1986

Aromatic amino acid requirements of the lactating sow

William Alfred Lellis
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agriculture Commons, and the Animal Sciences Commons

Recommended Citation
Lellis, William Alfred, "Aromatic amino acid requirements of the lactating sow " (1986). Retrospective Theses and Dissertations. 8266.
https://lib.dr.iastate.edu/rtd/8266

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

While the most advanced technology has been used to photograph and reproduce this manuscript, the quality of the reproduction is heavily dependent upon the quality of the material submitted. For example:

- Manuscript pages may have indistinct print. In such cases, the best available copy has been filmed.
- Manuscripts may not always be complete. In such cases, a note will indicate that it is not possible to obtain missing pages.
- Copyrighted material may have been removed from the manuscript. In such cases, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, and charts) are photographed by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each oversize page is also filmed as one exposure and is available, for an additional charge, as a standard 35mm slide or as a 17"x 23" black and white photographic print.

Most photographs reproduce acceptably on positive microfilm or microfiche but lack the clarity on xerographic copies made from the microfilm. For an additional charge, 35mm slides of 6"x 9" black and white photographic prints are available for any photographs or illustrations that cannot be reproduced satisfactorily by xerography.
Leilis, William Alfred

AROMATIC AMINO ACID REQUIREMENTS OF THE LACTATING SOW

Iowa State University

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106

Ph.D. 1986
Aromatic amino acid requirements
of the lactating sow

by

William Alfred Lellis

A Dissertation 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

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1986
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>2</td>
</tr>
<tr>
<td>Phenylalanine Requirements</td>
<td>2</td>
</tr>
<tr>
<td>History</td>
<td>2</td>
</tr>
<tr>
<td>Rats</td>
<td>3</td>
</tr>
<tr>
<td>Humans</td>
<td>4</td>
</tr>
<tr>
<td>Swine</td>
<td>6</td>
</tr>
<tr>
<td>Pigs</td>
<td>6</td>
</tr>
<tr>
<td>Gestating gilts</td>
<td>7</td>
</tr>
<tr>
<td>Lactating sows</td>
<td>7</td>
</tr>
<tr>
<td>Other species</td>
<td>8</td>
</tr>
<tr>
<td>Biochemistry of Phenylalanine</td>
<td>9</td>
</tr>
<tr>
<td>Synthesis</td>
<td>9</td>
</tr>
<tr>
<td>Metabolism</td>
<td>10</td>
</tr>
<tr>
<td>Catabolism</td>
<td>11</td>
</tr>
<tr>
<td>Evaluation Criteria</td>
<td>13</td>
</tr>
<tr>
<td>Milk yield and pig gain</td>
<td>14</td>
</tr>
<tr>
<td>Nitrogen retention</td>
<td>15</td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>16</td>
</tr>
<tr>
<td>Plasma free amino acids</td>
<td>18</td>
</tr>
<tr>
<td>SECTION I. TOTAL AROMATIC AMINO ACID REQUIREMENT OF THE LACTATING SOW</td>
<td>22</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>24</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>26</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>27</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>30</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>40</td>
</tr>
</tbody>
</table>
### SECTION II. PHENYLALANINE REQUIREMENT OF THE LACTATING SOW

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMARY</td>
<td>45</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>47</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>48</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>51</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>64</td>
</tr>
<tr>
<td>GENERAL SUMMARY</td>
<td>67</td>
</tr>
<tr>
<td>ADDITIONAL LITERATURE CITED</td>
<td>69</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>78</td>
</tr>
</tbody>
</table>
INTRODUCTION

The phenylalanine (Phe) and tyrosine (Tyr) requirement for the lactating sow has yet to be determined by direct experimentation. Based on the calculations of Reid (1961), Baker et al. (1970) and Speer (1975), a tentative dietary requirement of .85% total aromatic amino acids (TAAA) has been proposed for the mature lactating sow fed 5.5 kg/d of a grain-soybean meal diet (NRC, 1979). Estimates of the portion of the total requirement which must be supplied by Phe range from 30% in the adult human (Rose and Wixom, 1955) to 78% in the young pig (Bayley et al., 1981), but generally average 50% for most species studied. The purpose of these experiments was to determine the TAAA requirement for the lactating sow, and the portion of that requirement which must be supplied as Phe.
REVIEW OF LITERATURE

Phenylalanine Requirements

History

The fundamentals of protein nutrition research can perhaps be traced to its origins in the early 18th century, when Magendie (1816) reported that dogs fed diets completely lacking in nitrogen (N) soon died, and that this early mortality could be prevented by the addition of N-containing protein materials to the diet. The concept that these proteinaceous materials might be conglomerates of some sort of smaller components came a few years later, when Braconnor (1820) hydrolyzed gelatin with sulfuric acid and isolated the amino acids glycine and leucine. Tyrosine was isolated in a similar manner from casein (Bopp, 1849) in what is thought to be the first successful hydrolysis of protein using hydrochloric acid (Vickery and Schmidt, 1931). Phenylalanine was discovered in an alcohol extract of lupine seedlings (Schulze and Barbieri, 1881) and was chemically synthesized shortly thereafter (Erlenmeyer and Lipp, 1882). By the turn of the 19th century amino acid quantitation had begun (Kossel and Kutscher, 1899) and positive growth and N retention were obtained in animals fed only protein hydrolysates (Loewi, 1902).

The concept of removing one or more amino acids from a nutritionally adequate protein and then testing the effects of the partially deprived material on animal performance was first used by Abderhalden (1912), who removed tryptophan from a casein hydrolysate and found
that dogs fed this mixture lost both N and body weight. Supple-
mentation of this diet with tryptophan reversed the dogs' deprivation,
and in a like manner, proline removal produced no ill effects.
Tryptophan was thus deemed indispensable in the diet, whereas proline
was not.

With the fundamentals of protein nutrition now in place, researchers
began to concentrate their efforts on determining amino acid essentiality
and requirement. Totani (1916) of F. G. Hopkins' laboratory in
Cambridge removed Tyr from a nutritionally adequate protein and found
the residual material to be fully as effective as the original protein.
This finding came as quite a surprise at that time because Tyr had
been reported as essential for normal development (Abderhalden, 1913).
Reflecting upon this new concept, and relating it to the discovery
that Phe was converted to Tyr in the perfused liver (Embden and Baldes,
1913), Hopkins (1916) proposed the idea that "we may safely ascribe
growth in the absence of tyrosine in part, at least, to the circum-
stance that the phenylalanine, which was still present in the food,
is more or less equivalent to tyrosine, and can cover the essential
demands of the animal." This idea of Tyr replacement for Phe was
later confirmed by Lightbody and Kenyon (1928) and Alcock (1934) in
experiments with young rats.

Rats

In the early 1930s, W. C. Rose of the University of Illinois
began an historical series of experiments in which diets were con-
structured using mixtures of free amino acids as the sole source of protein. These diets initially proved quite inadequate for support of growth in the albino rat (Rose, 1931), and led to the first observation of depressed feed intake with an amino acid imbalance. The missing dietary components were later found to be isoleucine and threonine (Womack and Rose, 1935) and a new discovery, methionine (McCoy et al., 1935). These studies represent the first time that an artificial mixture of amino acids was successfully used to replace dietary protein, and allowed researchers the freedom to add or delete each nutritive element at will to test for quantitative and qualitative requirements.

In a series of experiments which followed, these purified diets were fed to young albino rats to demonstrate that 1) Phe was indispensable for growth (Womack and Rose, 1934), 2) D-Phe could replace nearly all of the L-isomer (Rose and Womack, 1946), 3) Phe requirement was between .7 and .9% of the diet in the absence of Tyr (Rose, 1937; Rose and Womack, 1946), and 4) that approximately half of the Phe could be composed of L-Tyr (Womack and Rose, 1946). These early estimates of the Phe requirement and Tyr replacement value for the rat were later confirmed by Williams et al. (1954), Rama Rao et al. (1961) and Stockland et al. (1971) who reported .86/44%, .72/42% and .69/45% for the requirement/replacement values respectively.

**Humans**

The indispensable nature of Phe for humans was first established by Rose et al. (1943) who measured N equilibrium in volunteer adult
males fed crystalline amino acid diets. Rose et al. (1955) later concluded that 1.1 g/d was the minimum quantity of dietary Phe required to produce a distinctly positive N balance in males, and that DL-Phe was capable of replacing an equal weight of the L-isomer in some of the subjects tested. In contrast to the earlier experiments with rats, Tyr was reported to have a replacement value as high as 70% in the human (Rose and Wixom, 1955). In further experiments, Albanese et al. (1946) reported no utilization of D-Tyr, Nakagawa et al. (1962) found a TAAA requirement of .8 g/d for the 10 to 12 year old boy and Tolbert and Watts (1963) reported a TAAA requirement of .8 to 1.2 g/d, with a Tyr replacement value of 70% for young women.

Contrary to the findings of the previously mentioned authors, Burrill and Schuck (1964) found an absolute TAAA requirement of .6 to .7 g/d for young women and .9 to 1.0 g/d for young college men, with a Tyr sparing effect of no more that 50%. This difference may be explained by the fact that the later authors used a much more stringent definition of 'requirement' than did the former, who may have overestimated both the requirement and replacement value by allowing for a "safer" dietary level.

In a somewhat unusual experiment from an ethical point of view, Snyderman et al. (1955) used growth curves as the criterion of measurement to assess the Phe requirement in human infants. Using six infants ranging from 12 d to 6 mo in age at the start of the experiments (which ran from one to four months in length), these authors estimated
a Phe requirement of 90 mg·kg\(^{-1}\)·d\(^{-1}\). In a somewhat longer and more complex study, Kindt et al. (1984) used plasma Phe levels in 16 children afflicted with phenylketonuria to estimate a need of about 39 mg·kg\(^{-1}\)·d\(^{-1}\) of dietary Phe for 1 to 12 mo old infants. No mention of Tyr is made in either paper, but if it is assumed that Snyderman et al. (1955) estimated the total aromatic requirement and Kindt et al. (1984) just the Phe portion, and if Tyr can spare approximately 50% of the Phe, then the estimated requirement for human infants would fall in the range of 78 to 90 mg·kg\(^{-1}\)·d\(^{-1}\) TAAA.

Swine

Pigs The first attempt to quantify the Phe requirement of the pig was made by Mertz et al. (1954) who reported that an 11 to 22 kg pig required .46% total digestible aromatic amino acids for maximum gain and feed efficiency. Interpolation of this figure is somewhat complicated in that the authors may have substantially underestimated the Phe (.41%) and Tyr (.26%) content of the corn used in their diets. If values of .5% Phe and .5% Tyr are used for ground corn containing approximately 9% protein (NRC, 1979), the actual TAAA content of their basal diet would increase to .66%. Adjusting for digestibility of the synthetic portion of their optimal diet (.66% from corn + .09 ÷ 78% from crystalline Phe), the actual determined TAAA requirement would increase to .78%, which is almost exactly the same as the .79% determined 23 years later by Robbins and Baker (1977). This value was extrapolated by NRC (1979) to estimate the requirements of various
size groups of pigs, based upon their crude protein requirement.

The extrapolated requirement for the 1 to 5 kg pig was confirmed by the work of Bayley et al. (1981) and Kim et al. (1983) from the University of Guelph. These authors measured the influence of dietary Phe on $^{14}$C-Phe oxidation to obtain a requirement of .9% TAAA with an absolute Phe need of .7%. Adding to this an adjustment for digestibility would increase the TAAA requirement to 1.15%, which corresponds very well with the extrapolated value of 1.18% in NRC (1979). It is interesting to note that Robbins and Baker (1977) reported a Tyr replacement value of 49% for the 12 kg pig, whereas Bayley et al. (1981) found a value of only 22% for the 3 kg pig.

**Gestating gilts** Very little information is available on the aromatic amino acid needs of adult swine. A maintenance requirement of only .049% TAAA was proposed by Baker et al. (1966), who observed that a complete dietary void of both Phe and Tyr did not cause negative N balance in the nonpregnant gilt. A tentative requirement of .30% Phe, and not more than .63% TAAA was proposed by Rippel et al. (1965) for the gestating gilt, based upon a few short-term N balance trials conducted during late gestation.

**Lactating sows** The Phe and Tyr requirement for the lactating sow has yet to be determined by direct experimentation. Reid (1961), Baker et al. (1970) and Speer (1975) have estimated the requirement using the factorial approach, in which the calculated amount of Phe and Tyr secreted in sow milk is added to the determined maintenance
requirement for the nonpregnant gilt. This figure is then adjusted for amino acid digestibility to obtain an estimate of the sows' total dietary need. Using this method, Baker et al. (1970) arrived at a TAAA requirement of 40.1 g/d, which was very similar to the 37.9 g/d figure calculated by Speer (1975). Baker, however, used a value of 4 kg/d feed intake to set the requirement at 1.00% of the diet, whereas Speers' higher estimate of 5.45 kg/d intake gave a requirement of .71%. NRC (1979) compromised these two figures and listed the requirement at .85% of the diet.

Other Species

The domestication of new species for both agricultural and research purposes has produced the need for chemically defined diets of known quality and composition to aid in defining specific nutrient roles, adequacies and interactions. Largely because of this need for reference diets, the aromatic amino acid requirements for a variety of species has now been examined. Some of the TAAA requirements and their respective Tyr replacement values obtained include; .87/42.5% for the chick (Sasse and Baker, 1972), .8/46% for the immature Beagle dog (Milner et al., 1984), 1.0/50% for the young kitten (Anderson et al., 1980), .6% for the growing rabbit (Adamson and Fisher, 1973), .4% for growing mice (Bell and John, 1981), 1.2/50% for the channel catfish (Robinson et al., 1980), 2.1% for the fingerling chinook salmon (Chance et al., 1964), 1.5% for the green sea turtle (Wood and Wood, 1977) and 1.0% for the honeybee (DeGroot, 1955).
The higher requirements for the catfish, salmon and turtle may reflect the higher protein levels consumed by these species. Expressed as a percentage of the dietary protein, the TAAA requirement is about 4.2% for the turtle, 4.7% for the pig, 5.0% for the catfish and salmon, 5.6% for the chick and 6.9% for the rat. The human seems to have a comparatively lower need of 3.0% for the infant and 1.0% for the older child.

Biochemistry of Phenylalanine

Synthesis

Although amino acids are of central importance in the metabolism of all organisms, different organisms vary considerably in their ability to manufacture them in a utilizable form. Vertebrates are capable of synthesizing only about half of the 20 or so amino acids required for protein synthesis, and must obtain the remainder (including Phe) from plants or bacteria (Lehninger, 1975). The biosynthesis of Phe was deduced from experiments with mutant strains of bacteria (Gibson and Pittard, 1968) and starts with the formation of shikimic acid from phosphoenolpyruvate and erythrose-4-phosphoric acid. Shikimic acid is then used as a precursor for a variety of biologically significant compounds, including lignin, ubiquinone (coenzyme Q), plastoquinone and chorismic acid. This later compound (Greek, "fork") represents an important metabolic branch point in which one fork leads to the formation of tryptophan through anthranilic
acid, whereas the other fork leads to phenylpyruvic acid, and thus to phenylalanine.

**Metabolism**

Ingested Phe is liberated from whole proteins through the combined efforts of the pancreatic and intestinal proteolytic enzymes and presented to the portal blood supply in the free form (Munro, 1976). Absorption across biological membranes is thought to involve a competitive Na-dependent active transport system common to the large neutral amino acids, which include Phe, Tyr, tryptophan, leucine, isoleucine, valine, histidine and methionine (Lines and Waisman, 1970; Oldendorf, 1971). Distribution of absorbed Phe from the liver into the systemic circulation may involve release of free Phe directly into plasma, synthesis and transport of small peptides or absorption and transport within red blood cells (Aoki et al., 1976). Besides being incorporated into the various body proteins, Phe serves as a precursor for certain biologically active compounds, including thyroxin, melanin and the catecholamines.

Catecholamine synthesis begins in the cytoplasm of adrenergic nerve endings with hydroxylation and decarboxylation of Tyr into dopa and dopamine, respectively (Ganong, 1979). Dopamine is then hydroxylated within the granulated vessels to form norepinephrine, which in turn can be methylated in the adrenal medulla to form epinephrine (adrenaline). Aside from being the principle neurotransmitters in most sympathetic ganglia, the catecholamines function in
the regulation of heart rate and blood pressure and stimulate lipid and glycogen breakdown while inhibiting the action of insulin (Tepperman, 1973). Thyroxin functions as a primary regulator of basal metabolism, and is formed through the iodization of two bound Tyr residues in the thyroid gland (Zapsalis and Beck, 1985).

**Catabolism**

Phenylalanine is one of the seven essential amino acids for which the main degradative pathway is located in the liver (Miller, 1962). Catabolism begins with the nonreversible hydroxylation of excess Phe to form Tyr, followed by a deamination to yield hydroxy-phenyl-pyruvate (Lehninger, 1975). The N fraction enters the urea cycle through glutamate and is incorporated into urea, while the remainder of the carbon skeleton is ultimately oxidized to carbon dioxide through acetoacetate and fumarate. Thus, Phe and Tyr are considered to be both ketogenic and glycogenic amino acids.

There are several inborn errors of metabolism in the Phe-Tyr pathway that are of particular interest to the human nutritionist. Phenylketonuria (PKU) is a hereditary disorder affecting 1 in 25,000 Americans (Munro, 1976) in which Tyr can not be formed from Phe due to the lack of the enzyme phenylalanine hydroxylase in the liver (Jervis, 1953). Excess Phe accumulates in the blood and cerebrospinal fluid and is excreted in the urine either unchanged or as phenyl-pyruvate, phenylacetate or phenyllactate (Jervis et al., 1940; Menkes, 1967). Untreated PKU children progressively develop a severe mental deficiency in which I.Q. rarely exceeds 50 (Jervis, 1937).
These children usually have fair hair and skin, a musty mouse-like odor, wide spacing of incisor teeth, microencephally, brisk reflexes, epileptic fits with hyperkinetic movements, stereotyped digital mannerisms, eczematoid rashes and disagreeable schizoid-like personalities (Bickel et al., 1954).

The exact reason as to why excessive levels of circulating Phe causes retarded brain development is unknown, but may be due to toxic effects of Phe or its byproducts (Bickel et al., 1954), excessive catecholamine production triggered by the large influx of Phe across the blood-brain barrier (Wurtman et al., 1974, Sved and Fernstrom, 1981) or to competitive inhibition of large neutral amino acid transport into the brain (McKean et al., 1968; Peng, 1973). Particular attention has focused on the role that tryptophan, and its' monoamine neurotransmitter serotonin might play in the expression of PKU abnormalities. Inhibition of both cerebral protein (Siegel et al., 1971) and serotonin (Fernstrom et al., 1983) synthesis have been correlated with reduced tryptophan transport across the blood-brain barrier during a Phe overload. The toxic effects of Phe can be partially (Elkin and Rogler, 1983) or wholly (Siegel et al., 1971) overcome with tryptophan supplementation. This led to the speculation that PKU abnormalities may be caused by a deficiency of tryptophan, rather than an excess of Phe, entering the brain (Siegel et al., 1971). When intervention occurs early enough in life, the deleterious effects of PKU have been successfully controlled with low Phe diets (Bickel et al., 1954; Lyman and
Two other noteworthy autosomal recessive disorders for Phe catabolism are alkaptonuria and albinism. Alkaptonuria originates from a homogentisic acid oxidase deficiency, in which the unmetabolized homogentisic acid accumulates in the blood and urine (Lehninger, 1975). This condition leads to abnormal pigmentation of connective tissue and degenerative arthritis in the advanced stages. In the normal human Tyr serves as a precursor of melanin, which is a polymer of indole nuclei produced in the melanocytes of skin, eye, mucous membranes and nervous tissue (Zapsalis and Beck, 1985). Graded degrees of pigmentation are caused by their melanin content, and albinism occurs when the enzyme tyrosine-3-monooxygenase is genetically lacking.

Evaluation Criteria

The quantitative determination of amino acid requirements for the lactating sow has received a good deal of attention over the past 15 years in an effort to subdivide the sows' protein requirement into its component fractions of specific amino acids and nonspecific N. Whereas the effect of amino acid supply on sow milk production, piglet gain and subsequent reproductive preparedness are the major economical considerations, the influence of diet on these criteria is often difficult to detect due to large variations among sows. Because the diets used in this type of research are semi-purified, and thus quite expensive, conducting large scale experiments to directly address
these factors is not feasible. Estimation of amino acid requirements must therefore be based upon more sensitive metabolic criteria, such as N balance, urea N excretion and plasma free amino acid curves.

**Milk yield and pig gain**

Lactational studies at Iowa State University with methionine (Ganguli et al., 1971), lysine (Lewis and Speer, 1973), tryptophan (Lewis and Speer, 1974a), threonine (Lewis and Speer, 1975a), isoleucine (Haught and Speer, 1977), leucine (Rousselow et al., 1979) and valine (Rousselow and Speer, 1980) have generally displayed a depression in both milk yield and pig gain during an amino acid deficiency in the sows' diet. Milk yield was found to be a more consistent predictor of requirement than was pig gain, with all of the amino acids except methionine producing some type of response to amino acid level in the diet. Prediction of requirements based on milk production is associated with large animal-to-animal variation and the tedious procedures involved with its measurement (Lewis and Speer, 1975b). The only reliable method of quantification is to weigh the litter before and after each nursing (Speer and Cox, 1984), which becomes increasingly more difficult as the pigs get older.

Piglet gain responded quadratically to levels of lysine, valine, isoleucine and methionine, but not to leucine, threonine or tryptophan. The effects of treatment on piglet gain are often complicated by the high incidence of scours that can afflict pigs during the second and third week of lactation.
Nitrogen retention

The study of N retention in relation to quantity and composition of ingested protein has contributed greatly towards our understanding of amino acid nutrition. It was Lavoisier in 1789 (see Kofranyi, 1972) who made the first basic observation in the field of N metabolism when he reported that the amount of N inhaled remains the same as when exhaled, and thus plays no part in respiration. Not long thereafter, N excretion in the urine and feces was shown to be correlated with the amount of N in the food (Magendie, 1816) and by the end of the 1800s N retention was being utilized to assess protein quality (Loewi, 1902). Rose et al. (1943), in their studies with humans, were the first to extensively use the criterion of N retention to determine the amino acid requirements of mature nongrowing animals, and Rippel et al. (1965) later applied these same techniques in studies on the pregnant gilt.

Although the measurement of N retention (Feed N - Fecal N - Urine N - Milk N) has experienced widespread usage in protein research, there are a number of technical difficulties in its application on the lactating sow (Lewis and Speer, 1975b). Nitrogen balance studies tend to overestimate N intake and underestimate N excretion, resulting in an overestimation of the total N retained (Wallace, 1959). Certain sows tend to be quite wasteful with their feed, and recovery of this waste tends to approach impossibility when dealing with highly water soluble diets. Consumption and waste by the baby pigs is difficult
to determine, especially as piglets get older and more adventurous. With the use of screens to separate piglet from sow feces and catheters to collect urine, quantitation of N output from these sources is quite acceptable, yet problems with exact measurement of milk output remains troublesome (Elsley, 1971). Another difficulty is that sows tend to lose large quantities of hair during lactation. A portion of this hair is unavoidably collected with the feces, resulting in increased estimates of actual fecal N (Lewis and Speer, 1975b).

Despite these inherent difficulties in estimating N retention in the lactating sow, the technique has proven useful in determining the requirements for lysine (Lewis and Speer, 1973), tryptophan (Lewis and Speer, 1974a), isoleucine (Haught and Speer, 1977) and valine (Rousselow and Speer, 1980) but not for methionine (Ganguli et al., 1971), threonine (Lewis and Speer, 1975a) or leucine (Rousselow et al., 1979).

Urea nitrogen

During amino acid catabolism two amino groups and one molecule of carbon dioxide are combined within the liver to form a molecule of urea, which is transported to the kidneys and excreted in the urine (Lehninger, 1975). Eggum (1970) fed rats varying proportions of casein and observed a linear rise in plasma urea with increased dietary protein. These urea levels increased quadratically after feeding to a plateau at 3 to 4 h, and were negatively correlated \( r=-.95 \) with the biological value of the dietary protein.
Rose et al. (1950) observed large increases in urinary N excretion among human subjects placed on amino acid deficient diets. By partitioning the urinary N into its various components, they were able to demonstrate that the majority of the increase came from urea.

Similar findings were made by Grosbach et al. (1985), who reported that urea N comprised nearly 77% of the total urine N from pigs fed isoleucine and threonine deficient diets, but only 26% when these amino acids were fed at adequate levels.

Kiriyama and Iwao (1969) fed rats diets containing a wide range of threonine levels and found that urine urea decreased linearly with increasing dietary threonine until the requirement was met, at which point urea either plateaued or started to increase again. Brown and Cline (1974) obtained similar results in pigs fed graded levels of lysine and tryptophan, and also found that urine urea excretion stabilized within 3 d after a dietary change. They suggested that as protein synthesis increases with supplementation of the first limiting amino acid, fewer nonlimiting amino acids are catabolized and urea synthesis decreases.

Blood urea N concentrations were found to be good indicators of amino acid adequacy in the lactating sow for lysine (Lewis and Speer, 1973), tryptophan (Lewis and Speer, 1974a), and threonine (Lewis and Speer, 1975a) but not for isoleucine (Haught and Speer, 1977), leucine (Rousselow et al., 1979) or valine (Rousselow and Speer, 1980). Because plasma urea N represents a 'snapshot' of urea concentration measured at a moment in time, whereas urine urea N is a quantitative
collection which can be related to N intake, urine urea may be the more accurate indicator of amino acid status in the sow.

**Plasma free amino acids**

The concentration of free amino acids in plasma (PFAA) at any given moment is a reflection of the balance between absorption from the gastrointestinal tract, removal by the tissues for protein synthesis, catabolism and excretion in the liver and kidneys and influx from tissues during protein turnover (Henry et al., 1979). The relationship between the dietary intake of a particular amino acid and its resulting concentration in the plasma (PFAA response curve) has been used extensively to estimate metabolic requirements. This technique is especially useful in mature lactating animals in which N balance and body weight change are confounded by milk secretion (Lewis and Speer, 1975b).

The use of amino acid response curves to estimate requirements began with the realization that postfeeding changes in PFAA concentrations were dependent upon the composition of the protein ingested (Longenecker and Hause, 1959; Hill et al., 1961; Morrison et al., 1961a). This finding led to the discovery that the plasma level of a limiting amino acid remains relatively low and constant until the animals' requirement is met, after which plasma levels increase proportionally with additional dietary increments (Morrison et al., 1961b; Zimmerman and Scott, 1965). This technique has been successfully used in the estimation of amino acid requirements of numerous species, including young pigs.
(Mitchell et al., 1968), humans (Young et al., 1971), chickens (Ishibashi, 1973), lactating sows (Lewis and Speer, 1974b), catfish (Robinson et al., 1980) and dogs (Milner et al., 1984).

The adequacy of intake of an amino acid can also be evaluated by studying the plasma levels of the other essential amino acids. Morrison et al. (1961b) observed an increased concentration of threonine in rat plasma when lysine was limiting in the diet. As the lysine deficiency was corrected, plasma threonine levels fell, reaching a minimum when the lysine requirement was met. This phenomena may be indicative of a poor utilization of essential amino acids for protein synthesis during an amino acid deficiency (Munro, 1976).

Although PFAA response curves are now widely used to establish amino acid requirements, little attention has been given to the factors other than diet composition which may influence the magnitude of their response. The length of time that a diet must be fed before eliciting a stable response was examined by Gray et al. (1960) who observed similar PFAA patterns in chicks fed lysine deficient diets for 2, 7, 14 or 28 d. Likewise, Morrison et al. (1961b) found no difference in the response of rats to amino acid deficient diets after 1, 3, or 7 d, but did find a difference between these values and those found just 2 h after a dietary change. Ganguli et al. (1971) reported that although sows fed diets containing graded levels of methionine displayed higher levels of some PFAA at 21 d postfeeding than at 14 d, the response pattern to treatment was unchanged. In a more extensive study involving two 5 x 5 Latin-square experiments to determine the
lysine and tryptophan requirements of the lactating sow, Lewis and Speer (1974b) analyzed blood samples taken every 5 d during a 27 d lactation and concluded that stage of lactation did not significantly affect PFAA response curves. From these studies it would seem that PFAA concentrations adjust rapidly to supply, and probably stabilize within 3 d after a dietary change.

If PFAA are to provide meaningful information about dietary adequacy, then blood samples should be obtained after the majority of amino acids have been absorbed from the gastrointestinal tract, but before contributions from tissue catabolism become so large as to mask treatment effect (Typpo et al., 1970). Early indications of this came by way of Zimmerman and Scott (1967) who found a much different PFAA pattern 30 m after chicks were fed an amino acid test diet that at 6 h. In a footnote to a study on the methionine requirement of the lactating sow, Ganguli et al. (1971) state that "in a study with lactating sows fed the experimental diet (0.55% sulfur amino acids), the plasma free methionine concentration remained nearly the same between 1 and 9 hr postfeeding with a maximum concentration between 4 and 6 hr." These authors also found that, except for a lower concentration of plasma lysine, there was no difference between any of the PFAA levels at 5 than at 1 h postfeeding.

Two comprehensive investigations into the effects of time and site of blood withdrawal on PFAA concentrations in pigs were reported by Nordstrom et al. (1970) and Typpo et al. (1970) from the University
of Minnesota, and Shimada and Zimmerman (1973) from Iowa State University. In a series of several experiments conducted by the Minnesota group, 23 kg barrows were fed 16% protein corn-soybean meal diets and PFAA concentrations were monitored from several different blood vessels for up to 36 h postfeeding. The authors found that concentrations of all PFAA, except cystine, increased sharply within 40 min after feeding and reached a peak at 2 to 4 h. Following the initial surge, amino acid levels declined linearly to their lowest baseline levels at 6 to 12 h, then increased gradually over the next 18 h. The same postfeeding response curves were observed for samples taken from the portal vein, abdominal aorta, posterior vena cava and jugular, although levels in the portal vein were generally higher. The authors concluded that during the period of 8 to 12 h postfeeding PFAA were most stable and were least influenced by the effects of absorption, anabolism and catabolism. Similar trends in PFAA concentrations were reported by Shimada and Zimmerman (1973) who found that portal to systemic (anterior vena cava) PFAA differences were greatest between 1 and 2 h postfeeding, and that these differences disappeared after 6 h of fast. They also found that concentrations of essential PFAA in blood were highly correlated (r= .92) with dietary levels at 1 h postprandial, and that more efficient absorption of amino acids occurred in heated than in raw soybean meal.
SECTION I. TOTAL AROMATIC AMINO ACID REQUIREMENT
OF THE LACTATING SOW
TOTAL AROMATIC AMINO ACID REQUIREMENT
OF THE LACTATING SOW

W. A. Lellis and V. C. Speer

Iowa Agricultural and Home Economics Experiment Station,
Ames, Iowa 50011
SUMMARY

Six mature Yorkshire X Landrace sows were randomly assigned to a 6 x 6 Latin-square experiment on d 5 of lactation to determine the total phenylalanine (Phe) and tyrosine (Tyr) requirement. A control diet of corn sugar, cornstarch, whey, L-glutamic acid, Solka floc, soybean oil, amino acids, minerals and vitamins was supplemented with L-Phe to provide .30 (basal), .45, .60, .75, .90 and 1.05% total aromatic amino acids (TAAA). Each diet was fed to a maximum of 5.5 kg/d within each of six 10-d periods. Feed intake, average pig weight gain and sow milk yield decreased linearly (P<.01) with increasing period. Sow milk yield was maximized at .75% TAAA (quadratic, P<.10), but average pig weight gain did not reflect the higher yield. Urea nitrogen decreased quadratically with increasing dietary Phe in both plasma (P<.05) and urine (P<.01) to a breakpoint at .56% TAAA. Plasma Phe increased (quadratic, P<.01) as dietary TAAA increased but no clear inflection point was obtained. A sharp rise (quadratic, P<.001) in plasma Tyr occurred at .73% dietary TAAA. Plasma lysine decreased (linear, P<.001) to a low level at .75% TAAA, but plasma methionine was unaffected by treatment. Urine allantoin/urea X protein intake was maximized at .61% TAAA (quadratic, P<.01). Nitrogen (N) intake varied among diets (quartic, P<.05), but fecal N was not altered by TAAA level. Urine N decreased quadratically (P<.01) with increasing Phe, yielding maximum (P<.01)
percentage N retention (excluding milk N) at .58% TAAA. Because daily milk N secretion also increased (linear, P<.01) with dietary TAAA, there were no treatment differences in overall N balance (including milk N). If equal weight is given to all response criteria, .65% TAAA seems to meet the dietary requirement for the lactating sow. In a diet containing natural ingredients, the requirement would increase to .75% of the diet, or 41.2 g/d for sows fed 5.5 kg/d.

(Key Words: Pigs, Lactation, Phenylalanine, Tyrosine, Blood Plasma, Nitrogen.)
INTRODUCTION

The phenylalanine (Phe) and tyrosine (Tyr) requirement for the lactating sow has yet to be determined by direct experimentation. Reid (1961), Baker et al. (1970) and Speer (1975) have estimated the requirement by adding the calculated amount of Phe and Tyr secreted in sow milk to the determined requirement for a nonpregnant adult gilt and adjusting this value by a coefficient for amino acid digestibility. Their estimates for the total aromatic amino acid (TAAA) requirement range from .70 to 1.04% of the diet. NRC (1979) recommends .85% (46.8 g/d) for a mature lactating sow fed 5.5 kg/d of a grain-soybean meal diet. The present study was conducted to establish more clearly the aromatic amino acid requirement of the lactating sow.
EXPERIMENTAL PROCEDURE

Six Yorkshire X Landrace crossbred sows (fifth through seventh parity) selected on the basis of previous satisfactory reproductive performance were randomly assigned to a 6 X 6 Latin-square experiment (Cochran and Cox, 1957) on d 5 of lactation. Corn sugar, cornstarch, whey, vitamins, minerals and indispensable amino acids were used to formulate a basal diet (Tables 1 and 2) containing .30% TAAA. This diet was supplemented with L-Phe to provide levels of .30 (basal), .45, .60, .75, .90 and 1.05% TAAA. Corn sugar and L-glutamic acid were varied to make the diets isonitrogenous. The basal diet was calculated to contain all essential nutrients, except Phe and leucine (Rousselow et al., 1979), at recommended levels (NRC, 1979). Each diet was fed to a maximum of 5.5 kg/d (in two equal feedings) within each of six 10-d lactational periods. During the first 5 d of lactation, all sows were fed 5.5 kg/d of a 13%-protein corn-soybean meal diet.

Sows and their litters were maintained in raised farrowing crates with free access to water, but creep feed was not offered. Facilities and management practices were approved for humane treatment of large animals by the Iowa State University attending veterinarian. Litter size was standardized to nine pigs and maintained by replacing any dead pig with a pig of similar age. Litters were weaned at the end of periods 2 and 4 and replaced with a new 7-d-old litter. Pig
weights were recorded at the beginning and end of each period. Sow milk yield was measured on d 8 of each period by weighing litters before and after suckling at hourly intervals for 5 h (Speer and Cox, 1984). The first 2 h were used for adaptation; the final 3-h values to estimate daily milk yield.

Nitrogen balance trials were conducted from d 5 to 10 of each period by using total fecal and urine collection. Urine was collected via a bladder catheter into carboys containing 40 ml of 1.16 N HCl. A .5% subsample was taken daily, pooled and frozen at -20 C until analyzed. Feces were collected on a fine-mesh screen, frozen and pooled. Recognizable piglet feces were hand-sorted from the sow fecal collection. A sample was then oven-dried at 55 C and ground in a Wiley mill. Milk and blood samples were obtained 6 h after feeding on d 8 of each period. Blood samples were taken via vena cava puncture. The heparinized samples were centrifuged, and the plasma was frozen at -20 C. Milk samples were obtained by manual expression after injection of 10 IU oxytocin into the vena cava.

Samples were analyzed for total N by micro-Kjeldahl (AOAC, 1975). Urine and plasma urea were determined by the method of Marsh et al. (1965), and urine allantoin by the method of Vogels and VanDerDrift (1970). Feed samples were hydrolyzed in 5 N HCl under nitrogen for 22 h at 110 C (Kaiser et al., 1974). Feed and
plasma were then analyzed for amino acids by gas-liquid chromatography (Frank et al., 1980) after removal of plasma impurities by the method of Adams (1973).

Data were analyzed by the method of least-squares, with orthogonal polynomials used to partition treatment effects into single-degree-of-freedom contrasts (SAS, 1982). Minimum amino acid requirements were estimated by fitting the data to several least-squares models, including quadratic and sigmoidal exponentials (Robbins et al., 1979; SAS, 1982), NLIN (SAS, 1982) and broken-line analysis (Draper and Smith, 1981). The most adequate model was considered to be that yielding the smallest residual sums of squares. The requirement was taken as the abscissa of the breakpoint in broken-line analysis or as 95% of the upper or lower asymptote in nonlinear models (Robbins et al., 1979).
RESULTS AND DISCUSSION

Feed intake, average pig weight gain, sow milk yield and plasma and urine constituents are presented in Table 3. Feed intake varied among dietary treatments (quartic, $P<.05$), but decreased linearly ($P<.01$) with increasing lactational period (5.41, 5.36, 5.23, 4.68, 5.04 and 4.54 kg/d). Possible explanation for this decrease may be a decreased energy requirement as milk production declined, or the long-term consumption of highly synthetic diets. Depressed feed intake has been reported in young pigs fed high levels of glutamic acid (Robbins and Baker, 1977), but this occurred at levels of inclusion greater than those used in the present experiment. The ratio of N contributed by indispensable amino acids to N contributed by dispensable amino acids (I:D ratio) also has been implicated in feed consumption and optimal N retention (Mitchell et al., 1968; Robbins and Baker, 1977). Although the I:D ratio of the basal diet (.31:1) fell below the suggested range of .67:1 to 1:1, the effect on long-term feed consumption is unknown. Sows did, however, increase feed intake when offered a normal corn-soybean meal diet at the conclusion of the experiment.

Average pig weight gain did not respond to level of Phe, but decreased linearly ($P<.01$) with increasing period (1.34, 1.74, 1.13, 1.14, 1.10 and .77 kg/period). This decrease was correlated with milk yield ($r=.62$), which also declined linearly ($P<.001$) with
period (7.71, 6.99, 5.49, 3.95, 3.12 and 2.16 kg/d). Sow milk yield was maximized at .75% TAAA (quadratic, P<.10). In previous trials with other amino acids, increasing the respective amino acid to the estimated requirement improved both milk yield and pig gain (Lewis and Speer, 1973, 1974, 1975a; Haught and Speer, 1977; Rousselow and Speer, 1980). Perhaps the shorter duration of feeding the experimental diets in the present experiment did not allow for expression of pig weight gain.

Minimal levels of urea N have been associated with optimal amino acid balance (Brown and Cline, 1974) and have been observed in previous lactation studies (Lewis and Speer, 1973, 1974, 1975a). In the present experiment, plasma urea decreased from .30 to .60% TAAA (quadratic, P<.05). When expressed as a percentage of N intake to correct for varying feed consumption, urinary urea also decreased rapidly with increasing Phe (44.1, 34.7, 27.2, 28.4, 28.0 and 28.3%; quadratic, P<.01) to .60% TAAA. Broken-line analysis estimated the TAAA requirement to be .54% and .56% based upon plasma urea and urine urea values, respectively.

Plasma Phe increased quadratically (P<.05) as dietary TAAA increased, but no clear point of inflection was obtained within the range of diets tested. This finding is contrary to previous amino acid studies with sows in which the plasma levels of the test amino acid remained low and constant until the estimated requirement was met, then increased as the dietary levels surpassed the requirement
(Lewis and Speer, 1973, 1974; Haught and Speer, 1977). Studies with the rat (Stockland et al., 1971) and catfish (Robinson et al., 1980), however, also have demonstrated an inconsistent response in plasma Phe as a function of dietary Phe. Stockland et al. (1971) have suggested plasma Tyr to be a better indicator of dietary aromatic amino acid adequacy. A more typical response of plasma Phe to increased dietary intake was seen in the beagle (Milner et al., 1984) and the chick (Ishibashi, 1973).

Plasma Tyr rose slightly between .30 and .75% TAAA, then increased sharply with further additions of Phe to the diet (quadratic, P<.001). Because it is the first product formed in the catabolism of excess dietary Phe (Munro, 1976), plasma Tyr may be the strongest indicator of TAAA requirement. Plasma methionine was unaffected by dietary treatment. Plasma lysine decreased rapidly between .45 and .75% TAAA (linear, P<.001), with no further reduction at the higher levels of Phe intake. Breakpoint analysis estimated the TAAA requirement to be .73% and .76%, based upon plasma Tyr and plasma lysine, respectively.

Excretion of urinary allantoin increased slightly from .30 to .60% TAAA, then decreased at intakes above .75%. This trend, however, was not significant. Kiriyama et al. (1967) observed that urinary allantoin excretion increased as threonine deficiency was corrected in the rat. Because this increase in allantoin accompanied a simultaneous decrease in urine urea, the authors considered the ratio
of allantoin:urea X protein intake (A/U X IP) to be the most sensitive indicator of protein quality. Because this test is simple and complete in collection, Lewis and Speer (1975b) proposed its application to amino acid studies with the sow. In the present experiment, A/U X IP increased sharply to a maximum at .60% TAAA (quadratic, P<.01), then declined slightly with each additional increment of Phe. Broken-line analysis indicated a requirement of .61% TAAA. Any conclusions made regarding the TAAA requirement with use of this index must be approached with caution, however, because urine allantoin was not affected by diet, whereas urine urea was. Thus, the index A/U X IP actually reflects the inverse of urine urea X protein intake. Further evaluation of this index in sow research will be necessary to determine its value as a predictor of amino acid requirement.

Nitrogen balance data of sows are presented in Table 4. Nitrogen intake varied inconsistently among treatments (quartic, P<.05), being greatest at the .75% TAAA level. Fecal N was unaffected by diet. Total urine N decreased quadratically (P<.01) with increasing Phe and as expected, was highly correlated with urine urea (r=.98). When expressed as a percentage of N intake, urine N decreased rapidly to .60% TAAA (55.9, 45.8, 38.4, 40.0, 38.8 and 41.4%; quadratic, P<.01). Percentage of N retained increased (quadratic, P<.01) with increasing dietary Phe (38.4, 47.8, 55.0, 54.1, 56.0 and 51.8%) and also plateaued at .60%
TAAA. Total daily secretion of milk N was maximized at .75% TAAA (linear, P<.01), but percentage of N in milk was not significantly altered by diet. Thus, milk N (g/d) was highly correlated with (r=.93) and reflected the differences in milk yield. All levels of TAAA supported positive N balance, and there were no differences between treatments when balance was expressed as g/d or as a percentage of N intake. Broken-line analysis predicts a dietary requirement of .56% TAAA with percentage urine N and .58% TAAA with percentage N retained.

When residual sums of squares were used as the means of comparison, 10 of the 11 variables analyzed that were significantly affected by diet at the quadratic level were best fit by a broken-line model. This finding is in contrast to that of Robbins et al. (1979), who obtained a better overall fit with nonlinear models. This discrepancy may be attributed to the nature of the data being analyzed, the greater number of diets in the experiments of Robbins et al. (1979) or to the fact that breakpoint analysis was not forced to a horizontal plane in the present experiment. When both the linear and nonlinear models were fit to an upper or lower plateau, the two models generally fit the data equally well.

With equal weight given to all the response criteria evaluated, .65% TAAA would seem to meet the dietary requirement for lactating sows fed semipurified crystalline amino acid diets. Nitrogen excretion and retention were optimized at .56% TAAA, with no further
Improvement at higher dietary levels. There was an indication of depressed N retention at the 1.05% TAAA level. Plasma Tyr, plasma lysine and milk yield seem to indicate a higher requirement, with plasma Tyr being especially strong in this regard. Assuming that crystalline L-Phe is 100% available and that naturally occurring Phe is about 87% available (Sauer et al., 1977; Rudolph et al., 1983) the TAAA requirement would increase to .75%, or 41.2 g/d for sows fed 5.5 kg/d of a diet containing all natural ingredients.
Table 1. Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn sugar</td>
<td>28.62</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>25.00</td>
</tr>
<tr>
<td>Whey, dried</td>
<td>25.00</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>9.75</td>
</tr>
<tr>
<td>Solka floc</td>
<td>3.00</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.00</td>
</tr>
<tr>
<td>Amino acid premix(^b)</td>
<td>2.44</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.70</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.35</td>
</tr>
<tr>
<td>Vitamin premix(^c)</td>
<td>1.00</td>
</tr>
<tr>
<td>Trace mineral premix(^d)</td>
<td>.14</td>
</tr>
<tr>
<td><strong>Total</strong>(^e)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

\(^a\) L-Phenylalanine added to provide .30 (basal diet), .45, .60, .75, .90 and 1.05% total phenylalanine and tyrosine in the experimental diets. Diets were maintained isonitrogenous by varying L-glutamic acid and corn sugar.

\(^b\) Contributed .32% L-Arg, .20% L-His, .19% L-Ile, .25% L-Leu, .38% L-Lys, .22% DL-Met, .10% L-Phe, .21% L-Thr, .07% L-Trp and .37% L-Val.

\(^c\) Contributed 2,000 IU vitamin A, 200 IU vitamin D\(_3\), 10 IU vitamin E, 3 mg riboflavin, 10 mg niacin, 12 mg pantothenic acid, 15 \(\mu\)g vitamin B\(_{12}\), 1 mg vitamin B\(_6\), 1 mg thiamin, .1 mg biotin, .6 mg folacin and 1250 mg choline per kg of diet.

\(^d\) Contributed the following in mg/kg of diet: Zn, 100; Fe, 50; Mn, 27.5; Cu, 5.0; I, .75; Se, .14; Mg, 100.

\(^e\) Analyzed 11.63% crude protein (air dry).
Table 2. Essential amino acid composition (%)\(^a\)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Supplied by</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whey</td>
<td>Premix</td>
<td>Total</td>
</tr>
<tr>
<td>Arginine</td>
<td>.08</td>
<td>.32</td>
<td>.40</td>
</tr>
<tr>
<td>Histidine</td>
<td>.05</td>
<td>.20</td>
<td>.25</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>.20</td>
<td>.19</td>
<td>.39</td>
</tr>
<tr>
<td>Leucine</td>
<td>.30</td>
<td>.25</td>
<td>.55</td>
</tr>
<tr>
<td>Lysine</td>
<td>.22</td>
<td>.38</td>
<td>.60</td>
</tr>
<tr>
<td>Methionine &amp; Cystine</td>
<td>.14</td>
<td>.22</td>
<td>.36</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>.11</td>
<td>.10</td>
<td>.21</td>
</tr>
<tr>
<td>Threonine</td>
<td>.22</td>
<td>.21</td>
<td>.43</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>.05</td>
<td>.07</td>
<td>.12</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>.09</td>
<td>.00</td>
<td>.09</td>
</tr>
<tr>
<td>Valine</td>
<td>.18</td>
<td>.37</td>
<td>.55</td>
</tr>
</tbody>
</table>

\(^a\)Lysine, methionine, phenylalanine and tyrosine values are based on analysis. All others are calculated from NRC (1979).
Table 3. Effect of level of phenylalanine on sow feed intake, pig weight gain, milk yield and plasma and urine measurements

<table>
<thead>
<tr>
<th>Item</th>
<th>.30</th>
<th>.45</th>
<th>.60</th>
<th>.75</th>
<th>.90</th>
<th>1.05</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total aromatic amino acids, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed intake, kg/d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.22</td>
<td>4.77</td>
<td>5.22</td>
<td>5.41</td>
<td>4.86</td>
<td>4.77</td>
<td>.17</td>
</tr>
<tr>
<td>Avg pig wt gain, kg</td>
<td>1.30</td>
<td>.94</td>
<td>1.28</td>
<td>1.30</td>
<td>1.18</td>
<td>1.22</td>
<td>.19</td>
</tr>
<tr>
<td>Milk yield, kg/d&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.88</td>
<td>4.23</td>
<td>5.21</td>
<td>5.75</td>
<td>4.99</td>
<td>5.37</td>
<td>.42</td>
</tr>
<tr>
<td>Plasma, mg/100 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea N&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.8</td>
<td>11.8</td>
<td>10.4</td>
<td>10.2</td>
<td>10.6</td>
<td>9.5</td>
<td>.67</td>
</tr>
<tr>
<td>Phenylalanine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.30</td>
<td>1.78</td>
<td>2.18</td>
<td>3.36</td>
<td>3.77</td>
<td>6.24</td>
<td>.44</td>
</tr>
<tr>
<td>Tyrosine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>.33</td>
<td>.30</td>
<td>.41</td>
<td>.50</td>
<td>1.27</td>
<td>1.93</td>
<td>.14</td>
</tr>
<tr>
<td>Methionine</td>
<td>.96</td>
<td>1.07</td>
<td>1.10</td>
<td>1.03</td>
<td>.97</td>
<td>1.02</td>
<td>.07</td>
</tr>
<tr>
<td>Lysine&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.21</td>
<td>4.28</td>
<td>3.50</td>
<td>2.38</td>
<td>2.73</td>
<td>2.52</td>
<td>.38</td>
</tr>
<tr>
<td>Urine, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea N&lt;sup&gt;h&lt;/sup&gt;</td>
<td>42.9</td>
<td>31.0</td>
<td>26.7</td>
<td>28.6</td>
<td>25.6</td>
<td>25.3</td>
<td>2.03</td>
</tr>
<tr>
<td>Allantoin</td>
<td>1.43</td>
<td>1.45</td>
<td>1.58</td>
<td>1.58</td>
<td>1.40</td>
<td>1.39</td>
<td>.11</td>
</tr>
<tr>
<td>Allantoin/urea ratio&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>20.2</td>
<td>26.0</td>
<td>35.6</td>
<td>34.4</td>
<td>34.0</td>
<td>32.7</td>
<td>2.42</td>
</tr>
</tbody>
</table>

<sup>a</sup>Six observations per mean.

<sup>b</sup>All diets contained .09% tyrosine.

<sup>c</sup>Treatment effect quartic (P<.05).

<sup>d</sup>Treatment effect linear (P<.01) and quadratic (P<.10).

<sup>e</sup>Treatment effect linear (P<.001) and quadratic (P<.05).

<sup>f</sup>Treatment effect linear (P<.001) and quadratic (P<.001).

<sup>g</sup>Treatment effect linear (P<.001).

<sup>h</sup>Treatment effect linear (P<.001), quadratic (P<.01) and cubic (P<.05).

<sup>i</sup>Calculated as: (Allantoin/urea N) x protein intake (g).

<sup>j</sup>Treatment effect linear (P<.001) and quadratic (P<.01).
Table 4. Nitrogen balance of sows fed different levels of phenylalanine (g/d)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Item</th>
<th>.30</th>
<th>.45</th>
<th>.60</th>
<th>.75</th>
<th>.90</th>
<th>1.05</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N intake\textsuperscript{c}</td>
<td>97.2</td>
<td>88.7</td>
<td>97.2</td>
<td>100.6</td>
<td>90.4</td>
<td>88.7</td>
<td>3.24</td>
</tr>
<tr>
<td>Fecal N</td>
<td>5.6</td>
<td>5.7</td>
<td>6.4</td>
<td>5.9</td>
<td>4.9</td>
<td>6.1</td>
<td>.59</td>
</tr>
<tr>
<td>Urine N\textsuperscript{d}</td>
<td>54.4</td>
<td>41.1</td>
<td>37.6</td>
<td>40.2</td>
<td>35.3</td>
<td>36.4</td>
<td>2.28</td>
</tr>
<tr>
<td>N retention\textsuperscript{e}</td>
<td>37.2</td>
<td>42.0</td>
<td>53.2</td>
<td>54.5</td>
<td>50.2</td>
<td>46.2</td>
<td>3.85</td>
</tr>
<tr>
<td>Milk N\textsuperscript{f}</td>
<td>29.0</td>
<td>26.6</td>
<td>34.8</td>
<td>41.8</td>
<td>39.6</td>
<td>40.5</td>
<td>3.55</td>
</tr>
<tr>
<td>N balance</td>
<td>8.2</td>
<td>15.4</td>
<td>18.4</td>
<td>12.7</td>
<td>10.6</td>
<td>5.7</td>
<td>4.46</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Six observations per mean.
\textsuperscript{b}All diets contained .09% tyrosine.
\textsuperscript{c}Treatment effect quartic (P<.05).
\textsuperscript{d}Treatment effect linear (P<.001) and quadratic (P<.01).
\textsuperscript{e}Treatment effect linear (P<.01) and quadratic (P<.01).
\textsuperscript{f}Treatment effect linear (P<.01).
LITERATURE CITED

the determination of amino acid profiles in serum and urine.
Perkin-Elmer Corp., Instrument Division, Norwalk, CT.

AOAC. 1975. Official Methods of Analysis. 12th ed. Association of
Official Analytical Chemists, Washington, DC.

Reproductive performance and progeny development as influenced by
As-655e. Univ. of Illinois, Urbana.

104:542.


Haught, D. G. and V. C. Speer. 1977. Isoleucine requirement of


Amino acid analysis: Hydrolysis, ion-exchange clean-up, derivati-
Biochem. Lab. Inc., Columbia, MO.

Kiriyama, S., Y. Yagishita, T. Suzuki and H. Iwao. 1967. The surveys
on the urea and allantoin excretion and other criteria from rats
fed "threonine imbalanced" or "corrected" diet. Agric. Biol. Chem.
31:743.


SECTION II. PHENYLALANINE REQUIREMENT OF THE LACTATING SOW
PHENYLALANINE REQUIREMENT OF THE
LACTATING SOW

W. A. Lellis and V. C. Speer

Iowa Agricultural and Home Economics Experiment Station,
Ames, Iowa 50011
SUMMARY

Six mature Yorkshire X Landrace sows were randomly assigned to a 6 X 6 Latin-square experiment on d 3 of lactation to determine the phenylalanine (Phe) requirement in the presence of excess tyrosine (Tyr). A control diet of corn sugar, cornstarch, whey, L-glutamic acid, Solka floc, soybean oil, amino acids, minerals and vitamins was supplemented with L-Phe to provide .175, .250, .325, .400, .475 and .550% Phe and .47% Tyr. Each diet was fed to a maximum of 5.5 kg/d within each of six 7-d periods. Sow milk yield and average pig weight gain decreased (cubic, P<.01) with increasing period. Feed intake and average pig weight gain were depressed (quadratic, P<.05) on the lowest Phe diet, but milk yield was not significantly affected. Nitrogen (N) intake was lowest (quadratic, P<.01) for the .175% Phe diet, but fecal N was not affected. Percentage urine N decreased (quadratic, P<.001) and percentage N retained (without milk) increased with increasing Phe to .288 and .296% dietary Phe, respectively. Total daily secretion of milk N was not affected by diet. Percentage overall N balance (with milk) increased with increasing Phe to .307%. Urea N decreased with increasing dietary Phe in both plasma (linear, P<.01) and urine (quadratic, P<.001) to a breakpoint at .285% Phe. A sharp rise (quadratic, P<.01) in plasma Phe occurred at .299% dietary Phe. Plasma Tyr increased (quadratic, P<.05) as dietary Phe increased, but no clear inflection point was obtained. Plasma lysine
and leucine decreased, and plasma isoleucine and valine increased with increasing Phe to plateaus at .317, .303, .367 and .359% Phe, respectively. If equal weight is given to the N balance and plasma amino acid data, .30% Phe seems to meet the dietary requirement for the lactating sow. In a diet containing natural ingredients, the requirement would increase to .35%, or 18.7 g/d for sows fed 5.5 kg/d. It was suggested that Tyr could supply 48 to 54% of the total aromatic amino acid requirement of the lactating sow.

(Key Words: Pigs, Lactation, Phenylalanine, Plasma amino acids, Nitrogen.)
INTRODUCTION

The total aromatic amino acid (TAAA) requirement of the lactating sow has been determined to be approximately 0.65% of the diet (Lellis and Speer, 1985). This estimate was based upon the response of sows to diets containing the majority of the TAAA as phenylalanine (Phe). Estimates of the portion of the TAAA requirement which can be supplied as tyrosine (Tyr) are 42 to 47% for the chick (Sasse and Baker, 1972, 1973), 45% for the rat (Stockland et al., 1971), 46% for the dog (Milner et al., 1984), 49% for the pig (Robbins and Baker, 1977) and 50% for the catfish (Robinson et al., 1980). The purpose of the present experiment was to determine the Phe requirement of the lactating sow in the presence of excess Tyr, and in combination with our previous study (Lellis and Speer, 1985), obtain an estimate of the portion of the TAAA requirement that can be supplied by Tyr.
EXPERIMENTAL PROCEDURE

Six Yorkshire X Landrace crossbred sows (fourth through tenth parity) selected on the basis of previous satisfactory reproductive performance were randomly assigned to a 6 X 6 balanced Latin-square experiment (Cochran and Cox, 1957) on d 3 of lactation. Corn sugar, cornstarch, whey, vitamins, minerals and indispensable amino acids were used to formulate a basal diet (Tables 1 and 2) containing .10% Phe and .47% Tyr. This diet was supplemented with L-Phe to provide levels of .175, .250, .325, .400, .475 and .550% total dietary Phe. Corn sugar and L-glutamic acid were varied to make the diets isonitrogenous. The basal diet was calculated to contain all essential nutrients, except Phe and leucine (Rousselow et al., 1979), at recommended levels (NRC, 1979). Dietary lysine was increased slightly above the NRC (1979) recommended level. Each diet was fed to a maximum of 5.5 kg/d (in two equal feedings) within each of six 7-d lactational periods. During the first 2 d of lactation all sows were fed 5.5 kg/d of a 13% protein corn-soybean meal diet.

Sow and litter management was similar to that of Lellis and Speer (1985), except that litters were replaced only at the end of period 3, sow milk yield was measured on d 5 and nitrogen (N) balance trials were conducted from d 3 to 7 of each period. Blood samples were obtained immediately before feeding and at .5 h intervals for 6 h, then 1 h intervals until 9 h postfeeding on d 7
of each period via an indwelling cannula fitted through an ear vein into the anterior vena cava. The method of cannulation was similar to that described by Bate and Hacker (1985), except that PE-90 tubing\(^1\) was used only for the internal portion of the cannula, while a more flexible medical-grade tubing\(^2\) (size 1.02 mm id x 2.15 mm od) was used for the external portion. The free end of the cannula was contained within a 5 X 10 cm denim pouch sutured to the back of the sows' neck. When cannula failure occurred on designated bleeding days, sows were bled via vena cava puncture 2 and 5 h postfeeding. Heparinized blood samples were immediately centrifuged and the plasma was frozen at -20 C until analysis. Milk samples were obtained 6 h postfeeding on d 5 of each period by manual expression after injection of 10 IU oxytocin into the vena cava.

Samples were analyzed for total N by micro-Kjeldahl (AOAC, 1975) and urea N by a modified carbamido-diacetyl reaction (Marsh et al., 1965). Feed samples were hydrolyzed in 6 N HCl under nitrogen for 22 h at 110 C (Kaiser et al., 1974), then analyzed for amino acids by gas-liquid chromatography (Frank et al., 1980) after removal of impurities by cation-exchange (Adams, 1973). Plasma was deproteinized with two volumes of ice cold acetonitrile and analyzed for free amino

\(^1\)Clay Adams, Parsippany, NJ.
\(^2\)Silastic, Dow Corning Corp., Midland, MI.
acids via reverse-phase HPLC using the procedure described by Bidlingmeyer et al. (1984). Separation was achieved with a slightly convex gradient curve of 10% to 58% buffer B in 21 min.

Data were analyzed by the method of least-squares, with orthogonal polynomials used to partition treatment effects into single-degree-of-freedom contrasts (SAS, 1982). Minimum amino acid requirements were estimated using broken-line analysis (Draper and Smith, 1981) with the requirement taken as the abscissa of the breakpoint.
RESULTS AND DISCUSSION

Feed intake, sow milk yield, average pig weight gain, N balance and urine urea N data are presented in Table 3. Feed intake was depressed when sows were fed less than .250% dietary Phe (quadratic, P<.01), and total feed refusal occurred within four days when diets contained less than .175% (W. A. Lellis, unpublished data). Reduced intake during a Phe deficiency has been observed in the pig (Mertz et al., 1954; Robbins and Baker, 1977), rat (Stockland et al., 1971), chick (Sasse and Baker, 1972), rabbit (Anderson and Fisher, 1973), mouse (Bell and John, 1981) and dog (Milner et al., 1984), and may be related to increased competition among the large neutral amino acids for transport into the brain (Tews and Harper, 1985; Choi and Pardridge, 1986).

Sow milk yield did not respond to level of Phe, but decreased with increasing period (5.30, 6.19, 5.85, 4.01, 3.24 and 2.84 kg/d; cubic, P<.01). This decline was correlated with average pig weight gain (r=.76, P<.001), which also decreased with advancing period (.84, .95, .78, .52, .50 and .59 kg/pig; cubic, P<.01). Pig weight gain was maximized at .475% Phe (quadratic, P<.05). In a previous trial conducted to determine the TAAA requirement (Lellis and Speer, 1985), milk yield was affected by dietary amino acid level, whereas pig gain was not. The shorter duration of feeding in the present study may have limited the response of milk yield to dietary treatment.
Nitrogen balance data are calculated as a percentage of N intake to correct for varying feed consumption. Fecal N was unaffected by diet. As level of Phe increased in the diet, total urine N decreased (quadratic, \( P<.001 \)), and N retained increased (quadratic, \( P<.001 \)), both to a plateau at .325% Phe. Although daily secretion of milk N was lower when sows were fed the lowest Phe diet, the difference among treatments was not significant. All levels of Phe supported positive N balance, which increased quadratically (\( P<.01 \)) with increasing levels of Phe to .325%. Broken-line analysis predicted a dietary requirement of .288% Phe with urine N, .296% Phe with N retained and .307% with N balance as the response criteria.

Minimal levels of urea N have been associated with optimal amino acid balance (Brown and Cline, 1974) and were observed in the TAAA requirement study (Lellis and Speer, 1985). In the present experiment, urine urea N excretion, as a percentage of N intake, decreased quadratically (\( P<.001 \)) with increasing Phe to an estimated break-point at .285%. Plasma urea N (Table 4) also decreased with additional dietary Phe, reaching minimum levels at .325%. Because plasma urea N represents urea concentration at a moment in time, whereas urine urea N is a quantitative collection which can be related to N intake, urine urea N may be the more accurate indicator of amino acid status in the sow.

The effect of level of Phe on the concentration of plasma free
amino acids (PFAA) at 2 and 5 h postprandial is shown in Table 4. Plasma levels of limiting amino acids are reported to remain relatively low and constant until the animals' requirement is met, then increase proportionally with additional dietary increments (Zimmerman and Scott, 1965). Conversely, the plasma concentrations of the other essential amino acids are reported to display the opposite pattern, and fall as the amino acid imbalance is corrected (Lewis and Speer, 1973). In the present experiment, plasma Phe rose slightly between .175 and .325% Phe, then increased sharply with further additions of Phe to the diet (quadratic, P<.01). Broken-line analysis predicted a requirement of .295% and .303% Phe based upon the 2 and 5 h postfeeding values, respectively. Although plasma Tyr increased linearly (P<.01) with increasing dietary Phe at h 2, and quadratically (P<.05) at h 5, no clear point of inflection was obtained.

Both plasma lysine (h 2 and 5) and leucine (h 5) decreased quadratically with increasing Phe, and show breakpoints at .317 and .303% Phe, respectively. Plasma isoleucine and valine, however, responded opposite to expectation based upon previous amino acid studies (Lewis and Speer, 1973, 1975), and increased from a low level at the lowest Phe diet to an upper plateau when the apparent Phe requirement was met. To the authors knowledge this relationship between Phe and the branch chain amino acids has not been reported previously, but a similar relationship was observed between lysine
and histidine (Woerman and Speer, 1976). Broken-line analysis indicated a Phe requirement of .367% with plasma isoleucine and .359% with plasma valine. Plasma histidine and methionine were unaffected by dietary treatment, as were the nonessential amino acids, aspartic acid, glutamic acid, glutamine, glycine, proline and hydroxyproline. Plasma levels of both alanine and taurine increased with increasing dietary Phe, whereas plasma serine levels fell.

The effect of time after feeding on concentrations of plasma Phe is shown in figure 1. Plasma Phe rose sharply after feeding for all dietary levels tested, reaching an initial postprandial peak at .5 to 1.0 h. Response to diet during this period was linear (r=.84) and may directly reflect absorption (Shimada and Zimmerman, 1973). All plasma levels decreased between 1.0 and 2.0 h postfeeding, after which a differential response to diet was observed. Postprandial Phe levels from diets containing less than .250% Phe fell below prefeeding levels after 1.5 h, and remained low and constant throughout the postfeeding period of 2.0 to 9.0 h. A postfeeding to fasting amino acid ratio of less than 1.0 is considered indicative of an amino acid deficiency (Longenecker and Hause, 1959), and has been observed in gestating gilts after a 24 h fast (Meisinger and Speer, 1979; Leonard and Speer, 1983). When sows received the .325% Phe diet, plasma Phe fell slightly below fasting levels between 2.0 and 4.5 h postprandial, but generally remained at or above prefeeding
levels throughout the remainder of the postfeeding period. In sows fed diets containing more than .400% Phe, plasma Phe increased sharply after the initial 1.0 to 2.0 h postfeeding depression and reached a second peak at 2.5 to 4.5 h postprandial before declining towards fasting levels.

Because minimal and maximal plasma Phe concentrations were attained between 2.0 and 5.0 h postfeeding, the median of this period (3 to 4 h) may be considered the most appropriate time for withdrawal of a single blood sample to test for dietary amino acid adequacy. A similar conclusion was reached by Ball and Bayley (1985), who measured CO₂ expiration in pigs fed lysine and tryptophan deficient diets and found that measurements of dietary effectiveness were most sensitive in the fourth hour following ingestion of the test meal. This response, however, may be specific for the amino acid being studied, inasmuch as not all amino acids reacted as did Phe in this experiment (figure 2). This concept is supported by the work of Shimada and Zimmerman (1973), Typpo et al. (1970) and Ganguli et al. (1971) who found postfeeding PFAA concentrations to peak at 1, 2 and 5 h, respectively. McLaughlan et al. (1963) reported maximal PFAA levels at 1 to 2 h postprandial in humans, and this was affected by source of protein and amount of dietary lipid.

With equal weight given to the N balance and PFAA data, .30% Phe would seem to meet the dietary requirement for lactating sows fed semipurified crystalline amino acid diets. Nitrogen excretion,
retention and balance were optimized at .297% Phe and plasma Phe displayed a break-point at .299%. Urine urea N excretion was minimized at a slightly lower level of .285% Phe, whereas the other essential amino acids indicate a slightly higher requirement. Considering the close agreement between the plasma Phe and N balance data in the present experiment, plasma Tyr may have overestimated the TAAA requirement in the previous study (Lellis and Speer, 1985). If the TAAA requirement is taken as .58 to .65% of the diet, the Tyr replacement value for the lactating sow would be estimated at 48 to 54%. This is in close agreement with Robbins and Baker (1977), who found a replacement value of 49% for the weanling pig. Assuming that crystalline L-Phe is 100% available and that naturally occurring Phe is about 87% available (Rudolph et al., 1983), the Phe requirement would increase to .34%, or 18.7 g/d for sows fed 5.5 kg/d of a corn-soybean meal diet.
Table 1. Composition of basal diet<sup>a</sup>

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn sugar</td>
<td>29.54</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>25.00</td>
</tr>
<tr>
<td>Whey, dried</td>
<td>23.81</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>9.48</td>
</tr>
<tr>
<td>Solka floc</td>
<td>3.00</td>
</tr>
<tr>
<td>Soybean oil&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00</td>
</tr>
<tr>
<td>Amino acid premix</td>
<td>2.93</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.73</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.37</td>
</tr>
<tr>
<td>Vitamin premix&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00</td>
</tr>
<tr>
<td>Trace mineral premix&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.14</td>
</tr>
<tr>
<td><strong>Total&lt;sup&gt;e&lt;/sup&gt;</strong></td>
<td>100.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>L-Phenylalanine added to provide .175, .250, .325, .400, .475 and .550% total phenylalanine in the experimental diets. Diets were maintained isonitrogenous by varying L-glutamic acid and corn sugar.

<sup>b</sup>Contributed .33% L-Arg, .20% L-His, .20% L-Ile, .27% L-Leu, .44% L-Lys, .23% DL-Met, .22% L-Thr, .08% L-Trp, .41% L-Tyr and .38% L-Val.

<sup>c</sup>Contributed 2,000 IU vitamin A, 200 IU vitamin D<sub>3</sub>, 10 IU vitamin E, 2 mg vitamin K, 3 mg riboflavin, 19 mg niacin, 12 mg pantothenic acid, 15 µg vitamin B<sub>12</sub>, 1 mg vitamin B<sub>6</sub>, 1 mg thiamin, .1 mg biotin, .6 mg folacin and 1.25 g choline per kg of diet.

<sup>d</sup>Contributed the following in mg/kg of diet: Mg, 100; Zn, 100; Fe, 50; Mn, 28; Cu, 5; I, .75; Se, .14.

<sup>e</sup>Analyzed 11.42% crude protein (air dry).
Table 2. Essential amino acid composition of basal diet (%)\(^a\)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Supplied by</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whey</td>
<td>Premix</td>
<td>Total</td>
</tr>
<tr>
<td>Arginine</td>
<td>.07</td>
<td>.33</td>
<td>.40</td>
</tr>
<tr>
<td>Histidine</td>
<td>.05</td>
<td>.20</td>
<td>.25</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>.19</td>
<td>.20</td>
<td>.39</td>
</tr>
<tr>
<td>Leucine</td>
<td>.28</td>
<td>.27</td>
<td>.55</td>
</tr>
<tr>
<td>Lysine</td>
<td>.21</td>
<td>.44</td>
<td>.65</td>
</tr>
<tr>
<td>Methionine &amp; Cystine</td>
<td>.13</td>
<td>.23</td>
<td>.36</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>.10</td>
<td>.10</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>.21</td>
<td>.22</td>
<td>.43</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>.04</td>
<td>.08</td>
<td>.12</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>.06</td>
<td>.41</td>
<td>.47</td>
</tr>
<tr>
<td>Valine</td>
<td>.17</td>
<td>.38</td>
<td>.55</td>
</tr>
</tbody>
</table>

\(^a\)Lysine, methionine, phenylalanine and tyrosine values for whey are based on analysis. All others are calculated from NRC (1979).
Table 3. Effect of level of phenylalanine on sow feed intake, milk yield, pig weight gain, nitrogen balance and urine urea

<table>
<thead>
<tr>
<th>Dietary phenylalanine, %</th>
<th>.175</th>
<th>.250</th>
<th>.325</th>
<th>.400</th>
<th>.475</th>
<th>.550</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake, kg/d²</td>
<td>4.40</td>
<td>5.50</td>
<td>5.26</td>
<td>5.40</td>
<td>5.39</td>
<td>5.47</td>
<td>.14</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>3.96</td>
<td>4.96</td>
<td>4.54</td>
<td>4.71</td>
<td>4.71</td>
<td>4.81</td>
<td>.34</td>
</tr>
<tr>
<td>Avg pig wt gain, kg¹</td>
<td>.52</td>
<td>.72</td>
<td>.73</td>
<td>.72</td>
<td>.84</td>
<td>.67</td>
<td>.06</td>
</tr>
<tr>
<td>Nitrogen Intake, g/d²</td>
<td>80.5</td>
<td>100.5</td>
<td>96.2</td>
<td>98.7</td>
<td>98.5</td>
<td>99.9</td>
<td>2.57</td>
</tr>
<tr>
<td>% of N intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal</td>
<td>7.1</td>
<td>7.1</td>
<td>6.1</td>
<td>6.5</td>
<td>7.3</td>
<td>7.3</td>
<td>.49</td>
</tr>
<tr>
<td>Urine</td>
<td>61.4</td>
<td>48.9</td>
<td>41.6</td>
<td>42.1</td>
<td>41.5</td>
<td>39.6</td>
<td>1.96</td>
</tr>
<tr>
<td>Retained</td>
<td>31.5</td>
<td>44.0</td>
<td>52.3</td>
<td>51.4</td>
<td>51.2</td>
<td>53.1</td>
<td>1.86</td>
</tr>
<tr>
<td>Milk</td>
<td>29.1</td>
<td>33.0</td>
<td>34.3</td>
<td>34.0</td>
<td>34.7</td>
<td>34.8</td>
<td>2.15</td>
</tr>
<tr>
<td>Balance</td>
<td>2.4</td>
<td>11.0</td>
<td>18.0</td>
<td>17.4</td>
<td>16.5</td>
<td>18.3</td>
<td>2.26</td>
</tr>
<tr>
<td>Urine urea</td>
<td>44.1</td>
<td>32.0</td>
<td>26.5</td>
<td>24.8</td>
<td>25.7</td>
<td>24.6</td>
<td>1.94</td>
</tr>
</tbody>
</table>

ªLeast-square means of six observations.

ªAll diets contain .47% tyrosine.

²Treatment effect linear (P<.01) and quadratic (P<.01).

¹Treatment effect linear (P<.05) and quadratic (P<.05).

³Treatment effect linear (P<.001) and quadratic (P<.001).

⁴Retained = intake - (fecal + urine).

⁵Balance = retained - milk.
Table 4. Effect of level of phenylalanine on sow plasma urea N and free amino acid concentrations (mg/100 ml)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Item</th>
<th>Hour after feed</th>
<th>Dietary phenylalanine, %\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.175</td>
<td>.250</td>
</tr>
<tr>
<td>Urea N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{c}</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{d}</td>
<td>12.9</td>
</tr>
<tr>
<td>Phenylyalanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{e}</td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{e}</td>
<td>.16</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{c}</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{f}</td>
<td>3.87</td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{f}</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{e}</td>
<td>2.00</td>
</tr>
<tr>
<td>Leucine\textsuperscript{g}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{c}</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{c}</td>
<td>2.10</td>
</tr>
<tr>
<td>Isoleucine\textsuperscript{h}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{f}</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{c}</td>
<td>1.68</td>
</tr>
<tr>
<td>Valine\textsuperscript{h}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2\textsuperscript{e}</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{e}</td>
<td>9.1</td>
</tr>
<tr>
<td>Histidine\textsuperscript{h}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.72</td>
</tr>
<tr>
<td>----------</td>
<td>----</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.54</td>
</tr>
<tr>
<td>Methylone</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2^f</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>5^c</td>
<td>13.2</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2^f</td>
<td>.96</td>
</tr>
<tr>
<td></td>
<td>5^d</td>
<td>.95</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2^d</td>
<td>.52</td>
</tr>
<tr>
<td></td>
<td>5^c</td>
<td>.51</td>
</tr>
<tr>
<td>Taurine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^Least-square means of six observations.

b All diets contain .47% tyrosine.

c Treatment effect linear (P<.01).

d Treatment effect linear (P<.01).

e Treatment effect linear (P<.01) and quadratic (P<.01).

^f Treatment effect linear (P<.01) and quadratic (P<.05).

g Hour effect (P<.01).

h Hour effect (P<.01).
Figure 1. Effect of time after feeding on concentration of phenylalanine in plasma. Each point represents the mean of all complete bleedings within each diet.
Figure 2. Effect of time after feeding on the concentration of free amino acids and urea nitrogen (PUN) in plasma. Each point represents the mean of all complete bleedings (n=27) averaged across diet. GLY and GLN are presented together due to incomplete separation during chromatography. Note that scales differ for the different amino acids.
LITERATURE CITED

the determination of amino acid profiles in serum and urine.
Perkin-Elmer Corp., Instrument Division, Norwalk, CT.

103:1306.

of Official Analytical Chemists, Washington, DC.

Ball, R. O. and H. S. Bayley. 1985. Time course of the total and
radioactive carbon dioxide production by piglets receiving

Bate, L. A. and R. R. Hacker. 1985. Effect of cannulation and
environmental temperature on the concentration of serum cortisol

111:525.

analysis of amino acids using pre-column derivatization. J.
Chromatogr. 336:93.

Brown, J. A. and T. R. Cline. 1974. Urea excretion in the pig:
An indicator of protein quality and amino acid requirements.

Choi, T. B. and W. M. Pardridge. 1986. Phenylalanine transport
at the human blood-brain barrier. J. Biol. Chem. 261:6536.


GENERAL SUMMARY

Two separate 6 X 6 Latin-square experiments were conducted with mature Yorkshire X Landrace sows to determine the TAAA and Phe requirements during lactation. A basal diet of corn sugar, cornstarch, whey, L-glutamic acid, solka floc, soybean oil, amino acids, minerals and vitamins was supplemented with L-Phe to provide .30, .45, .60, .75, .90 and 1.05% TAAA in the first experiment, and .175, .250, .325, .400, .475 and .550% Phe in the second. Diets were formulated to contain a minimal amount of Tyr in the TAAA study (.09%), and an excessive amount in the Phe study (.47%). Sows were fed to a maximum of 5.5 kg/d during 10-d (TAAA study) and 7-d (Phe study) lactational periods.

In the first experiment, both plasma (P<.05) and urine (P<.01) urea N decreased quadratically with increasing dietary Phe, reaching minimum levels at .56% TAAA. This decline in urea excretion had a major influence on both total urine N excretion and retained N, which were optimized at .56 and .58% TAAA, respectively. A higher dietary TAAA requirement was indicated by plasma Tyr, which increased sharply at .73% TAAA. Dietary TAAA level had a less dramatic effect on the other response criteria measured, including plasma Phe, sow milk yield and baby pig weight gain.

In the second experiment, urea N decreased with increasing dietary Phe in both plasma (linear, P<.01) and urine (quadratic,
P<.001) to minimum levels at .285% Phe. This decrease was reflected in urine N excretion, N retained and overall N balance, which were optimized at .288, .296 and .307% Phe, respectively. Plasma Phe rose sharply (quadratic, P<.01) at .299% dietary Phe, but there was no clear inflection point in plasma Tyr. Plasma lysine and leucine decreased, and plasma isoleucine and valine increased with increasing Phe to plateaus at .317, .303, .367 and .359% dietary Phe, respectively.

With equal weight given to the N balance and plasma amino acid response criteria, .65% TAAA, with a minimum of .30% Phe, would seem to meet the sows' dietary requirements. This would increase to .75% (41.2 g/d) TAAA and .34% (18.7 g/d) Phe for sows fed 5.5 kg/d of a corn-soybean meal diet. Based upon these two experiments, the portion of the TAAA requirement which can be supplied as Tyr was estimated at 48 to 54%.
ADDITIONAL LITERATURE CITED

Abderhalden, E. 1912. Futterungsversuche mit vollstandig abgebauten

Abderhalden, E. 1913. Weitere versuche uber die synthetischen
fähigkeiten des organismus des hundes. Z. Phys. Chem. 83:444.

Adamson, I., and H. Fisher. 1973. Amino acid requirement of the
103:1306.

Albanese, A. A., V. Irby and M. Lein. 1946. The utilization of

Alcock, R. S. 1934. CLX. The non-essential nature of tyrosine.

Histidine, phenylalanine-tyrosine and tryptophan requirements for

Aoki, T. T., M. F. Brennan, W. A. Muller, J. S. Soeldner, J. S. Alpert,
Amino acid levels across normal forearm muscle and splanchnic bed

Reproductive performance and progeny development as influenced by
nutrition during pregnancy and lactation. Pork Industry Day
Rep. AS-655e. Univ. of Illinois, Urbana.

1966. Quantitative evaluation of the threonine, isoleucine, valine
88:391.

and tyrosine requirements of the young pig using the amino acid

111:525.

Bickel, H., J. Gerrard and E. M. Hickmans. 1954. The influence of
phenylalanine intake on the chemistry and behavior of a phenyl-
ketonuric child. Acta Ped. 43:64.


Siegell, F. L., K. Aoki and R. E. Colwell. 1971. Polyribosome dis- 
aggregation and cell-free protein synthesis in preparations from 
18:537.

Snyderman, S. E., E. L. Pratt, M. W. Cheung, P. Norton, L. E. Holt, Jr., 
A. E. Hansen and T. C. Panos. 1955. The phenylalanine requirement 

Speer, V. C. 1975. Amino acid requirements for the lactating sow. 


1971. L-phenylalanine and L-tyrosine requirements of the growing 

synthesis in the striatum: Studies with gamma - butyrolactone. 
Life Sci. 29:743.

Year Book Medical Pub., Chicago.

Tolbert, B. and J. H. Watts. 1963. Phenylalanine requirement of 
woman consuming a minimal tyrosine diet and the sparing effect of 

Totani, G. 1916. XXXII. Feeding experiments with a dietary in which 
tyrosine is reduced to a minimum. Biochem. J. 10:382.

Influence of time of fast on concentrations of free amino acids 
in plasma and total amino acids on liver protein of young swine. 

Vickery, H. B. and C. L. A. Schmidt. 1931. The history of the 

Wallace, W. M. 1959. Nitrogen content of the body and its relation 


ACKNOWLEDGEMENTS

To my wife, Kathy. You are truly my love and my life, nothing else matters. Our faith in God and love for each other has pulled us through some very trying times. I hope that some day soon you, me, the kids and Barney can stand amongst our fruit trees, look back, and say it was worth the effort. I thank you for your unwaivering support and for our children, Chris and Kim. They are my pride and joy and reason for living.

To Dr. Vaughn C. Speer, for your advice, guidance and friendship throughout my studies. You have always entrusted me with the utmost freedom to pursue my own interests and ideas, and I sincerely thank you for understanding. To aid in the financial support of your pending retirement, I leave you full patent rights to my Spring collection of Gucci Ultra-thin designer catheter pouches. May you reap fully the benefits you so justly deserve.

To Dr. Z, who has become a dear friend. I wish for you a 25 lb. Northern on 2 lb. line, masterfully subdued on a rod and jig crafted with your own hands. I would very much like to be sternman on that day.

Special thanks are expressed to Dr. John Nickum, who supported and encouraged Rick and myself through our first fish project. Your faith in us shall always be remembered.

Appreciation is also extended to Dr. Richard Ewan for assistance in the lab, and Drs. Jerry Young and Pilar Garcia for serving on my graduate committee. I would also like to thank my good friend,
John Simonson. When times were tough on the farm, he was always there.

Finally, I would like to express deepest regards to my parents, Alfred and Florence. Mom always taught us to reach for the stars, while Dad kept our feet on the ground. I love you both without measure.