravity values, PUN and SC concentrations were in the physiological ranges indicated by Altman and Dittmer (1961) and Finco (1989), respectively.
There is recent evidence of an inverse relationship between PUN concentration and lean growth (Gourley and Zimmerman, 1993; Whang et al., 1994a,b; Hahn and Baker, 1994). Concentrations of urea N in plasma are directly related to the rate of urea synthesis, and, therefore, inversely related to the efficiency of N deposition (i.e., lean growth). Gourley and Zimmerman (1993) indicated that carcass lean composition and PUN were inversely related with 65 to 75% of differences in carcass lean:fat being predicted from average PUN concentrations among pigs of two lean strains fed a common diet. Similarly, Whang et al. (1994a,b) and Hahn and Baker (1994) reported that the relationship of PUN with lean gain and G:F was negative and of a variable magnitude depending on sex and BW of the animals. But, in contrast with those previous studies, simple correlation coefficients describing the relationship of PUN concentrations with lean growth (DFFCL and DEBP) were positive and of small magnitude in the present study (Table 4). Fat deposition (DCF and DEBLI) was strongly and positively related to PUN. The total disagreement of our results with those obtained in the previous research was explained by a confounding effect of ADFI in the present study. Quantity of N intake (i.e., ADFI) has a positive relationship with PUN concentrations (Eggum, 1970), and, because ADFI was also directly related to lean growth, the expected inverse relationship between lean growth and PUN was not detected. However, when multivariate analysis procedures were used to remove the effect of ADFI (Table 5), partial correlation coefficients indicated a strong and inverse relationship between PUN and lean growth measurements, whereas the relationship of PUN with fat deposition remained positive. Plasma urea N concentrations were more closely related to the daily lean growth (corrected for ADFI) than to lean composition of the body at a specific BW (Table 6). A stronger relationship between PUN and lean:fat composition of the body was reported in the work of Gourley and Zimmerman (1993), wherein PUN
concentrations of two strains of pigs were highly correlated with ultrasound backfat (r = .90 and .81) and percentage of muscle (r = -.86 and -.80).

Based on these data, there is a potential value of PUN concentration as an indicator of the efficiency of lean tissue growth. For a given feed intake, PUN concentration decreases as rate of protein deposition increases; this inverse relationship, however, does not occur when rate of protein deposition increases mainly as a result of greater feed intake. On the other hand, the relationship between PUN concentrations and fat deposition after removing the ADFI effect remained positive indicating that less efficient protein deposition results in greater fat deposition.

**Variation in the plasma urea nitrogen response to dietary lysine**

Initial PUN was the pretreatment measurement most closely related to PUN1 (Table 7) and, thus, the most useful variable to correct PUN1 for variation not related to amino acid adequacy. More than 70% of differences between PUN1 values were predicted from PUN0. Small increases in R² and reduction of MSE were achieved by addition of other independent variables (ADFI, DFFCL, DCF, SG, and SC) to PUN0 in the model describing the PUN1 response to dietary lysine (Table 8). Addition of the other five variables only improved R² by 4.4%. Minimum MSE were achieved when four variables were used (4.9% reduction). Further addition of variables increased MSE, with only a 1.1% improvement being achieved when all six variables were used.

Plasma urea N concentrations corrected with either PUN0 or all six variables decreased linearly (P < .06) with increasing dietary lysine concentrations (Table 9). Estimates of the dietary lysine requirement obtained from the two corrected responses (Figure 1) were not (P > .05) different (.656 vs .678%). Initial and final BW of the gilts
in the treatment period were 103.3 ± .8 and 107.7 ± .9 kg, respectively.

In previous research, PUN concentrations have been used in short-term trials to accurately estimate the dietary lysine required by pigs of a specific body weight to maximize N utilization (Coma et al., 1995). In a total of five experiments summarized in that study, PUN0 was closely related (r > .65, P < .01) to PUN1, and, therefore, it was used as a covariate when describing the dietary lysine-PUN response curve. The results in the present study confirm the value of PUN0 to correct PUN1 for individual animal differences not related to amino acid adequacy.

Concentrations of urea (or urea N) and/or creatinine in blood and plasma have been classically used to assess renal function (Smith, 1951; Finco, 1989). Urea and creatinine in blood passively diffuse through the glomerulus and appear in the glomerular filtrate in the same concentrations as in plasma. Thus, rate of urea and creatinine clearance from plasma into the nephron are related to the glomerular filtration rate (GFR). Although a variable percentage of the filtered urea and creatinine are reabsorbed as the glomerular filtrate passes through the proximal tubules, blood concentrations of both urea and creatinine are considered to be crude estimates of GFR; and, therefore, effects of GFR on PUN concentrations should be also reflected on SC concentrations (Finco, 1989). However, the results of this experiment indicated a weak relationship between SC and PUN1. Creatinine is a waste product formed following dephosphorylation of phosphocreatinine to creatine in muscle (Novak, 1973), and, thus, it is released from muscle in amounts proportional to the muscle mass (Rassin and Bhatia, 1992). In an attempt to correct for this process, creatinine measurements were expressed as SC concentrations per unit of lean mass (SC / FFCL at d 35). But, again, no relationship was found between PUN0 and corrected SC (r = -.013, P = .93). The small value of SC when used to correct PUN1 for individual differences in
glomerular function could be explained by a lack of differences among pigs or the inability of SC to detect the variation, because normal SC concentrations have been reported in animals with 75% non-functional nephrons (Bernstein, 1965; Chew and DiBartola, 1986).

Plasma urea N concentration is affected by the water intake of the pig. Cai and Zimmerman (1991) reported that pigs provided a water intake of one and one-half times the feed intake had significantly greater PUN concentrations than those provided a water intake three times the feed intake. Physiological mechanisms of water homeostasis cause renal tubules to concentrate or dilute the urine, and this has a direct effect on PUN concentrations. As a result of the tubular action, amount of water ingested is inversely related to urine SG (Gans and Mercer, 1984). Therefore, the value of SG measurements in explaining some of the variation in PUN concentration may indicate individual differences in renal tubular function and/or in water intake.

As a result of the direct relationship between PUN concentrations and amount of N intake at concentrations above minimum N requirement (Eggum, 1970), daily feed intake (i.e., N intake) also was effective in explaining some of the variation in PUN1. Because animals with a great feed intake and small efficiency of N deposition have greater PUN concentrations, PUN1 was positively and strongly related to fat deposition (DCF). On the other hand, as previously discussed, the increasing effect of ADFI on PUN concentrations may explain the small value of daily lean gain (DFFCL) to correct PUN1 for individual differences.

The estimated lysine requirement of .65 to .68% for gilts weighing 103 to 108 kg is greater than NRC (1988) recommendations. The NRC (1988) recommends a dietary lysine concentration of .60% for pigs weighing 50 to 110 kg without sex distinction. As reported in previous research (Coma et al. 1995), lysine requirements estimated using PUN
as a criterion are greater than the NRC recommendations. Dietary lysine requirement estimated from PUN concentrations responds to maximum N utilization; whereas, NRC recommendations are mostly based on maximal growth performance. Moreover, gilts were used in this experiment, and it has been extensively reported that gilts have a greater lysine requirement than barrows (Baker et al., 1967; Watkins et al., 1977; Cromwell et al., 1990).

**Implications**

Plasma urea N concentrations have a strong relationship with rate of lean tissue growth when the effect of feed intake is removed. Further investigation of this relationship may provide an easy and nonintrusive method to assess the efficiency of lean tissue growth in pigs. Pretreatment PUN is a very useful measurement to explain variation in PUN not related to amino acid adequacy of dietary treatments. When assessing amino acid requirements by using PUN as a criterion, use of pretreatment PUN as a covariate greatly improves accuracy and precision of requirement estimates.

**Literature Cited**


Table 1. Composition (%) of the basal diet, as fed basis

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<tr>
<th>Item</th>
<th>Pretreatment</th>
<th>Treatmenta</th>
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<tbody>
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<td>Corn</td>
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<td>Dicalcium phosphate</td>
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<td>.25</td>
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<td>Antibiotic mixturec</td>
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<tr>
<td>Vitamin premixd</td>
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<td>.10</td>
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<td>Trace mineral mixe</td>
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<tr>
<td>L-lysine·HCl</td>
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<tr>
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<td>DL-methionine</td>
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<td>P, %</td>
<td>.54</td>
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<td>Lysine, %</td>
<td>.780</td>
<td>.475</td>
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aGraded concentrations of crystalline lysine were added to the basal diet to obtain the other 5 dietary treatments.

bPartly replaced by sodium carbonate when crystalline lysine was added, to maintain an identical electrolyte balance (Na+K-Cl).

cContributed per kilogram of diet: 33 mg of bacitracin activity from bacitracin methylene disaclylante.

dContributed per kilogram of diet: 4,400 IU of vitamin A; 1,100 IU of vitamin D3; 22 IU of vitamin E; 6.6 g of riboflavin; 18.0 mg of d-pantothenic acid; 33 mg of niacin; 10.0 μg of vitamin B12.

eContributed in parts per million of diet: Zn, 150; Fe, 175; Mn, 60; Cu, 17.5; I, 2.0.

fGradually removed when crystalline lysine was added, to maintain a constant dietary N content among diets.
Table 2. Body measurements and estimates of body composition of pigs (n = 48) at d 1 and 35 of the pretreatment period

<table>
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<td>Max.</td>
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<tr>
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<td>1.37</td>
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</tr>
<tr>
<td>BFL, cm</td>
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<td>2.29</td>
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<td>1.44</td>
<td>11.4</td>
<td>16.9</td>
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^aUltrasound measurements: LEA = 10th rib longissimus muscle area, BF = 10th rib backfat depth, BFL = last rib backfat depth.
^bFFCL = fat-free carcass lean; CF = total carcass fat; EBP = empty body protein; EBLI = empty body lipid.
Table 3. Growth performance, plasma urea N (PUN) concentrations, renal function measurements, and lean growth of pigs (n = 48) during the 35-d pretreatment period

<table>
<thead>
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<th>Measurement</th>
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<th>SD</th>
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<th>Maximum</th>
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<td>Gain / feed</td>
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<td>.05</td>
<td>.21</td>
<td>.55</td>
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<tr>
<td>Initial PUN&lt;sup&gt;a&lt;/sup&gt;:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN0A, mg/dL</td>
<td>7.7</td>
<td>1.9</td>
<td>4.5</td>
<td>12.4</td>
</tr>
<tr>
<td>PUN0B, mg/dL</td>
<td>9.8</td>
<td>2.7</td>
<td>5.0</td>
<td>17.4</td>
</tr>
<tr>
<td>PUN0C, mg/dL</td>
<td>10.1</td>
<td>2.8</td>
<td>5.1</td>
<td>18.7</td>
</tr>
<tr>
<td>Renal Function:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.018</td>
<td>.010</td>
<td>1.000</td>
<td>1.032</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.59</td>
<td>.19</td>
<td>1.30</td>
<td>2.05</td>
</tr>
<tr>
<td>Lean growth&lt;sup&gt;b&lt;/sup&gt;:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFFCL, g/d</td>
<td>322</td>
<td>49</td>
<td>222</td>
<td>450</td>
</tr>
<tr>
<td>DCF, g/d</td>
<td>250</td>
<td>71</td>
<td>65</td>
<td>382</td>
</tr>
<tr>
<td>DEBP, g/d</td>
<td>109</td>
<td>14</td>
<td>83</td>
<td>144</td>
</tr>
<tr>
<td>DEBLI, g/d</td>
<td>268</td>
<td>73</td>
<td>84</td>
<td>416</td>
</tr>
</tbody>
</table>

<sup>a</sup>PUN0A, PUN0B, and PUN0C = PUN concentrations at d 1, 20, and average of d 34 and 35 of the pretreatment period, respectively.

<sup>b</sup>DFFCL = daily fat-free carcass lean gain; DCF = daily total carcass fat; DEBP = daily empty body protein accretion; DEBLI = daily empty body lipid deposition.
Table 4. Simple correlation coefficients relating variables measured in the 35-d pretreatment period

<table>
<thead>
<tr>
<th>Variables</th>
<th>ADG</th>
<th>ADFI</th>
<th>G:F</th>
<th>PUN0</th>
<th>DFFCL</th>
<th>DCF</th>
<th>DEBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADFI</td>
<td>.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(.0001)(^b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>.03</td>
<td>-.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(.84)</td>
<td>(.0001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN0</td>
<td>.43</td>
<td>.50</td>
<td>-.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(.002)</td>
<td>(.0003)</td>
<td>(.18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFFCL</td>
<td>.72</td>
<td>.58</td>
<td>-.09</td>
<td>.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(.0001)</td>
<td>(.0001)</td>
<td>(.54)</td>
<td>(.28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCF</td>
<td>.85</td>
<td>.63</td>
<td>.03</td>
<td>.65</td>
<td>.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(.0001)</td>
<td>(.0001)</td>
<td>(.81)</td>
<td>(.0001)</td>
<td>(.009)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEBP</td>
<td>.78</td>
<td>.53</td>
<td>-.01</td>
<td>.10</td>
<td>.89</td>
<td>.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(.0001)</td>
<td>(.0001)</td>
<td>(.94)</td>
<td>(.51)</td>
<td>(.0001)</td>
<td>(.009)</td>
<td></td>
</tr>
<tr>
<td>DEBLI</td>
<td>.87</td>
<td>.66</td>
<td>.01</td>
<td>.67</td>
<td>.45</td>
<td>.99</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td>(.0001)</td>
<td>(.0001)</td>
<td>(.93)</td>
<td>(.0001)</td>
<td>(.001)</td>
<td>(.001)</td>
<td>(.001)</td>
</tr>
</tbody>
</table>

\(^a\)G:F = gain to feed ratio; PUN0 = initial PUN; DFFCL = daily fat-free carcass lean gain; DCF = daily total carcass fat; DEBP = daily empty body protein accretion; DEBLI = daily empty body lipid deposition.

\(^b\)Probability of observing a significant correlation of this magnitude if the true correlation is zero.
Table 5. Partial correlation coefficients (ADFI effect removed) relating variables measured in the 35-d pretreatment period

<table>
<thead>
<tr>
<th>Variables^a</th>
<th>ADG</th>
<th>G:F</th>
<th>PUN0</th>
<th>DFFCL</th>
<th>DCF</th>
<th>DEBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>G:F</td>
<td>.99</td>
<td>(.0001)^b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN0</td>
<td>-.52</td>
<td>-.50</td>
<td>(.23)</td>
<td>(.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFFCL</td>
<td>.71</td>
<td>.68</td>
<td>-.88</td>
<td>(.07)</td>
<td>(.09)</td>
<td>(.008)</td>
</tr>
<tr>
<td>DCF</td>
<td>.18</td>
<td>.20</td>
<td>.66</td>
<td>-.47</td>
<td>(.70)</td>
<td>(.67)</td>
</tr>
<tr>
<td>DEBP</td>
<td>.69</td>
<td>.65</td>
<td>-.91</td>
<td>.94</td>
<td>-.53</td>
<td>(.08)</td>
</tr>
<tr>
<td>DEBLI</td>
<td>.24</td>
<td>.24</td>
<td>.54</td>
<td>-.33</td>
<td>.93</td>
<td>-.33</td>
</tr>
</tbody>
</table>

^aG:F = gain to feed ratio; PUN0 = initial PUN; DFFCL = daily fat-free carcass lean gain; DCF = daily total carcass fat; DEBP = daily empty body protein accretion; DEBLI = daily empty body lipid deposition.

^bProbability of observing a significant correlation of this magnitude if the true correlation is zero.
Table 6. Partial correlation coefficients (ADFI removed) relating the average plasma urea N concentrations of d 34 and 35 (PUN0C) with estimates of body composition at d 35 of the pretreatment period

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFCL35</td>
<td>-.48</td>
<td>.27</td>
</tr>
<tr>
<td>CF35</td>
<td>.62</td>
<td>.14</td>
</tr>
<tr>
<td>EBP35</td>
<td>-.53</td>
<td>.22</td>
</tr>
<tr>
<td>EBLI35</td>
<td>.51</td>
<td>.24</td>
</tr>
</tbody>
</table>

*FFCL35 = fat-free carcass lean, CF35 = total carcass fat, EBP35 = empty body protein, EBLI35 = empty body lipid content at d 35 of the pretreatment period.

*Probability of observing a significant correlation of this magnitude if the true correlation is zero.
Table 7. Simple correlation coefficients relating the dependent variable (plasma urea N concentrations in the treatment period, PUN1) with different independent variables measured in the pretreatment period

<table>
<thead>
<tr>
<th>Variables&lt;sup&gt;a&lt;/sup&gt;</th>
<th>r</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUN0</td>
<td>.856</td>
<td>.0001</td>
</tr>
<tr>
<td>DCF</td>
<td>.476</td>
<td>.0006</td>
</tr>
<tr>
<td>SG</td>
<td>.375</td>
<td>.0086</td>
</tr>
<tr>
<td>ADFI</td>
<td>.352</td>
<td>.0141</td>
</tr>
<tr>
<td>DFFCL</td>
<td>.187</td>
<td>.2034</td>
</tr>
<tr>
<td>SC</td>
<td>.166</td>
<td>.2593</td>
</tr>
</tbody>
</table>

<sup>a</sup>PUN0 = initial PUN; DCF = daily total carcass fat deposition; SG = urine specific gravity; DFFCL = daily fat-free carcass lean gain; SC = serum creatinine.

<sup>b</sup>Probability of observing a significant correlation of this magnitude if the true correlation is zero.
Table 8. Best combinations of one to six variables to describe final plasma urea N concentrations (PUN1) obtained by using maximum $R^2$ improvement of SAS (1988)

<table>
<thead>
<tr>
<th>Combination</th>
<th>$R^2$</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUN0</td>
<td>.733</td>
<td>1.350</td>
</tr>
<tr>
<td>PUN0, DCF</td>
<td>.744</td>
<td>1.326</td>
</tr>
<tr>
<td>PUN0, ADFI, DFFCL</td>
<td>.756</td>
<td>1.290</td>
</tr>
<tr>
<td>PUN0, ADFI, DFFCL, DCF</td>
<td>.763</td>
<td>1.284</td>
</tr>
<tr>
<td>PUN0, ADFI, DFFCL, DCF, SC</td>
<td>.764</td>
<td>1.308</td>
</tr>
<tr>
<td>PUN0, ADFI, DFFCL, DCF, SG, SC</td>
<td>.765</td>
<td>1.335</td>
</tr>
</tbody>
</table>

$^a$PUN0 = initial PUN; DCF = daily total carcass fat deposition; SG = urine specific gravity; DFFCL = daily fat-free carcass lean gain; SC = serum creatinine.
Table 9. Least square means (n = 8) of plasma urea N (PUN) concentrations corrected with one variable (initial PUN = PUN0) or with six 6 variables

<table>
<thead>
<tr>
<th>Dietary lysine, %</th>
<th>PUN0</th>
<th>Six variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>.475</td>
<td>9.034</td>
<td>9.004</td>
</tr>
<tr>
<td>.550</td>
<td>8.812</td>
<td>8.666</td>
</tr>
<tr>
<td>.625</td>
<td>7.406</td>
<td>7.504</td>
</tr>
<tr>
<td>.700</td>
<td>7.375</td>
<td>7.177</td>
</tr>
<tr>
<td>.775</td>
<td>7.688</td>
<td>7.762</td>
</tr>
<tr>
<td>.850</td>
<td>7.839</td>
<td>8.023</td>
</tr>
<tr>
<td>Requirement(^b)</td>
<td>.656 ± .0178</td>
<td>.678 ± .0105</td>
</tr>
</tbody>
</table>

\(^a\)Initial PUN, ADFI, daily total carcass fat deposition, urine specific gravity, daily fat-free carcass lean gain, serum creatinine.

\(^b\)Lysine requirement (%) estimated by two-slope, broken-line model regression models (Figure 1).
Figure 1. Use of a two-slope, broken-line regression model (Robbins, 1986) to describe the response of plasma urea N (PUN) to dietary lysine concentrations using (1a) one (initial PUN) or (1b) six variables (initial PUN, ADFI, daily total carcass fat deposition, urine specific gravity, daily fat-free carcass lean gain, serum creatinine) as covariates. R = inflection point (% lysine), L = PUN (mg/dL) at inflection point, U = slope below inflection point, and V = slope above inflection point. Standard error of the pooled PUN least squares means = .386 (1a) and .397 (1b).
CHAPTER 5. INTERACTIVE EFFECTS OF FEED INTAKE AND STAGE OF GROWTH ON THE LYSINE REQUIREMENT OF PIGS

A paper submitted to the Journal of Animal Science

J. Coma, D. R. Zimmerman and D. Carrion

Abstract

The objective of this study was to evaluate the effect of a reduction in feed intake on the lysine requirement of pigs at two stages of growth: growing (G) and finishing (F) phases. Two sets of 40 individually penned crossbred barrows averaging 27.1 ± .2 (G) and 92.6 ± 1.1 (F) kg BW were fed five dietary treatments (G: .75, .90, 1.05, 1.20, 1.35%; and F: .45, .60, .75, .90, 1.05% lysine) at two feeding levels (equivalents to 100 and 80% ad libitum intakes) for 15 d. In both stages, pigs on the 80% feeding level had lower ADG (P < .001) and lower plasma urea N (PUN) concentrations (P < .10) than pigs on the 100% feeding level. In all instances, increasing dietary lysine caused ADG to increase (P < .05) and PUN to decrease (P < .10) linearly. An interaction was detected between the effects of growth stage and the effects of feeding level on the lysine requirements estimated by ADG (P < .19) and PUN (P < .10). In the F stage, pigs on the 80% feeding level required a greater dietary lysine concentration to maximize ADG and
minimize PUN (.85 and .87%, respectively) than pigs on the 100% feeding level (.61 and .68%, respectively). In the G stage, however, the dietary lysine concentrations required to maximize ADG (.97 to 1.01%) and minimize PUN (1.05 to 1.09%) were not different (P > .20) between feeding levels. It is concluded that the effect of a reduction in feed intake on the lysine requirement depends on the stage of growth of the pigs.

Key words: Pigs, Lysine, Feed Intake, Plasma Urea Nitrogen

Introduction

A number of physiological, environmental, and dietary factors influence daily feed intake and, therefore, daily nutrient intake (NRC, 1988). It is well established that protein deposition can be limited by an insufficient intake of protein, energy, or both (Black and Griffiths, 1975). Under conditions of protein adequacy, protein deposition is linearly related to energy intake from near maintenance to ad libitum intakes in young pigs (Campbell et al., 1975, 1986; Campbell and Dunkin, 1983a,b; Whittemore, 1986). But in later stages of growth (BW > 50 kg), protein deposition response to increasing energy intake reaches a plateau at energy intakes below the appetite level (Campbell et al., 1985b, Dunkin and Black, 1987). Because the relationship between energy intake and protein deposition depends on the stage of growth, we hypothesized that the effect of a reduction in feed intake (i.e., energy intake) on daily lysine required to maximize protein deposition might be different, depending on the BW of the animal.

The objective of this study was to evaluate the effect of a reduction in feed intake on the lysine requirement, estimated by ADG or plasma urea N (PUN) concentrations, of pigs at two stages of growth: growing phase and finishing phase.
Materials and Methods

Animals. Eighty individually penned barrows (Yorkshire x Landrace) x (Hampshire x Duroc) were used: 40 in the growing (G) stage (average BW of 23.3 ± .2 kg) and 40 in the finishing (F) stage (average BW of 84.8 ± .9 kg). Lean growth potential of this strain was previously calculated to be .30 to .35 kg/d by using NPPC (1983) formulas. Animals within stage were allotted to four blocks based on initial BW. Pigs in the G stage were placed in .6-m x 1.2-m raised-deck pens with woven-wire floors in a room kept at 21.5 ± 2.2°C. Pigs in the F stage were placed in .6-m x 2.4-m pens with steel-slatted flooring in a room kept at 17.0 ± 2.0°C. After a 7-d period of adjustment to the new environment, pigs within a block were randomly assigned to one of five experimental diets and one of two feeding levels and fed for 15 d. Average initial and final BW in the treatment period were 27.1 ± .2 and 35.4 ± .9 kg, respectively, for pigs in the G stage; and 92.6 ± 1.1 and 104.0 ± 1.5 kg, respectively, for pigs in the F stage. Animals were provided ad libitum access to water.

Feeding Level. Two feeding levels were studied: the equivalents of 100 and 80% ad libitum access to feed. The diets were given to the animals at a constant rate per kilogram of metabolic BW (BW^75). The rates were based on NRC (1987) formulas and previous measurements of ad libitum intakes of pigs at the two BW. Feed allowances of 130 and 116 g of feed/kg BW^75 were calculated to be the equivalent of 100% ad libitum access to feed for the G and F stages, respectively. For the 80% feeding level, the rates were 104 and 93 g of feed/kg BW^75 for G and F stages, respectively. The amount of feed per animal was corrected every 5 d and was based on the initial BW of the animal for that period. Feed was supplied once daily at 0800 h. Dietary energy consumptions averaged 6.4 and 4.4 Mcal of DE/d for the 100 and 80% feeding levels in the G stage, and 12.2 and 9.1 Mcal of DE/d
for the 100 and 80% in the F stage, respectively.

Experimental Diets. Dietary treatments consisted of two sets of five lysine concentrations: .75, .90, 1.05, 1.20, 1.35% for the G stage; and .45, .60, .75, .90, 1.05% for the F stage. Two basal corn-soybean meal diets were formulated to contain the lowest lysine concentrations fed in the G and F stage (Table 1). The two basal diets were supplemented with crystalline lysine to produce the other four experimental diets of each set of dietary treatments. Optimal ratios of essential amino acids to lysine (Chung and Baker, 1992) were calculated for the greatest lysine concentrations of the treatment sets: 1.35 and 1.05%, for G and F stages, respectively. The resulting amino acid concentrations were maintained constant in all diets by supplementation with crystalline methionine, threonine, isoleucine, tryptophan, histidine, and valine. Therefore, the only difference among experimental diets fed in each stage of growth was the dietary lysine concentration; all other amino acid concentrations were constant across treatments. Diets were made isonitrogenous by replacing crystalline lysine with L-glutamic acid.

Diets were formulated to have identical electrolyte balance (Na+K-Cl) by adjusting the crystalline lysine, sodium chloride and sodium carbonate concentrations. The reason for this modification was that recent research has indicated that PUN concentrations are responsive to alterations of the dietary electrolyte balance (Cai and Zimmerman, 1991; Cai et al., 1992).

Minerals and vitamins concentrations were formulated to provide the daily intakes recommended by NRC (1988) when diets were supplied at the 80% feeding level; thus, amounts provided at the 100% feeding level exceeded NRC (1988) recommendations. An antibacterial agent was included in the diets.

Sample Collection and Analyses. Blood samples were obtained the last 2 d of the
adjustment period and on d 4, 5, 14, and 15 of the treatment period. The samples were obtained between 0730 and 0800 h before the pigs were fed. No feed was left from the previous feeding in any pen. Blood was collected in heparinized tubes, via orbital sinus puncture in the G stage and via anterior vena cava puncture in the F stage. Plasma was separated by centrifugation and stored at -20°C until it was analyzed. The PUN concentrations were determined by colorimetrically measuring the product formed in the direct reaction of urea and diacetylmonoxime, as described by Marsh et al. (1965).

Statistical Analyses. Estimates of the lysine requirement were calculated for each group of five animals in the same feeding level within a block, by using ADG (over the 15-d treatment period), average PUN of d 4 and 5 of the treatment period (PUN1), and average PUN of d 14 and 15 of the treatment period (PUN2) as response criteria. Initial PUN (adjustment period) was used as a covariate (P < .01) to correct PUN1 and PUN2 for individual animal differences. Pretreatment PUN has been demonstrated to be very effective in correcting the response of PUN to dietary lysine treatments for variation not related to amino acid adequacy (Coma et al., 1994). A two-slope, broken-line regression model (Robbins, 1986) was used to obtain the requirement estimates (inflection point) from the ADG, PUN1, and PUN2 response curves to increasing dietary lysine. The estimated lysine requirements were considered the dependent variables. The independent variables (treatments) were stage of growth and feeding level. The treatments were arranged in a split-plot experiment, with stages of growth as the whole-plot treatments, and feeding levels as the subplot treatments (Figure 1). Data were analyzed by using the appropriate GLM procedures of SAS (1988). Pooled means of the effect of dietary lysine concentrations and feeding level on ADG and PUN concentrations were calculated for each stage of growth.
Results and Discussion

In all instances, increasing dietary lysine caused ADG (Figure 2) to increase ($P < .05$) and PUN (Figure 3) to decrease ($P < .10$) linearly. In both stages, pigs on the 80% feeding level had lower ADG ($P < .001$) and lower PUN concentrations ($P < .10$) than pigs in the 100% feeding level. As indicated in earlier research (Eggum, 1970), quality of dietary protein is inversely correlated with PUN concentrations. Thus, as dietary amino acid balance is improved by supplementation with the first-limiting amino acid, PUN concentrations are reduced (Kumta and Harper, 1961; Brown and Cline, 1974). On the other hand, quantity of dietary protein is directly related to PUN concentrations (Eggum, 1970). Therefore, reduced feed intake (e.g., protein intake) results in lower PUN concentrations.

The effects of stage of growth and feeding level on lysine requirement estimated from ADG, PUN1, and PUN2 are shown in Table 2. When using the response criteria to estimate the lysine requirement, the regression model did not yield a breakpoint for one dietary treatment set in each of the two feeding levels at the F stage. Therefore, the means of estimated lysine requirements in the F stage were each based on three values.

Average daily gain response tended ($P < .19$) to indicate a different effect of reducing feed intake on the lysine requirement, depending on the pig’s stage of growth. The interaction between stage and feeding level was significant ($P < .10$) when PUN1 concentrations were used to estimate the requirement, indicating that the effect of a reduction in feed intake (80% ad libitum equivalent) on the dietary lysine requirement was dependent on the BW of the animal. In the F phase, pigs on the 80% feeding level had a greater dietary lysine requirement (as percentage of the diet) than those on the 100% feeding level. In the G phase, however, the dietary lysine percentages required by pigs on both feeding levels were similar. No clear effects were observed when PUN2 was used as a
response criterion.

In previous research (Coma et al., 1994) PUN concentrations accurately estimated the dietary lysine required to maximize total N utilization in pigs at a specific BW. Body weight of the animals at the times of PUN1 and PUN2 determinations were 29 and 35 kg (G stage), and 96 and 104 kg (F stage), respectively. Thus, theoretically, lysine requirements estimated at the time of PUN2 determination should have been different from the estimates by PUN1. But, because of the characteristics of the dietary treatments, there was a large variation in BW at the time of PUN2 determination, and this variation may have had a confounding effect on PUN concentrations. Moreover, the large variation in BW and ADFI resulted in a smaller relationship of initial PUN with PUN2 (r = .19, P < .10) than with PUN1 (r = .59, P < .001). The smaller effectiveness of initial PUN as a covariate of PUN2 than of PUN1 data may have also contributed to the lack of differences.

Average daily gains were estimated to be optimized at lower dietary lysine concentrations than were PUN concentrations. It is well established that maximum protein accretion requires a greater intake of amino acids than does maximal rate of weight gain (NRC, 1988). Therefore, because PUN concentrations reflect the protein metabolism of the animal, estimates of the requirement assessed by PUN tend to be greater than when ADG is used as the criterion.

Separate protein- and energy-dependent phases of protein accretion have been demonstrated in pigs (Campbell et al. 1984, 1985a). Whittemore and Fawcett (1976) proposed that under conditions of protein adequacy, protein deposition in pigs responds linearly to increasing energy intake until a maximum deposition was attained at which point the response plateaus. For pigs above 50 kg BW, the maximal lean tissue growth (plateau) has been demonstrated to be reached at energy intakes below the appetite level (Campbell
et al. 1985b; Dunkin et al. 1986; Dunkin and Black, 1987). Because of their limited ingestive capacity, however, younger pigs do not consume sufficient energy to achieve their genetic potential for muscle growth (Campbell et al. 1975, 1986; Whittemore, 1986). Consequently, for young pigs, the relationship between energy intake and protein deposition is essentially linear; and thus, any factor that reduces feed intake or the utilization of dietary energy will restrict lean tissue growth. Because energy intake is the major determinant of maximal protein deposition, the constraint of lean tissue growth by reduced feed intake cannot be removed by simply increasing the intake of the first-limiting amino acid. Therefore, an increased percentage of dietary lysine is not required for lean growth when a reduction in feed intake occurs in G pigs; rather, the required daily intake of dietary lysine is decreased.

Growing and F pigs have a different partitioning of consumed energy among maintenance, protein deposition, and lipid deposition (Fuller and Livingstone, 1978). In the F pig, because of its high voluntary feed intake, the proportion of consumed energy directed to lipid deposition is greater than in the younger pig. Thus, when energy intake is reduced, there still is adequate energy to maintain protein deposition at the expense of lipid deposition and lipid stores. Protein deposition in the F stage is constrained to a lesser extent (if constrained) by reduced feed intake than it is in the G stage. Dunkin and Black (1987), using restricted energy intakes similar to those in this experiment, reported that N balance (g/d) was reduced by approximately 15% in pigs weighing 30 kg, but it was not affected in pigs weighing 90 kg BW. Therefore, when feed intake is reduced in the F stage, the dietary amino acid percentage needs to be increased to supply the daily intakes of amino acids required to support protein deposition. These results are in agreement with data reported by Campbell and King (1982), Campbell et al. (1984) and Batterham et al. (1985).
In those studies, a significant protein by feeding level interaction was found for pigs weighing above 45 kg, indicating that pigs on a low feeding level (approx. 2.5 times maintenance energy) required a higher protein concentration in the diet (grams/kilogram) than when on a high feeding level (approx. 3.2 times maintenance energy). As summarized in SCA (1987), the interaction between energy intake and protein requirement is most likely to occur at BW above rather than below 50 kg, in females and barrows rather than in males, and also in pigs of low- rather than of high-genetic potential for lean gain.

When the lysine requirement was expressed as intake per day, the amount of lysine required by pigs in the F stage was relatively constant and not related to energy intake (22.7 and 23.1 g/d for the 100 and 80% feeding levels, respectively); however, in the younger pig, it was related to the energy intake (17.5 and 14.1 g/d for the 100 and 80% feeding levels, respectively). At the G stage, the lysine:energy ratios were 3.09 and 3.20 g of lysine/Mcal of DE for the 100 and 80% feeding levels, respectively. The concept of expressing amino acid requirements relative to energy concentration is valid only if the relationship between energy intake and rate of protein deposition is linear, as described in pigs weighing less than 45 kg (SCA, 1987). NRC (1988) and ARC (1981) recommended total lysine requirements for pigs of approximately 20 to 50 kg as 2.22 and 3.51 g/Mcal of DE, respectively. Recent experiments with animals in a similar BW range suggest requirements of 2.85 (Yen et al. 1986), 2.94 (Campbell et al. 1988), 3.34 (Rao and McCracken, 1990), 3.00 (Chiba et al. 1991), and 3.02 (Bikker et al., 1994) g of lysine/Mcal of DE. The large differences among the proposed lysine:energy ratio requirements may be because of variations in sex, genetic capacity (Campbell et al., 1985b), energy intake level (Campbell et al., 1984), and ratios, availability, and digestibility of dietary amino acids (ARC, 1981).
It is concluded that, in the growing pig, a reduction in feed intake results in no change in the lysine required to maximize ADG or minimize PUN, when expressed as a percentage of the diet. But when expressed as daily lysine intake, the requirement is reduced by feed restriction. In the finishing pig, reduced feed intake results in an increase in the lysine requirement expressed as a percentage of the diet but no change in the requirement expressed as grams of lysine per day.

Implications

This study indicates the different effect of a reduction in feed intake on the lysine requirement of pigs depending on their stage of growth. Data suggest that some of the depression in lean growth caused by decreased feed intake can be overcome by increasing amino acid concentrations in diets for finishing pigs, whereas adjustments of both dietary energy and amino acids might be needed in growing pigs.

Literature Cited


Table 1. Composition (%) of basal diets\(^a\), as-fed basis

<table>
<thead>
<tr>
<th>Ingredients:</th>
<th>Growth stage</th>
<th>Finishing stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Growing</td>
<td>Finishing</td>
</tr>
<tr>
<td>Corn</td>
<td>75.82</td>
<td>87.10</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>19.19</td>
<td>8.35</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.63</td>
<td>1.15</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.86</td>
<td>.88</td>
</tr>
<tr>
<td>Sodium chloride(^b)</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Antibiotic(^c)</td>
<td>.04</td>
<td>.04</td>
</tr>
<tr>
<td>Vitamin premix(^d)</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Trace mineral mix(^e)</td>
<td>.06</td>
<td>.06</td>
</tr>
<tr>
<td>L-glutamic acid(^f)</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>.24</td>
<td>.17</td>
</tr>
<tr>
<td>L-threonine</td>
<td>.22</td>
<td>.19</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>.01</td>
<td>.07</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>.09</td>
<td>.09</td>
</tr>
<tr>
<td>L-histidine</td>
<td>.04</td>
<td>.06</td>
</tr>
<tr>
<td>L-valine</td>
<td>.10</td>
<td>.14</td>
</tr>
</tbody>
</table>

Calculated analysis:
<table>
<thead>
<tr>
<th>Item</th>
<th>Growing</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>16.40</td>
<td>12.00</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3,261</td>
<td>3,271</td>
</tr>
<tr>
<td>Ca, %</td>
<td>.75</td>
<td>.63</td>
</tr>
<tr>
<td>P, %</td>
<td>.63</td>
<td>.50</td>
</tr>
<tr>
<td>Lysine(^g), %</td>
<td>.75</td>
<td>.45</td>
</tr>
</tbody>
</table>

- \(^a\) Graded concentrations of crystalline lysine were added to the basal diet to obtain the other dietary treatments.
- \(^b\) Partly replaced by sodium carbonate when crystalline lysine was added to maintain an identical electrolyte balance (Na+K-Cl).
- \(^c\) Contributed per kilogram of diet: 41 mg of bacitracin activity from bacitracin methylene disalicylate.
- \(^d\) Contributed per kilogram of diet: 5,500 IU of vitamin A; 1,370 IU of vitamin D3; 28 IU of vitamin E; 8.3 g of riboflavin; 23.0 mg of d-pantothenic acid; 41.2 mg of niacin; 12.5 μg of vitamin B\(_{12}\).
- \(^e\) Contributed in parts per million of diet: Zn, 180; Fe, 220; Mn, 75; Cu, 21.9; I, 2.5.
- \(^f\) Partly removed when crystalline lysine was added to obtain isonitrogenous diets.
- \(^g\) Analyzed values were: .76 and .46% lysine in the growing and finishing stages, respectively. The values were determined by ion exchange chromatography after acid hydrolysis as described by Gehrke et al. (1985).
Table 2. Effects of stage of growth (SG) and feeding level (FL) on the lysine requirement (expressed as a percentage of the diet) of barrows, estimated by a two-slope, broken-line regression model (Robbins, 1986) from ADG and from plasma urea N concentrations on d 4 and 5 (PUN1) and on d 14 and 15 (PUN2) of the treatment period.

<table>
<thead>
<tr>
<th>SG</th>
<th>BW, kg</th>
<th>FL</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>ADG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>27.1 ± .2d</td>
<td>.97</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>92.6 ± 1.1</td>
<td>.61</td>
<td>.85</td>
</tr>
<tr>
<td>F</td>
<td>29.2 ± .3d</td>
<td>1.05</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>96.3 ± 1.0</td>
<td>.68</td>
<td>.87</td>
</tr>
<tr>
<td>PUN1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>35.4 ± .9d</td>
<td>1.06</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>104.0 ± 1.5f</td>
<td>.79</td>
<td>.84</td>
</tr>
<tr>
<td>PUN2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.1 ± .2d</td>
<td>.97</td>
<td>1.01</td>
</tr>
<tr>
<td>PUN1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>92.6 ± 1.1</td>
<td>.61</td>
<td>.85</td>
</tr>
<tr>
<td>PUN2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.4 ± .9d</td>
<td>1.06</td>
<td>1.11</td>
</tr>
</tbody>
</table>

*Growing stage.
*bFinishing stage.
*cMean BW on d 1 of the treatment period.
*d,e,fMeans based on 40, 39, and 38 pigs, respectively.
<table>
<thead>
<tr>
<th>Feeding Level</th>
<th>Growing</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.75 .90 1.05 1.20 1.35</td>
<td>.45 .60 .75 .90 1.05</td>
</tr>
<tr>
<td>100%</td>
<td>X X X X X  ➔ Lys Req 1</td>
<td>X X X X X  ➔ Lys Req 2</td>
</tr>
<tr>
<td></td>
<td>X X X X X  ➔ Lys Req 3</td>
<td>X X X X X  ➔ Lys Req 4</td>
</tr>
<tr>
<td>80%</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Figure 1. Diagram of the statistical design to evaluate the effects of stage of growth and feeding level on lysine requirement of pigs, estimated from ADG and plasma urea N data. Each x represents an individually penned barrow. Estimates of the lysine requirement (Lys Req) were calculated for each group of five animals (fed five dietary lysine concentrations) in the same feeding level within a 10-animal block by using a two-slope, broken-line regression model (Robbins, 1986).
Figure 2. Effect of dietary lysine concentrations on ADG of pigs on two feeding levels (equivalent to 100 and 80% ad libitum access to feed) at two stages of growth (growing and finishing). A two-slope, broken-line regression model (Robbuis, 1986) was used to estimate the pooled optimum lysine concentrations (indicated by arrows) that maximized ADG for each stage of growth at each different feeding level. Standard error of the pooled ADG least squares means = 34.
Figure 3. Effect of dietary lysine concentrations on plasma urea N (PUN) concentrations (average of d 4 and 5 of the treatment period) of pigs on two feeding levels (equivalent to 100 and 80% ad libitum access to feed) at two stages of growth (growing and finishing). A two-slope, broken-line regression model (Robbins, 1986) was used to estimate the pooled optimum lysine concentrations (indicated by arrows) that minimize PUN concentrations for each stage of growth at each different feeding level. Standard error of the pooled PUN least squares means = .598.
CHAPTER 6. LYSINE REQUIREMENT OF THE LACTATING SOW DETERMINED
BY USING PLASMA UREA NITROGEN AS A RAPID RESPONSE CRITERION\textsuperscript{1,2}

A paper to be published in the Journal of Animal Science

J. Coma\textsuperscript{3}, D. R. Zimmerman\textsuperscript{4}, and D. Carrion

Abstract

The dietary lysine intake required to minimize body protein mobilization by adult sows nursing 10 pigs was estimated by using plasma urea nitrogen (PUN) concentration as a rapid response criterion. A preliminary experiment indicated that a 3-d feeding time is required to re-equilibrate PUN concentrations after a change in the dietary concentration of lysine. In the main experiment, a total of 12 sows (BW = 219 ± 5 kg; parity = 4.5 ± .3; backfat = 21.3 ± .9 mm) were used in two 6x6 Latin squares. Treatments consisted of dietary lysine intakes (30.2, 36.9, 43.6, 50.3, 57.0 and 63.7 g/d) that were assigned to six 4-d periods and to six sows in each square. Diets provided adequate levels of all nutrients other than lysine. The treatment periods started on d 5 of lactation and ended on d 29. Plasma urea N concentrations were determined on the third and fourth day of each of the six 4-d treatment periods. Plasma urea N concentrations decreased quadratically (P < .02)

\textsuperscript{1}Journal Paper no. J-a of the Iowa Agriculture and Home Economics Experiment Station, Ames; Project b.
\textsuperscript{2}The authors wish to express appreciation to Heartland Lysine, Chicago, IL for providing amino acids used in this study.
\textsuperscript{3}Supported by a Fulbright/Ministry of Education and Science (Spain) grant.
\textsuperscript{4}To whom correspondence should be addressed.
with increasing dietary lysine intakes. A two-slope, broken-line regression model was used
to estimate the dietary lysine intake that minimize PUN concentrations. The results indicate
that adult sows nursing 10-pig litters with an average growth of 2.22 kg/d required 55.3 g/d
of dietary total lysine to minimize PUN concentrations and, therefore, minimize muscle
protein mobilization.

Key words: Sows, Lactation, Lysine, Blood Plasma, Urea Nitrogen

Introduction

There is considerable variation in the estimates of the lysine requirement of the
lactating sow. Recent reports (Johnston et al., 1991; Stahly et al., 1992; Laurin et al. 1993;
Monegue et al., 1993; Knabe et al., 1993; King et al., 1993; Sauber et al. 1994a) have
suggested that the daily lysine requirement for maximum lactational performance of sows
nursing more than nine pigs is greater than ARC (1981) and NRC (1988) recommendations.
Moreover, the lactating sow requires a greater daily lysine intake to maximize N balance
than to maximize lactational performance (King et al., 1993). Restricted amino acid intake
and excessive body protein mobilization (i.e., negative N balance) during lactation have
been associated with the occurrence of post-weaning anestrus in sows (King and Dunkin,
1986; Brendemuhl et al., 1987; King and Martin, 1989; Jones and Stahly, 1995).

Nitrogen balance and plasma urea nitrogen (PUN) concentrations can be used as
indicators of protein mobilization in the lactating sow. Both response criteria have been
extensively used to determine amino acid requirements of lactating sows (Lewis and Speer,
1973, 1974, 1975; Chen et al., 1978; King et al., 1993), and they yield similar estimates
of the requirement when used simultaneously (Lewis and Speer, 1973, 1974, 1975). In those
studies, PUN concentrations were used during long-term treatment periods. But, because
N metabolism has a rapid response to changes in dietary amino acid concentrations (Brown and Cline, 1974; Fuller et al., 1979), PUN concentration can be used as a rapid response criterion in the determination of amino acid requirements of the lactating sow, as reported by Wilkinson et al. (1982). The value of PUN concentration as a rapid response criterion to accurately estimate the dietary lysine required to maximize N balance has been demonstrated in growing pigs (Coma et al., 1995).

In the present study, the dietary lysine intake required by adult sows nursing 10 pigs to minimize body protein mobilization was estimated by using PUN concentration as a rapid response criterion.

Materials and Methods

The study consisted of two experiments. A preliminary experiment was conducted to determine the feeding time required in adult sows for PUN concentrations to plateau after a change in the dietary concentration of the first-limiting amino acid. In the main experiment, the response of PUN concentrations to dietary lysine was investigated in order to estimate the lysine requirement of lactating sows nursing 10-pig litters.

General. Fifteen sows between third and sixth parity (average 4.5 ± .3) farrowing a minimum of eight live pigs and nursing 10 pigs (natural or via transfer) were used in the study. Lean growth potential of the strain was previously calculated to be .30 to .35 kg/d during the 50 to 110 kg BW period by using NPPC (1983) formulas. Sows were individually-penned in farrowing crates (2.1 x 6 m) with a woven-wire flooring that had a .6-m x .6-m rubber mat centered below the sows' shoulders. Pigs were provided a .3-m x 1.3-m electric heating pad for supplemental heat. Room temperature was maintained at 19 ± 2°C. Litter size was standardized to 10 pigs by fostering pigs within 2 d of parturition.
Healthy pigs that died before d 4 due to crushing were replaced with pigs of similar size and BW. Within 2 d of birth, each pig was given 100 mg of iron, teeth were clipped, and tails docked. Pigs had access to water but not to creep feed throughout lactation. Sows were provided ad libitum access to water. From breeding to farrowing, sows had received 1.8 kg of a corn-soybean meal diet formulated to meet their needs during the gestation period (Table 1). Throughout lactation, each sow was allocated 6.7 kg of feed daily that was provided in two meals (0700 and 1500 h). The amount of feed was calculated to supply the energy (21.5 Meal DE/d) required for maintenance and milk production by a mature sow nursing a 10-pig litter. Calculations assumed a maintenance requirement of 110 kcal·kg⁻⁰·⁷⁵·d⁻¹ and an estimated milk energy output of 175 kcal·kg⁻⁰·⁷⁵·d⁻¹ (Noblet et al., 1986) with an utilization of 65%. A common diet (Table 1) was fed to all sows during the first 6 and 4 d of lactation in the preliminary and main experiments, respectively, to allow the sows to recover their appetite before feeding the treatment diets.

Dietary treatments. Dietary treatments consisted of six lysine concentrations (.45, .55, .65, .75, .85 and .95%). As previously discussed, sows were provided 6.7 kg of feed/d, therefore, daily lysine intakes were 30.2, 36.9, 43.6, 50.3, 57.0 and 63.7 g. The corn-soybean meal diet formulated to contain .45% lysine was supplemented with crystalline lysine to obtain the other five experimental diets. Optimal ratios of essential amino acids to lysine were calculated for the greatest dietary lysine concentration treatment (.95%). The optimal digestible amino acid ratios to lysine were: arginine .67, histidine .45, isoleucine .71, leucine 1.15, lysine 1.00, methionine + cysteine .55, phenylalanine + tyrosine 1.16, threonine .70, tryptophan .20, valine 1.04. The resulting amino acid concentrations were maintained constant in all diets by supplementation with crystalline methionine, threonine, tryptophan, isoleucine, histidine, valine and phenylalanine. Therefore, the only difference
among experimental diets was the dietary lysine concentration; all other amino acid concentrations were constant across treatments. A recent study (Richert et al., 1994) has suggested that lactating sows have a dietary valine requirement of at least 117% of lysine, much greater than current NRC (1988) or ARC (1981) recommendations. Valine was formulated to be 104% of lysine in the present study. But, because a dietary lysine concentration of .95% was used in the calculations and because of the great feed intake of the sows in the present study, the daily intake of valine in all experimental diets (66.2 g/d) was similar to the recommendation of Richert et al. (1994). Diets were made isonitrogenous by replacing crystalline lysine with L-glutamic acid.

Diets were formulated to have identical electrolyte balance (Na+K-Cl) by modifying the crystalline lysine, sodium chloride and sodium carbonate concentrations in the diet. The reason for this modification was that PUN has been shown to be responsive to alterations of the dietary electrolyte balance (Cai and Zimmerman, 1991; Cai et al., 1992). Minerals and vitamins were added to meet NRC (1988) recommendations.

Preliminary Experiment. Three sows were used in a 3x3 Latin square. Three dietary treatments providing 30.2, 50.3, and 63.7 g/d of lysine were assigned to three 6-d periods and to the three sows. Diets were randomized to sows within each period with the constraint that sows were fed each dietary treatment in only one period. Thus, each animal received all three treatments, each treatment was fed for 6 d, and each of the sows received a different treatment in a same period. The treatment periods started on d 7 of lactation and ended on d 24.

Blood samples were obtained daily at 0700 h before feeding the sows from d 5 to d 24 of the lactation period. Blood was collected via ear vein puncture with a 23 gauge needle and transferred to heparinized tubes. Plasma samples were separated by centrifugation
and stored at -20°C until they were analyzed. The PUN concentrations were determined by colorimetrically measuring the product formed in the direct reaction of urea and diacetylmonoxime, as described by Marsh et al. (1965). Average of PUN concentrations in d 5 and 6 (pre-treatment) was designated initial PUN.

**Main Experiment.** A total of 12 sows were used in two 6x6 Latin squares. Treatments consisted of the six dietary lysine concentrations (.45, .55, .65, .75, .85 and .95%) that were assigned to six 4-d periods and to six sows. Diets were randomized as described in the preliminary experiment. Two squares were needed for optimum statistical power, given the expected effect of dietary lysine on PUN concentrations and the expected variation in the measurement. The 4-d treatment periods started on d 5 of lactation and ended on d 29.

Sow and pig BW were recorded on d 0 (within 15 h postpartum) and at the beginning and end of each treatment period, that is on d 5, 9, 13, 17, 21, 25 and 29. Backfat depth was measured by using an ultrasonic probe (Renco Lean-Meter, Renco, Minneapolis, MN) on d 0 and 29 of lactation. Average of the backfat depths 5 cm off midline over the first, tenth, and last rib, and last lumbar vertebrae were calculated. Plasma urea nitrogen concentrations were obtained using the procedure described in the preliminary experiment on d 4 and 5 of lactation, before starting the treatment periods, (initial PUN) and on the third and fourth day of each of the six 4-d treatment periods. All procedures were approved by the Committee on Animal Care at Iowa State University.

**Statistical Analyses.** Data of both experiments were analyzed as Latin square designs with sows as columns and time periods as rows using the appropriate GLM procedure of SAS (1988). Because there was no effect (P > .20) of square in the main experiment, data of both 6x6 Latin squares were combined in a common analysis. Initial PUN was used as
a covariate (P < .001) to correct for individual animal differences. The response of PUN to dietary lysine in the main experiment (n = 72) was fitted by a two-slope, broken-line regression model (Robbins, 1986) to estimate the optimum lysine intake (requirement) that minimized PUN concentrations.

**Results and Discussion**

**Preliminary Experiment**

The response of PUN concentrations to the changes in dietary lysine intake is shown in Figure 1. No values for the last 2 d of the treatment period are reported in sow number two because the sow was accidentally allowed access to feed before bleeding on those days and, therefore, PUN concentrations were much greater than those of the fasted animal in previous days. Means of PUN concentrations decreased (P < .03) with increasing daily lysine intakes (14.1, 7.7 and 4.9 ± 1.0 mg/dL for the 30.2, 50.3 and 63.4 g/d intakes of lysine, respectively). Plasma urea nitrogen concentrations responded to the dietary treatments in less than 24 h (P < .05). A new equilibrium in PUN concentration was reached 2 to 3 d after changing the dietary lysine concentration (P < .05). These data are in agreement with results reported by Kaji and Furuya (1987) and Coma et al. (1995) for growing pigs. Thus, the rapidity of re-equilibration of PUN concentrations after a change in the dietary concentration of the first-limiting amino acid is similar in growing pig and adult sows despite the differences in feed intake and body size. Based on these data, it was concluded that a feeding time of 3 d is required to obtain a constant PUN concentration in a lactating sow after a change in the dietary concentration of lysine.
Main Experiment

A major factor influencing milk yield is energy intake. Milk yield is dependent on both lysine and energy intake. Because the sow is often unable to consume sufficient feed to satisfy the needs to support milk production, body protein and fat are catabolized to maintain a normal level of milk production. However, if the dietary under-supply of nutrients is severe, the sow is unable to supply sufficient nutrients from body tissues and milk production will decline (Whittemore et al., 1988). Therefore, inadequate energy intake can limit the ability of the sow to utilize high levels of dietary lysine for milk production, as reported by Tokach et al. (1992) and Sauber et al. (1994a). In the present study, sows had considerable fat reserves at parturition, as indicated by the backfat measurement. Because of the high daily dietary energy intake, there was a small loss of backfat during lactation (Table 2). These observations and the litter growth performance (Figure 2) indicate that energy intake did not limit the lactational performance in the present study.

Plasma urea N concentrations decreased quadratically (P < .02) with increasing dietary lysine intakes (Figure 3). Based on the broken-line regression analysis, the minimum PUN concentration was obtained at a dietary lysine intake of 55.3 g/d. The sow factor (P < .01), but not the period factor (P > .50), affected PUN concentration.

Quality of dietary protein is inversely related with PUN concentrations (Eggum, 1970). Therefore, early research demonstrated that, as dietary amino acid balance is improved by supplementation with the first-limiting amino acid, PUN concentrations are reduced (Kumta and Harper, 1961; Brown and Cline, 1974). With increasing dietary concentrations of the first-limiting amino acid, PUN reaches a minimum when the requirement is met, and then plateaus (Brown and Cline, 1974) or slightly increases (Lewis et al., 1977, 1980, Coma et al., 1995) at greater concentrations.
The results of the present study indicated that a 215-kg sow, nursing a 10-pig litter with an average growth of 2.22 kg/d needs 55 g/d of total lysine to minimize PUN concentrations. This estimate is considerably greater than ARC (1981) and NRC (1988) recommendations, but it compares well with some of the more recent estimates that range from 42.5 to 57.0 g/d (Johnston et al., 1991; Stahly et al., 1992; King et al., 1993; Laurin et al., 1993; Sauber et al. 1994a). Milk yields of 5 to 7 kg/d in sows nursing seven to eight pigs were used to estimate the requirements recommended by ARC (1981) and NRC (1988). However, modern sows nursing >9 pigs are capable of producing much greater daily milk yields (King et al. 1989; Schoenherr et al., 1989; Sauber et al., 1994b). Using a conversion ratio of milk into pig of 3.8 g/g (Noblet and Etienne, 1986), the estimated milk yield of the sows in the present study was 8.2 kg/d. Pettigrew (1993) suggested that 26 g of total lysine were required in the lactating sow for each kilogram of litter growth. This value indicates a requirement of 57.7 g of lysine for milk production in the present study. The daily total lysine requirement for maintenance was estimated to be 2.6 g (48 mg/kg\textsuperscript{75}; BW = 215 kg; Fuller et al., 1989). Therefore, the lysine need for milk production plus maintenance would be 60.3 g/d if no body protein was mobilized.

When lactating sows are fed dietary amino acid concentrations below the requirement, body protein tissue is catabolized in an attempt to supply the nutrients to maintain milk production (Etienne et al., 1985). As dietary concentration of the first-limiting amino acid increases, muscle tissue loss decreases (Etienne et al., 1989) and, therefore, N balance increases until a plateau is reached (Lewis and Speer, 1973, 1974, 1975; King et al., 1993). In a previous study (Coma et al., 1995), PUN concentration and N balance were demonstrated to be optimized at the same dietary lysine concentration in growing pigs. Lewis and Speer (1973, 1974, 1975) reported that the dietary lysine required to maximize
N balance in the lactating sow was similar to that estimated by using PUN concentration as response criterion. Therefore, the requirement estimated in the present study is assumed to be the dietary lysine intake required by lactating sows to maximize N balance and, therefore, minimize the amount of muscle catabolism.

Lower lysine requirements than that estimated in the present study have been reported for lactating sows with similar litter growths when lactational performance was used as criteria (Stahly et al., 1992; Laurin et al., 1993; Knabe et al., 1993; King et al., 1993). However, King et al. (1993) reported that lactational performance criteria such as litter growth and measured milk yield reached a plateau at a lower dietary lysine intake (40.0 g/d) than those required to maximize N balance (48.1 g/d). Johnston et al. (1991) and Laurin et al. (1993) indicated that sows required greater dietary lysine concentrations to minimize BW loss (65.0 and > 50 g/d, respectively) than to maximize optimum litter weight gain (55.0 and 42.5 g/d, respectively). Because lysine from mobilized muscle protein contributes to the total needs for milk production (Etienne et al., 1985), lactational performance can be maximized even if the intake of dietary lysine is below the total needs for milk production. As previously discussed, N balance and PUN concentrations are assumed to estimate the dietary lysine required by the sow to minimize the amount of muscle catabolism. Therefore, because the contribution of lysine from muscle to milk production needs is minimized, the dietary lysine requirement estimated from these measurements should be greater than when lactational performance is used as the criterion.

The magnitude of the difference among the requirements estimated by the different criteria depends on the amounts of muscle protein mobilized by the lactating sow. Excessive body protein mobilization in lactating sows is undesirable because of potential effects on subsequent reproductive performance. Restricted amino acid intake (< 22 g/d of lysine)
and, consequently, great mobilization of body protein mobilization (i.e., negative N balance) during lactation has been associated with the occurrence of post-weaning anestrus in first-litter sows (King and Dunkin, 1986; Brendemuhl et al., 1987; King and Martin, 1989; Jones and Stahly, 1995). In the work of King and Martin (1989) and Jones and Stahly (1995), the prolonged days-to-estrus interval has been associated with lower mean plasma concentrations of luteinizing hormone (LH) in sows fed deficient concentrations of amino acids.

Implications

The rapid response of N metabolism to changes in the dietary concentrations of the first-limiting amino acid allows the use of PUN concentrations to efficiently estimate the dietary lysine requirement of the lactating sow with a small number of animals. Adult sows nursing 10-pig litters with an average growth of 2.2 kg/d required 55 g/d of dietary total lysine to minimize PUN concentrations and, therefore, to minimize muscle protein mobilization.

Literature Cited


Table 1. Composition (%) of diets, as-fed basis

<table>
<thead>
<tr>
<th>Item</th>
<th>Gestation</th>
<th>d 0 to 4</th>
<th>Basal treatment(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>81.73</td>
<td>74.71</td>
<td>85.47</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>15.00</td>
<td>21.00</td>
<td>7.80</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.50</td>
<td>2.48</td>
<td>2.77</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.94</td>
<td>1.00</td>
<td>.91</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>.50</td>
<td>.50</td>
<td>.50(^b)</td>
</tr>
<tr>
<td>Vitamin premix(^c)</td>
<td>.20</td>
<td>.20</td>
<td>.20</td>
</tr>
<tr>
<td>Choline chloride (60%)</td>
<td>.08</td>
<td>.06</td>
<td>.06</td>
</tr>
<tr>
<td>Trace mineral mix(^d)</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>L-glutamic acid(^e)</td>
<td></td>
<td></td>
<td>1.03</td>
</tr>
<tr>
<td>DL-methionine</td>
<td></td>
<td></td>
<td>.09</td>
</tr>
<tr>
<td>L-threonine</td>
<td></td>
<td></td>
<td>.23</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td></td>
<td></td>
<td>.22</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td></td>
<td></td>
<td>.07</td>
</tr>
<tr>
<td>L-histidine</td>
<td></td>
<td></td>
<td>.12</td>
</tr>
<tr>
<td>L-valine</td>
<td></td>
<td></td>
<td>.40</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td></td>
<td></td>
<td>.08</td>
</tr>
</tbody>
</table>

**Calculated analysis:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Gestation</th>
<th>d 0 to 4</th>
<th>Basal treatment(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>13.57</td>
<td>15.91</td>
<td>12.60</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3,302</td>
<td>3,265</td>
<td>3,190</td>
</tr>
<tr>
<td>Ca, %</td>
<td>.75</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>P, %</td>
<td>.60</td>
<td>.80</td>
<td>.80</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>.64</td>
<td>.80</td>
<td>.45</td>
</tr>
</tbody>
</table>

\(^a\)Graded concentrations of crystalline lysine·HCl were added to the basal diet to obtain the other dietary treatments.

\(^b\)Partly replaced by sodium carbonate when crystalline lysine·HCl was added to maintain an identical electrolyte balance (Na+K-Cl).

\(^c\)Contributed per kilogram of diet: 4,400 IU of vitamin A; 1,100 IU of vitamin D\(_3\); 22 IU of vitamin E; 6.6 mg of riboflavin; 18.0 mg of d-pantothenic acid; 33.1 mg of niacin; 22.0 \(\mu\)g of vitamin B\(_12\).

\(^d\)Contributed in parts per million of diet: Zn, 75.0; Fe, 87.5; Mn, 30.0; Cu, 8.8; I, 1.0.

\(^e\)Partly removed when crystalline lysine·HCl was added to obtain isonitrogenous diets.
Table 2. Means (n = 12) of sow and litter traits at the beginning (d 0) and end (d 29) of the lactation period

<table>
<thead>
<tr>
<th>Item</th>
<th>d 0</th>
<th>d 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow BW, kg</td>
<td>219 ± 5</td>
<td>204 ± 7</td>
</tr>
<tr>
<td>Sow backfat, mm</td>
<td>21.3 ± .9</td>
<td>18.7 ± 1.6</td>
</tr>
<tr>
<td>Litter size</td>
<td>10 ± 0</td>
<td>9.8 ± .1</td>
</tr>
<tr>
<td>Litter weight, kg</td>
<td>14.8 ± 1.2</td>
<td>79.2 ± 2.4</td>
</tr>
</tbody>
</table>
Figure 1. Response of plasma urea N (PUN) concentrations to changes in the dietary lysine intake (30.2, 50.3, or 63.4 g/d of lysine) of three lactating sows (Preliminary Experiment). Vertical dotted lines indicate the dietary changes. Plasma urea N concentrations were affected (P < .03) by dietary lysine intakes over the total lactation period.
Figure 2. Changes in average (n = 12) live BW of the sows and daily growth of the litter throughout the lactation period in the main experiment. The litter growth data represents the daily growth during each 4-d period. Standard errors of sows' BW and litter growth means were 6 and .09, respectively.
Figure 3. Response of plasma urea N (PUN) concentrations to dietary lysine intake in lactating sows (d 5 to 29). Standard error of PUN least square mean (n = 12) was .48. There was a quadratic effect (P < .001) of dietary lysine on PUN concentrations. A two-slope, broken-line regression model (dotted line) was used to describe the lysine-PUN response (n = 72). R = inflection point (lysine intake, g/d), L = PUN (mg/dL) at inflection point, U = slope below inflection point, and V = slope above inflection point. The estimated lysine requirement (R) was 55.3 g/d. Standard error of the estimate was 1.2.
CHAPTER 7. GENERAL SUMMARY

Nine experiments were conducted to evaluate the use of plasma urea nitrogen concentration (PUN) as a rapid response criterion for determination of amino acid requirements of growing and lactating pigs.

Two preliminary trials indicated that a 3-d feeding time was required to re-equilibrate PUN after a change in the dietary concentration of lysine. Based on these observations, three short-term trials were conducted to estimate the dietary lysine requirement of growing pigs at different specific body weights. The response of PUN to dietary lysine concentrations was assessed at the end of 5-d treatment periods. Increasing lysine caused PUN to decrease quadratically (P < .01). A two-slope, broken-line regression model had the highest R² and lowest MSE when compared with three other models as means for estimating the dietary lysine concentration that minimized PUN (i.e., lysine requirement). Lysine requirements were estimated to be .85, .76, .69, and .75 for 36-kg pigs, 49-kg pigs, 74-kg barrows, and 74-kg gilts, respectively. The validity of using PUN as a rapid response criterion was verified by comparing the estimated lysine requirement based on PUN with the requirement determined in a 7-d nitrogen balance period. The estimated lysine concentrations that maximized rates of nitrogen retention and minimized PUN in 25-kg pigs (1.03 vs 1.05%, respectively) were not different (P > .10). From these results, it was concluded that PUN concentrations can be used in short-term trials to accurately estimate the dietary lysine required to maximize total nitrogen utilization in pigs at a specific body weight.

An experiment was conducted to improve accuracy and precision of lysine requirements estimated from PUN data. The value of selected pretreatment variables to
explain variation not related to lysine adequacy in the lysine-PUN response was assessed in 108-kg gilts. In the model describing the PUN response to lysine, pretreatment PUN (PUNO) had the greatest $R^2$ and the lowest MSE when compared with daily feed intake, urine specific gravity, serum creatinine, daily fat-free carcass lean and daily total carcass fat depositions. The estimated lysine requirements from the PUN response corrected with either PUNO or with the combination of all pretreatment measurements were .66 and .68%, respectively, and these estimates were not different ($P > .05$). Therefore, pretreatment PUN is a very useful variable to correct treatment PUN for variation not related to amino acid adequacy. In addition, the relationship between pretreatment PUN and pretreatment lean growth was investigated in this experiment. Partial correlation coefficients (feed intake effect removed) indicated a strong and inverse relationship between the two measurements ($r > -.88$, $P < .01$). These results indicated that PUN concentrations have a potential value as an easy and nonintrusive indicator of the efficiency of lean tissue growth.

The effect of two feeding levels (100% and 80% of ad libitum) on the lysine requirement of pigs was evaluated at two stages of growth: growing (G; 29 kg) and finishing (F; 96 kg) phases. An interaction was detected between the effects of feeding level and the effects of growth stage on the lysine requirement estimated by ADG ($P < .19$) and PUN ($P < .10$). In the F stage, barrows on the 80% feeding level required a greater dietary lysine concentration to maximize ADG and minimize PUN (.85 and .87%, respectively) than barrows on the 100% feeding level (.61 and .68%, respectively). In the G stage, however, the dietary lysine concentrations required to maximize ADG (.97 to 1.01%) and minimize PUN (1.05 to 1.09%) were not different ($P > .20$) between feeding levels. Therefore, these data suggested that some of the depression in lean growth caused by decreased feed intake can be overcome by increasing dietary amino acid concentrations in
finishing pigs, whereas adjustments of both dietary energy and amino acids might be needed in early growing pigs.

The dietary lysine intake required by lactating sows was estimated by using plasma urea nitrogen (PUN) concentration as a rapid response criterion. Adult sows nursing 10-pig litters with an average growth of 2.2 kg/d require 55 g/d of dietary total lysine to minimize PUN concentrations. This estimate was assumed to be the dietary lysine required to minimize body protein mobilization. Lower lysine requirements are needed to optimize lactational performance. However, minimum body protein mobilization in lactating sows might be beneficial to prevent the occurrence of post-weaning anestrus.
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I would also like to thank my friends in both sides of the Atlantic Ocean for their support and friendship.

My deepest appreciation is expressed to my parents for their support and love.
APPENDIX. EQUATIONS TO ESTIMATE BODY COMPOSITION

In Chapter 4, body composition of pigs was estimated from ultrasound measurements and live animal weight (BW) by using equations that were developed by Dr. Allan Schinckel (Purdue University).

The estimated components of body composition were:

FFCL = fat-free carcass lean
EBP = empty body protein
CF = total carcass fat
EBLI = empty body lipid

Real-time ultrasound was used to measure:
LEA = 10th rib longissimus muscle area
BF = 10th rib backfat depth
BFL = last rib backfat depth

The equations were:

For pigs weighing from 63 to 82 kg

\[ FFCL = 2.086 + .112 \times BW - 6.297 \times BF + 2.164 \times LEA \]
\[ EBP = -.051 + .069 \times BW - 2.212 \times BF \]
\[ CF = -8.969 + .104 \times BW + 7.049 \times BF + 3.491 \times BFL \]
\[ EBLI = -11.531 + .122 \times BW + 5.462 \times BF + 3.078 \times BFL \]

For pigs weighing 100 to 127 kg

\[ FFCL = -.145 + .138 \times BW - 9.197 \times BFL + 2.073 \times LEA \]
\[ EBP = 1.222 + .055 \times BW - 2.642 \times BFL + .318 \times LEA \]
\[ CF = -16.349 + .142 \times BW + 3.337 \times BF + 10.469 \times BFL - .582 \times LEA \]
\[ EBLI = -22.138 + .155 \times BW + 4.758 \times BF + 7.933 \times BFL \]