

2005

# Amino acid requirements and low crude protein, amino acid supplemented diets for swine and poultry

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AMINO ACID REQUIREMENTS AND LOW CRUDE PROTEIN, AMINO ACID  
SUPPLEMENTED DIETS FOR SWINE AND POULTRY

A Dissertation  
Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

in

The Department of Animal Sciences

by  
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May 2005

## **ACKNOWLEDGEMENTS**

I have enjoyed my time at LSU both as a graduate student and as a member of the faculty. There are many people that I need to thank for their support and guidance.

The opportunity to work with Dr. Southern has been a great experience. He is someone I respect greatly for his abilities as a scientist, and he is also a great person that I consider a friend. I also would like to thank Dr. Bidner for being a mentor of mine while I have been at LSU. I always knew that I could talk to Dr. Bidner about anything that I needed and he would give me his honest opinion and guide me in the right direction. Also, thanks to Dr. Franke, Dr. Lavergne, Dr. Rowntree, and Dr. Greenway for serving on my graduate committee.

Thanks to Dr. Humes and all of the animal science faculty for allowing me the flexibility to work on my Ph.D. while coaching the judging team and teaching classes. Dr. Humes has been supportive in everything that I have done at LSU and I appreciate his help.

I would like to thank Rebecca and Chip and everyone that I have worked with at the swine unit for their help with projects. Thanks to Bobby, Jerry, and Curt and everyone that works at the poultry unit for their help.

Thanks to Boo and John and the workers in the meats lab. Boo, I consider you a great friend and I will come back to visit you and your family and I hope that you will come and visit me wherever I may end up.

There have been many graduate students that I have worked with over the past five years that I need to thank. Rob Payne and Jason Shelton are two people that I can't thank enough for their help and friendship. I miss our discussions and debates about nutrition and about life. They helped push me to be better than I would have been in

their absence. I also want to thank Neal, LeAnne, Amy, Joy, Syrena, Melanie, Ria, Jenny, Danielle, and Jeanne for their help and thanks to all the other graduate students in the department that I have gotten to know over the past five years.

I appreciate the support of my mom and dad for always trusting and supporting the decisions I have made. My success has been a result of how they raised me and the opportunities that they encouraged me to participate in.

When I finished my masters degree, I stated that I was told at a young age that surrounding yourself with good people is one of the keys to success. Max King told me that when I was a young kid. I can not say enough about the things I learned from Max and Harry King, there is no doubt in my mind that some of my success can be traced back to them.

I have been fortunate to be surrounded by many good people all my life. Kyle Whittaker, Gary Harvey, Bob Hines, and Scott Schaaque are four people that I have to thank for guiding me in the right direction along the way. Jason King has been a good friend to me since I met him in high school. We have always pushed each other to excel in whatever we were involved in.

I have met a lot of people in Louisiana and had an opportunity to see and do many interesting things. Thanks to everyone who made me welcome in Louisiana and helped me finish my doctoral degree.

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## ABSTRACT

The purpose of this research was to determine requirements for amino acids that limit the use of crystalline amino acids, to determine the effects of reducing crude protein, and to evaluate the problems associated with low crude protein diets for swine and poultry. Four experiments were conducted to determine the Lys and sulfur amino acid requirements for 5- to 10-kg pigs. The results of these experiments suggest that pigs in this weight range require a diet containing 1.40% true digestible (TD) Lys and the ratio of sulfur amino acids to Lys is not greater than 0.54. Two experiments were conducted to determine the requirement of Lys and sulfur amino acids in 90-kg barrows using plasma urea nitrogen as the response. These experiments indicate a TD Lys requirement of 0.57% and there was no response to TD sulfur amino acid concentrations above 0.27%. Six experiments were conducted to evaluate the Ile requirement of 80- to 120-kg barrows. These experiments indicate a requirement of 0.34% TD Ile in a corn-blood cell diet; however, the requirement may not be greater than 0.24% in barrows fed a corn-soybean meal diet. Two experiments were conducted to evaluate the effects of soybean meal and lowering crude protein in diets for late-finishing barrows. Results suggest that reductions in soybean meal are not the cause of increased carcass fat in pigs fed low crude protein diets. Furthermore, the fortification of a corn diet with crystalline Lys, Trp, and Thr will not support optimal growth or carcass composition. Five experiments were conducted to determine the effects of lowering crude protein in diets for broilers and to evaluate limiting essential and nonessential amino acids. Results indicate that low crude protein diets can support optimal growth of broilers when surfeit Gly is supplied in the diet. The requirement of Gly + Ser seems to be not less than 2.14% from d 0 to 17 posthatching.

## CHAPTER 1

### INTRODUCTION

The use of crystalline amino acids (AA) has become an important part of diet formulation within the swine and poultry industries. Crystalline AA are relatively purified sources of AA that can be added to diets to meet an AA requirement. Their use not only allows for producers to lower feed costs per unit of gain but also may help producers in their efforts to manage nutrients more effectively and prevent damage to the environment caused by excess nitrogen (N) in manure.

Unfortunately, there are problems that limit the use of crystalline AA. Specifically, rate and efficiency of growth, and carcass composition is usually negatively affected when crude protein (CP) is decreased by more than three percent (Lewis and Southern, 2001). The causes for these negative effects are unknown, but may be a result of one or several factors. When diets are formulated with crystalline AA, the requirement estimates for more of the AA become crucial, because their concentrations are decreased and more likely to be nearing a deficiency. Furthermore, there is a lack of information regarding AA requirements in modern genetic strains at varying stages of growth. There is also the possibility that AA that have been thought of as nonessential in the past may become essential when dietary CP is reduced.

The objective of this research was to determine requirements of the most limiting AA in diets for swine and poultry and to determine possible reasons for the negative effects on growth and carcass composition when CP is reduced.

**CHAPTER 2**  
**REVIEW OF LITERATURE**  
**CRYSTALLINE AMINO ACIDS**

There are four commercially available crystalline amino acids (AA) (Lys, Met, Thr, and Trp) that are commonly used in diets for swine and poultry. Their use is largely dependant upon feed ingredient prices such as corn and soybean meal. Specifically, as the price of soybean meal increases the use of crystalline AA becomes more economical. Conversely, when corn prices are high the economics of using crystalline AA are decreased. In most diets for swine, Lys is the first limiting AA. Therefore, the use of other crystalline AA may be dependent upon the price of Lys. Furthermore, the price of other crystalline AA directly affect how much crystalline Lys will be used. In most poultry diets, Met is the first limiting AA and Lys is the second limiting AA.

Lysine is most commonly sold commercially as L-Lys monohydrochloride (L-lysine•HCl). There are no known mammals that are able to utilize D-Lys and therefore only L-Lys is considered to have bioavailability in swine and poultry (Lewis and Southern, 2001). Feed grade Lys contains a minimum of 98.5% L-Lys•HCl. This is equivalent to 78.8% actual Lys.

Methionine seems to be equally available for use in swine and poultry in the D- or L-form (Waldroup et al., 1981; Reifsnyder et al., 1984; Chung and Baker, 1992). Feed grade sources of Met are available as DL-Met (99% pure) and as Met hydroxy analog (a liquid that contains 88% Met hydroxy analog). There is considerable controversy over the relative bioactivity of Met hydroxy analog; however, previous research has indicated

that it is equivalent to DL-Met on an equal molar basis (Waldroup et al., 1981; Reifsnyder et al., 1984; Chung and Baker, 1992).

The bioactivity of D-Trp varies between species and may vary from 60 to 100% in the pig (Lewis and Southern, 2001). Almost all feed-grade Trp is available as L-Trp (98.5% pure).

Threonine has four chemical isomers: D- and L-Thr and D- and L-allothreonine (Lewis and Southern, 2001). Poultry can only utilize L-Thr (Kidd, 1996). It is also assumed that pigs can only utilize L-Thr, because of an inability for transamination to occur (Lewis and Southern, 2001). Thus, commercially available Thr is in the L-form (98.5% pure).

The use of other crystalline AA in diets for swine and poultry will be dependant upon the extent to which CP can be lowered without affecting growth and carcass traits. It seems likely that in the future, crystalline forms of other essential AA will be produced commercially and priced for inclusion into swine and poultry feeds.

### **LOW CRUDE PROTEIN DIETS FOR SWINE**

Increasing environmental concerns related to N concentrations of swine manure has generated interest in the use of crystalline AA to lower the CP of swine diets. Kerr and Easter (1995) estimated that each one percentage unit reduction in dietary CP results in 8% less N excreted in manure. However, there are some inconsistencies in the literature as to the extent CP can be lowered without affecting growth performance and carcass traits. Most reports suggest reducing CP by more than 3 to 4% leads to a reduction in rate and efficiency of growth, even when all known nutrient requirements are met (Tuitoek et al., 1997a,b; Shelton et al., 2001; Gomez et al., 2002a,b).

Furthermore, reductions in CP with the use of crystalline AA often lead to increased fat deposition (Tuitoek et al., 1997a,b; Knowles et al., 1998; Shelton et al., 2001; Figueroa et al., 2002; Gomez et al., 2002a,b).

Knowles et al. (1998) suggested that pigs fed low CP crystalline AA diets should have a lower energy need for deamination of excess AA and lower pancreatic activity. Therefore, the NE of the diet increases and this leads to fatter carcasses. However, the authors concluded that reduction of NE in low CP AA supplemented diets was not an effective means of reducing fat in finishing pigs. Other reports suggest that formulating with the NE system will prevent fatter carcasses (Le Bellego et al., 2001, 2002).

Liu et al. (2001) reported that a 9.55% CP corn diet fortified with crystalline Lys, Thr, Trp, Met, Ile, and Val supported growth and carcass composition equal to gilts fed a 15.17% CP C-SBM diet. These authors also reported that deletion of crystalline Ile or Val reduced growth performance of gilts. Shelton et al. (2001) evaluated nine different protein sources for grow-finish pigs. Diets for their experiment were formulated to meet all NRC (1998) AA requirements and they maintained an equivalent Lys:calorie ratio. They reported feeding a 9.35% CP corn diet with crystalline AA reduced performance during the grower and early-finishing periods, but not during the late-finishing period.

According to NRC (1998), the limiting order of AA in a corn diet is as follows: Lys, Trp, Thr, Ile, Val, and Met. Eliminating soybean meal (SBM) in diets for late-finishing barrows results in close to a 5% reduction in CP. This amount of CP reduction may lead to a deficiency in dispensable N. Kendall et al. (2004) concluded that adding Gln and Gly together or Glu improved performance of nursery pigs fed low CP diets. However, growth studies conducted by Zimmerman (1975), Taylor et al. (1981), Russell et al.

(1987), and Kephart and Sherritt (1990) have all shown no effect on growing pig performance to the addition of dispensable N. Nevertheless, there still may be specific nonessential AA that become essential when CP is reduced.

### **LOW CRUDE PROTEIN DIETS FOR BROILERS**

The use of crystalline AA to lower the CP of diets for broilers has been shown to effectively reduce N excretion (Ferguson et al., 1998; Bregendahl et al., 2002; Si et al., 2004a,b). However, rate and efficiency of growth is typically lower in broilers fed diets where CP has been lowered by more than 3%, even when all known nutrient requirements are met (Fancher and Jensen et al, 1989a,b; Pinchasov et al., 1990; Aletor et al., 2000; Waldroup, 2000; Bregendahl et al., 2002).

Researchers have evaluated many potential reasons for the reduction in performance. These include potassium concentrations and dietary electrolyte balance (Fancher and Jensen, 1989a,b; Waldroup, 2000), concentrations of essential AA (Fancher and Jensen, 1989a,b; Kidd and Kerr, 2000; Waldroup et al., 2000), ratios of essential AA to level of dietary CP (Pinchasov et al., 1990; Cabel et al., 1991), additions of nonessential AA (Fancher and Jensen, 1989b; Pinchasov et al., 1990; Bregendahl et al., 2002), the ratio of Trp to other large neutral AA (Waldroup, 2000), and the addition of Cys to reduce Met concentrations (Waldroup, 2000).

Dietary manipulations related to the proposed problems have been unsuccessful in improving performance of broilers to a level equal to that of chicks fed a positive control diet. One exception is that Hahn et al. (1992) demonstrated that additions of essential AA and Glu to a 19% CP diet supported equal performance of both slow and fast growing chicks when compared to a 23% CP diet. However, it should be noted that

the level of CP reduction in this experiment is marginal and the results may have been different if CP had been lowered more significantly.

### **CONCLUSIONS**

A reduction of CP of more than 3% in diets for either swine or poultry often leads to reductions in performance and/or negative effects on carcass composition. It is the purpose of the research contained herein to evaluate the limits of lowering CP, determine the requirements of the most limiting AA, and determine possible reasons for the negative effects of lowering CP on swine and poultry.



## CHAPTER 3

### LYSINE AND TOTAL SULFUR AMINO ACID REQUIREMENTS OF 5- TO 10-KILOGRAM PIGS

#### INTRODUCTION

To economically formulate low crude protein (CP), amino acid (AA)-supplemented diets for young pigs, one needs to know precisely the AA requirement for the typical phase feeding periods of growth. Based on NRC (1998) requirement estimates, the first and second limiting AA in a typical diet for 6- to 12-kg pigs are Lys and Met, respectively.

Recent estimates of the true digestible (TD) Lys requirement (Table 3.1) of 1.39% in modern high lean growth pigs weighing from 6- to 14-kg are considerably higher than the NRC (1998) estimate of 1.19% for 5- to 10-kg pigs (Broekman et al., 1997; Gaines et al., 2003a). Regarding the total sulfur AA (TSAA), considerable variation exists in the recent requirement estimates (Table 3.2) with values ranging from 0.51% to 0.72% TD TSAA (Chung, et al., 1992b; Owen, et al., 1995b; Matthews, et al., 2001b). All of these recent TSAA requirement estimates are lower than the NRC (1998) requirement of 0.76%. When these TSAA requirement estimates are used to calculate ratios of TSAA to 1.39% TD Lys, the resulting ratios range from 0.37 to 0.52. The NRC (1998) suggests a higher ratio of 0.57. In a review of the Lys requirement data in pigs, Kerr et al. (2002) calculated the amount of Lys needed per gram of average daily gain (ADG) and derived an average value of 18.77 mg Lys/g ADG when needs for both maintenance and growth are considered. When we make similar calculations with the recent Lys and TSAA estimates, we obtain average values of 17.59 and 9.31 (Tables 3.1 and 3.2), respectively. These estimates result in a TSAA:Lys ratio of 0.53.

Table 3.1. Lysine requirement estimates of young swine

BW, kg			Growth performance			Lysine that maximized performance				Reference
Mean	Initial	Final	ADG, g	ADFI, g	G:F	Total, % <sup>a</sup>	Dig, % <sup>b</sup>	Dig, g/d <sup>c</sup>	mg/g ADG <sup>d</sup>	
9.4	6.3	12.4	432	514	0.84	1.50	1.32	6.79	15.72	Broekman et al., 1997 <sup>e</sup>
10.4	7.4	13.4	427	528	0.81	1.61	1.42	7.50	17.56	Gaines et al., 2003a <sup>f</sup>
10.9	7.6	14.1	463	557	0.83	1.61	1.42	7.91	17.08	Gaines et al., 2003a <sup>g</sup>
11.0	6.3	15.7	335	604	0.55	1.20	1.06	6.40	19.10	Martinez et al., 1990 <sup>h</sup>
11.4	8.5	14.2	400	531	0.75	1.61	1.42	7.54	18.85	Gaines et al., 2003a <sup>i</sup>
11.8	7.1	16.5	334	565	0.59	1.10	0.97	5.48	16.41	Mahan et al., 1993 <sup>j</sup>
14.0	8.0	20.0	608	948	0.64	1.31	1.10	10.43	17.15	Campbell et al., 1988 <sup>k</sup>
15.5	11.0	20.0	526	687	0.77	1.50	1.32	9.07	17.24	Kendall et al., 2002 <sup>l</sup>
15.7	10.2	21.1	518	710	0.73	1.59	1.40	9.94	19.19	Lenahan et al., 2003 <sup>m</sup>
16.5	10.9	22.0	599	919	0.65	1.15	1.01	9.28	15.49	Hansen et al., 1999 <sup>n</sup>
18.4	12.2	24.5	586	889	0.66	1.48	1.30	11.56	19.73	Gaines et al., 2004a <sup>o</sup>
									17.59	Overall mean

<sup>a</sup>When Lys values were not given on a total basis, values were calculated from the true ileal digestible (TD) Lys by dividing by 0.88.

<sup>b</sup>When possible, digestibility values were taken from the articles; if they were not reported, a digestibility value of 88% was used to estimate the TD Lys.

<sup>c</sup>Calculated grams of TD Lys consumed per d.

<sup>d</sup>Calculated milligrams of Lys required for 1 g of BW gain.

<sup>e</sup>Broekman et al. (1997). Calculated d 0 to 14 growth performance from the 0 to 7 and 7 to 14 d data reported. Gain:feed reached a plateau at 1.32% TD Lys.

<sup>f</sup>Gaines et al. (2003a). Experiment 3, ADG and G:F seemed to be reaching a plateau at 1.42% TD Lys.

<sup>g</sup>Gaines et al. (2003a). Experiment 1, ADG and G:F were maximized at 1.42% TD Lys.

<sup>h</sup>Martinez et al. (1990). Feed efficiency reached a plateau at 1.06% TD Lys. Break point analysis predicted a requirement of 1.02% TD Lys.

<sup>i</sup>Gaines et al. (2003a). Experiment 2, ADG and G:F seemed to plateau at 1.42% TD Lys.

<sup>j</sup>Mahan et al. (1993). In corn-soybean meal diets with dried whey, pigs responded to Lys additions up to 0.97% TD Lys.

<sup>k</sup>Campbell et al. (1988). Taken from Kerr et al. (2002).

(Table 3.1 continued)

<sup>l</sup>Kendall et al. (2002). Gain reached a plateau at 1.32% TD Lys; however G:F improved to 1.41% TD Lys, which was the highest level fed.

<sup>m</sup>Lenahan et al. (2003). Gain and G:F were maximized at 1.40% TD Lys.

<sup>n</sup>Hansen et al. (1999). Gain:feed was maximized at 1.01% TD Lys.

<sup>o</sup>Gaines et al. (2004a). Gain was maximized at 1.30% TD Lys, break point analysis suggested a requirement of 1.27% TD Lys.

Table 3.2. Sulfur amino acid requirement estimates of young swine

BW, kg			Growth performance			Dietary		TSAA				Reference
Mean	Initial	Final	ADG, g	ADFI, g	G:F	Lys, % <sup>a</sup>	Cys, %	Total, % <sup>b</sup>	Dig, % <sup>c</sup>	Dig, g/d <sup>d</sup>	ADG <sup>e</sup>	
4.8	3.5	6.1	200	222	0.88	1.80/1.57	0.62	0.96	0.88	1.95	9.75	Owen, et al., 1995a <sup>f</sup>
5.9	3.8	7.9	291	323	0.90	1.40/1.18	0.62	0.77	0.66	2.13	7.32	Owen, et al., 1995a <sup>g</sup>
6.0	3.8	8.1	329	325	1.01	1.80/1.57	0.62	0.99	0.88	2.86	8.69	Owen, et al., 1995a <sup>h</sup>
7.2	4.9	9.4	318	318	1.00	1.60/1.41	0.52	0.80	0.70	2.23	7.01	Owen, et al., 1995b <sup>i</sup>
8.1	5.8	10.3	302	493	0.62	1.29/1.14	1.00	0.62	0.55	2.71	8.97	Chung, et al., 1992b <sup>j</sup>
8.3	5.8	10.7	351	617	0.57	1.29/1.14	1.00	0.58	0.51	3.15	8.97	Chung, et al., 1992b <sup>k</sup>
8.7	5.6	11.8	432	703	0.62	1.30/1.14	0.46	0.66	0.62	4.36	10.09	Owen, et al., 1995b <sup>l</sup>
10.6	7.4	13.8	440	639	0.69	1.56/1.37	0.41	0.79	0.64	4.09	9.30	Matthews, et al., 2001b <sup>m</sup>
11.6	8.3	14.8	463	604	0.77	1.50/1.32	NA	0.86	0.76	4.59	9.91	Gaines, et al., 2004b <sup>n</sup>
13.3	9.6	16.9	518	860	0.60	1.29/1.14	1.00	0.58	0.51	4.39	8.47	Chung, et al., 1992b <sup>o</sup>
13.6	9.9	17.3	528	898	0.60	1.34/1.34	0.40	0.54	0.54	4.85	9.19	Chung, et al., 1992a <sup>p</sup>
17.0	10.2	23.8	542	930	0.58	1.25/1.10	NA	0.69	0.61	5.67	10.46	De La Llata, et al., 1998 <sup>q</sup>
17.7	10.6	24.8	645	1174	0.55	1.29/1.14	1.00	0.62	0.55	6.46	10.02	Chung, et al., 1992b <sup>r</sup>
18.5	12.3	24.6	586	872	0.67	1.59/1.40	NA	0.88	0.77	6.71	11.45	Gaines, et al., 2004a <sup>s</sup>
16.8	11.4	22.1	509	761	0.67	1.31/1.15	NA	0.77	0.68	5.18	10.18	Gaines, et al., 2003b <sup>t</sup>
19.6	12.8	26.3	642	1041	0.62	1.19/1.05	NA	0.65	0.57	5.93	9.24	Gaines, et al., 2003b <sup>u</sup>
											9.31	Overall mean

<sup>a</sup>Values before the back slash (/) are total AA and after the slash are estimates or actual values for digestibility.

<sup>b</sup>Calculated by multiplying the total Met concentration times two. Assumes cystine can only provide up to 50% of TSAA requirement.

<sup>c</sup>When possible, digestibility values were taken from the articles; if they were not reported a digestibility value of 88% was used to estimate the true digestible TSAA.

<sup>d</sup>Calculated grams of TD TSAA consumed per d.

<sup>e</sup>Calculated milligrams of TSAA required for 1 g of BW gain.

(Table 3.2 continued)

<sup>f</sup>Owen et al. (1995a). Experiment 1, maximal gain and the lowest PUN were at 0.88% TD TSAA.

<sup>g</sup>Owen et al. (1995a). Experiment 2, 1.4% Lys, gainfeed was maximized with an apparent plateau for gain and PUN at 0.66% TD TSAA.

<sup>h</sup>Owen et al. (1995a). Experiment 2, 1.8% Lys, maximal gain and the lowest PUN were at 0.88% TD TSAA.

<sup>i</sup>Owen et al. (1995b). Experiment 1, G:F was maximized and PUN was the lowest at 0.70% TD TSAA. Break point analysis estimated the requirement at 0.72% TD TSAA.

<sup>j</sup>Chung et al. (1992b). Experiment 2, the largest improvements in gain and G:F occurred at levels up to 0.55% TD TSAA.

<sup>k</sup>Chung et al. (1992b). Experiment 3, the authors concluded 0.51% TD TSAA supported maximal gain, however G:F was improved further at 0.55% TD TSAA.

<sup>l</sup>Owen et al. (1995b). Experiment 2, breakpoint analysis suggested an estimate of 0.62% TD TSAA for G:F.

<sup>m</sup>Matthews et al. (2001b). Experiment 2, 0.64% TD TSAA was estimate from broken line regression for ADG and PUN.

<sup>n</sup>Gaines et al. (2004). Experiment 2, maximal gain and G:F was at the 0.76% TD TSAA level, however performance was similar at the 0.70% TD TSAA. Authors reported that their analysis suggested the requirement was 0.76% TD TSAA.

<sup>o</sup>Chung et al. (1992b). Experiment 5, maximal G:F was reached at 0.51% TD TSAA, however ADG improved to 0.55% TD TSAA.

<sup>p</sup>Chung et al. (1992a). Experiment 1, maximal G:F was reached at 0.54% TD TSAA, however broken line regression estimated the requirement at 0.46% TD TSAA for gain.

<sup>q</sup>De La Llata. (1998). Maximal G:F was reached at 0.61% TD TSAA.

<sup>r</sup>Chung et al. (1992b). Experiment 4, maximal G:F was reached at 0.55% TD TSAA.

<sup>s</sup>Gaines et al. (2004a). Experiment 2, Broken line estimates for ADG and G:F were 0.72 and 0.84% for TD TSAA.

<sup>t</sup>Gaines et al. (2003b). Experiment 1, maximal gain was reached at 0.68% TD TSAA, G:F was maximized at 0.74% TD TSAA.

<sup>u</sup>Gaines et al. (2003b). Experiment 2, gain and G:F seemed to plateau at 0.57% TD TSAA. Ratio study.

The economic importance of further evaluation of these requirements seem obvious considering they dictate the amount of intact protein sources and crystalline AA used during this phase of growth. Thus, we conducted four experiments to evaluate the TD Lys and TSAA requirements of pigs ranging in weight from 6- to 12-kg.

## **MATERIALS AND METHODS**

### General

All methods used in these experiments related to animal care were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee. Yorkshire, Yorkshire x Landrace, or Yorkshire x Landrace x Duroc pigs from the LSU Agricultural Center were used in each experiment. Pigs were housed in an environmentally controlled modular building in 0.97- x 1.47-m pens on hard plastic slotted floors with an under-floor flush system. Pigs and feeders were checked twice daily. Feed in mash form and water were provided on an *ad libitum* basis throughout all experiments. The pigs were allotted to dietary treatment on the basis of weight and ancestry in randomized complete block designs. Diets (Table 3.3) were formulated to meet or exceed AA requirements (with the exception of Lys or TSAA) for pigs ranging in weight from 5- to 10-kg (NRC, 1998). The diets were formulated to contain 0.90% Ca and 0.80% P. Amino acid, mineral, and ME values for feed ingredients were based on NRC (1998). Diets were kept isonitrogenous by altering concentrations of cornstarch and L-Glu as L-Lys•HCl or DL-Met was added to basal diets.

The AA composition of the basal diets were determined after acid hydrolysis (AOAC, 2000; Method 982.30 E[a]). Total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2000; Method 982.30 E[b]).

Table 3.3. Basal diet composition, as-fed basis<sup>a</sup>

Ingredient	Experiment 1		Experiment 2		Experiments 3 and 4		
	Positive control	1.10%	Positive control	1.10%	Positive control	0.62%	0.52%
		TD Lys		TD Lys		TD TSAA	TD TSAA
Corn	39.79	52.58	40.26	44.98	47.28	53.03	60.48
Soybean meal, 47.5% CP	36.52	19.36	37.86	23.72	29.42	21.50	20.18
Whey, dried	10.00	10.00	10.00	10.00	10.00	10.00	7.00
Fishmeal, menhaden	4.50	4.50	3.00	3.00	4.50	4.50	---
Corn gluten meal	---	---	---	6.54	---	---	---
Dry fat <sup>b</sup>	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Cornstarch	---	2.11	---	1.48	---	1.50	1.00
AP 920 <sup>c</sup>	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Monocalcium phosphate	0.96	1.35	1.13	1.39	1.09	1.28	1.98
Limestone	0.79	0.76	0.91	0.91	0.79	0.78	1.17
Antibiotic <sup>d</sup>	0.75	0.75	0.10	0.10	0.10	0.10	0.10
Vitamins <sup>e</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium bentonite <sup>f</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Zinc oxide	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Trace minerals <sup>g</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Flavor <sup>h</sup>	0.08	0.80	0.08	0.08	0.08	0.08	0.08
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine·HCl	---	0.12	0.04	---	0.10	0.36	0.67
L-glutamate	---	0.43	---	0.80	---	0.20	0.20
L-threonine	---	0.21	---	0.18	0.03	0.14	0.26
DL-methionine	0.18	0.32	0.19	0.23	0.18	---	---
L-valine	---	0.14	---	0.08	---	0.06	0.20
L-isoleucine	---	0.07	---	0.01	---	---	0.13
L-tryptophan	---	0.06	---	0.07	---	0.04	0.08
L-histidine	---	0.01	---	---	---	---	0.04
Calculated nutrient composition, %							
ME, kcal/kg	3409	3400	3422	3424	3430	3428	3390
CP	26.01	19.90	25.78	24.39	23.37	20.57	18.02
Ca	0.90	0.90	0.90	0.90	0.90	0.90	0.90
P	0.80	0.80	0.80	0.80	0.80	0.80	0.80

(Table 3.3 continued)

Lys	1.62	1.22	1.62	1.24	1.50	1.48	1.47
Trp	0.34	0.29	0.33	0.33	0.29	0.28	0.28
Thr	1.05	0.98	1.04	1.11	0.97	0.95	0.94
Met	0.60	0.65	0.59	0.64	0.56	0.34	0.26
TSAA	1.04	0.99	1.04	1.06	0.97	0.70	0.59
Lys, analyzed	1.60	1.23	1.56	1.28	1.52	1.44	1.39
Trp, analyzed	0.32	0.27	0.34	0.33	0.31	0.29	0.29
Thr, analyzed	1.11	0.84	1.01	1.06	0.96	0.85	0.82
Met, analyzed	0.54	0.58	0.52	0.62	0.56	0.35	0.27
TSAA, analyzed	0.92	0.87	0.90	1.02	0.96	0.68	0.56
TD Lys	1.45	1.10	1.45	1.10	1.35	1.35	1.35
TD Trp	0.30	0.27	0.30	0.30	0.26	0.26	0.26
TD Thr	0.91	0.87	0.90	0.98	0.84	0.84	0.84
TD Met	0.56	0.61	0.56	0.60	0.53	0.31	0.24
TD TSAA	0.94	0.91	0.94	0.96	0.88	0.62	0.52
TD Ile	1.00	0.77	0.99	0.87	0.88	0.74	0.74
TD Val	1.11	0.95	1.10	1.06	1.00	0.92	0.92
TD Leu	1.94	1.50	1.93	2.10	1.78	1.57	0.93
TD Arg	1.56	1.04	1.55	1.22	1.35	1.11	1.39
TD His	0.62	0.45	0.62	0.53	0.55	0.47	0.43
TD Phe + Tyr	1.95	1.38	1.95	1.84	1.73	1.46	1.27

<sup>a</sup>TD = true digestible; CP = crude protein.

<sup>b</sup>Fat Pak 100, Milk Specialties Co., Dundee, IL.

<sup>c</sup>Spray-dried animal plasma, American Protein Corporation, Ames, IA.

<sup>d</sup>Neo-Terra 10/10 for EXP 1 and TM 50 for EXP 2 through 4, Oxytetracycline (from oxytetracycline quaternary salt) equivalent to oxytetracycline hydrochloride 22 g/kg (Nutra Blend Corporation, Neosho, MO).

<sup>e</sup>Provided the following per kilogram of diet: vitamin A, 11,023 IU; vitamin D<sub>3</sub>, 3,307 IU; vitamin E, 88 IU; menadione (menadione pyrimidinol bisulfite) 8.3 mg; riboflavin, 13 mg; pantothenic acid, 50 mg; niacin, 88 mg; vitamin B<sub>12</sub>, 61 µg; biotin, 441 µg; folic acid, 3.3 mg; pyridoxine, 4.41 mg; thiamin, 4.41 mg; and vitamin C, 110 µg.

<sup>f</sup>AB-20, provided by Prince Agri Products, Inc, Quincy, IL.

<sup>g</sup>Provided the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, ethylenediamine dihydroiodide, respectively with calcium carbonate as the carrier.

<sup>h</sup>Dried strawberry, Feed Flavors, Inc., Wheeling, IL.



Tryptophan content was determined after alkaline hydrolysis (AOAC, 2000; Method 982.30 E[c]).

#### Experiment 1

One hundred twenty weanling pigs were allotted to six dietary treatments. Each treatment was replicated five times with four pigs per replicate. Average initial and final BW were  $6.8 \pm 0.1$  and  $12.3 \pm 0.2$  kg, respectively. Pigs were weaned at an average age of 20 d and were allotted to treatment diets after a 6-d adjustment period. Pigs were bled and weighed at the end of the 13-d experiment. Dietary treatments included a positive control (PC; 1.45% TD Lys) and a basal experimental diet (Table 3.3) containing 1.10% TD Lys and supplemented with crystalline Lys to provide TD Lys levels of 1.100, 1.175, 1.250, 1.325, and 1.400%. The PC and basal diet were formulated to a TSAA:Lys ratio of 0.65 to ensure adequacy. All other AA were based on NRC (1998) ratios.

#### Experiment 2

One hundred fifty weanling pigs were allotted to six dietary treatments. Each treatment was replicated five times with five pigs per replicate. Average initial and final BW were  $6.3 \pm 0.1$  and  $10.8 \pm 0.2$  kg, respectively. Pigs were weaned at an average age of 20 d and were allotted to treatment diets after a 7-d adjustment period. Pigs were bled and weighed at the end of the 13-d experiment. Dietary treatments included a PC (1.45% TD Lys) and a basal experimental diet (Table 3.3) containing 1.10% TD Lys and supplemented with crystalline Lys to provide TD Lys levels of 1.10, 1.20, 1.30, 1.40, and 1.50%. The PC and basal diet were formulated to a TSAA:Lys ratio of 0.65 to ensure adequacy. All other AA were based on NRC (1998) ratios to 1.50% TD Lys.

### Experiment 3

One hundred fifty weanling pigs were allotted to six dietary treatments. Each treatment was replicated five times with five pigs per replicate. Average initial and final BW were  $6.7 \pm 0.1$  and  $11.7 \pm 0.2$  kg, respectively. Pigs were weaned at an average age of 21 d and were allotted to treatment diets after a 7-d adjustment period. Pigs were weighed at the end of the 14-d experiment. Dietary treatments included a PC (1.35% TD Lys) and a basal experimental diet (Table 3.3) containing 0.62% TD TSAA and supplemented with crystalline DL-Met to provide TD TSAA levels of 0.62, 0.68, 0.74, 0.80, and 0.86%. The PC and basal diet were formulated to 1.35% TD Lys with TD TSAA concentrations of 0.88 and 0.62%, respectively. All other AA were based on NRC (1998) ratios to 1.35% TD Lys.

### Experiment 4

One hundred sixty-eight weanling pigs were allotted to six dietary treatments. Each treatment was replicated five times with five or six pigs per replicate. Average initial and final BW were  $6.4 \pm 0.1$  and  $11.2 \pm 0.1$  kg, respectively. Pigs were weaned at an average age of 21 d and were allotted to treatment diets after a 7-d adjustment period. Pigs were bled and weighed on d 13. Dietary treatments included a PC (1.35% TD Lys) and a basal experimental diet (Table 3.3) containing 0.52% TD TSAA and supplemented with crystalline DL-Met to provide TD TSAA levels of 0.52, 0.58, 0.64, 0.70, and 0.76%. The PC and basal diet were formulated to 1.35% TD Lys with TD TSAA concentrations of 0.88 and 0.52%, respectively. All other AA were based on NRC (1998) ratios to 1.35% TD Lys.

## Blood Sampling

Blood was collected via the anterior vena cava and placed into 4-ml tubes (Monoject, Sherwood Medical, St. Louis, MO) containing 10.0 mg of sodium fluoride and 8.0 mg of potassium oxalate. Samples were placed on ice before centrifugation at 1,500  $\times g$  at 4°C for 20 min. Plasma was collected after centrifugation and samples were frozen until analysis for plasma urea N (PUN) concentrations by the methods of Laborde et al. (1995).

## Statistical Analysis

Data were analyzed by ANOVA procedures using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) as random complete block designs. The statistical model included treatment and replication for all experiments. Orthogonal contrasts were used to determine linear and quadratic effects for Lys or TSAA. The NLIN procedure of SAS (break point analysis) was used to estimate AA requirements when responses were observed (Robbins et al., 1979). Treatment differences were considered significant at  $\alpha = 0.10$ . The pen of pigs was the experimental unit for all data.

## **RESULTS AND DISCUSSION**

In EXP 1 (Table 3.4), pigs fed the PC diet had higher ADG ( $P < 0.05$ ) compared with pigs fed the other diets, except those fed 1.175% TD Lys. Gain:feed was decreased ( $P < 0.05$ ) in pigs fed all concentrations of Lys compared with pigs fed the PC. Incremental Lys addition did not affect ADG, average daily feed intake (ADFI) or gain:feed (G:F). Plasma urea N was decreased linearly ( $P < 0.001$ ) as Lys concentration increased in the diet. Break point analysis of PUN suggested a TD Lys requirement of 1.34%.

Table 3.4. Growth performance and plasma urea nitrogen concentrations of pigs fed graded concentrations of lysine in Experiment 1<sup>a</sup>

Response	Positive control	1.100% TD Lys	1.175% TD Lys	1.250% TD Lys	1.325% TD Lys	1.400% TD Lys	SEM
Lys intake, g/d	9.96	7.43	8.25	7.90	8.78	9.69	---
Lys, mg/g ADG	20.97	17.99	18.29	20.31	21.11	22.12	---
Initial BW, kg	6.74	6.74	6.78	6.67	6.76	6.79	0.06
Final BW, kg	12.90	12.11	12.64	11.72	12.16	12.48	0.19
Growth performance							
ADG, g	475	413	451	389	416	438	11
ADFI, g	687	675	702	632	663	692	22
G:F, g/g	0.691	0.612	0.644	0.609	0.627	0.632	0.016
Plasma							
Urea N, mmol/L <sup>b, c</sup>	4.97	2.79	2.14	1.68	1.32	1.14	0.25

<sup>a</sup>Data are means of five replicates with four pigs per pen.

<sup>b</sup>Lys linear ( $P < 0.001$ ).

<sup>c</sup>Break point analysis of PUN estimated a Lys requirement of  $1.34 \pm 0.02\%$  for the single slope method.

The sporadic growth performance of pigs fed TD Lys levels ranging from 1.10 to 1.40% in EXP 1 and failure of pigs fed the basal diet supplemented with Lys to have growth performance similar to pigs fed the PC diet, led us to reformulate our basal diet and conduct EXP 2.

In EXP 2 (Table 3.5), incremental addition of Lys increased G:F (linear,  $P = 0.008$ ), ADG (linear,  $P < 0.001$ ; quadratic,  $P = 0.05$ ), and ADFI (linear,  $P = 0.007$ ; quadratic,  $P = 0.06$ ). Growth performance of pigs fed the three highest levels of Lys was not different from pigs fed the PC. Plasma urea N was decreased linearly ( $P < 0.001$ ) with the addition of Lys. Break point analysis estimated requirements of 1.35, 1.40, and 1.43% for ADG, G:F, and PUN (Table 3.6), respectively. The milligrams of Lys needed per gram of ADG was calculated to be 18.8 for pigs fed 1.40% TD Lys.

Our review of estimates of the Lys requirement of young pigs (Table 3.1) resulted in three estimates from refereed journal articles (Campbell et al., 1988; Martinez et al., 1990; Mahan et al., 1993) and eight estimates from abstracts (Broekman et al., 1997; Hansen et al., 1999; Kendall et al., 2002; Gaines et al., 2003a, 2004a; Lenehan et al., 2003). Researchers used various techniques to estimate plateaus and break points in these studies. To be more consistent, we used our own assessment regarding the Lys level that optimized growth performance. In some cases, break point analysis was performed on treatment means to estimate requirements when the authors used some other statistical method. Because many of these experiments were based on total Lys rather than on TD Lys, a digestibility coefficient of 88% was used to calculate a TD Lys value when none was reported or when it was impossible to calculate the value from dietary ingredients. Growth performance values of pigs at or above the estimated

Table 3.5. Growth performance and plasma urea nitrogen concentrations of pigs fed graded levels of lysine in Experiment 2<sup>a</sup>

Response	Positive control	1.10% TD Lys	1.20% TD Lys	1.30% TD Lys	1.40% TD Lys	1.50% TD Lys	SEM
Lys intake, g/d	7.32	4.90	5.86	6.67	7.14	7.62	---
Lys, mg/g ADG	20.33	16.90	19.03	18.58	18.84	21.11	---
Initial BW, kg	6.34	6.31	6.31	6.35	6.31	6.36	0.03
Final BW, kg <sup>b, c</sup>	11.00	10.09	10.31	11.02	11.24	11.05	0.18
Growth performance							
ADG, g <sup>b, d</sup>	360	290	308	359	379	361	13
ADFI, g <sup>b, c</sup>	505	445	488	513	510	508	15
G:F, g/g <sup>b</sup>	0.717	0.650	0.628	0.704	0.743	0.714	0.026
Plasma							
Urea N, mmol/L <sup>e</sup>	6.62	8.71	7.40	5.92	4.69	4.23	0.43

<sup>a</sup>Data are means of five replicates with five pigs per pen.

<sup>b</sup>Lys linear ( $P < 0.05$ ).

<sup>c</sup>Lys quadratic ( $P < 0.10$ ).

<sup>d</sup>Lys quadratic ( $P < 0.05$ ).

<sup>e</sup>Lys linear ( $P < 0.001$ ).

Table 3.6. Break point estimates of the percent lysine requirement of pigs in Experiment 2

Response	One slope estimate	Two slope estimate
Final BW	1.34 ± 0.05	1.44 ± 0.02
Growth performance		
ADG	1.35 ± 0.05	NE <sup>a</sup>
ADFI	1.25 ± 0.01	1.26 ± 0.01
G:F	1.40 ± 0.18	NE <sup>a</sup>
Plasma urea N	1.43 ± 0.01	NE <sup>a</sup>

<sup>a</sup>NE = Not able to be estimated.

requirement were used to calculate Lys intake and the milligrams of Lys required per gram of ADG. The average estimate from Table 1 is 17.59 mg Lys/g ADG. Kerr et al. (2002) indicated a requirement of 18.77 mg Lys/g ADG in a review of research that included pigs ranging in weight from 8 to 90 kg. Our estimate of 18.8 mg Lys/g ADG from data in EXP 2 agrees closely with recent research with pigs in the same weight range (Gaines et al., 2003a, 2004a; Lenehan et al., 2003).

Experiment 3 was conducted to evaluate the TSAA requirement of pigs in the weight range of 5- to 10-kg. In EXP 3 (Table 3.7), there was no response of increasing TSAA from 0.62 to 0.86%. Furthermore, pigs fed all levels of TSAA had growth performance that was not different from pigs fed the PC diet. When the milligrams of TSAA needed per gram ADG was calculated, a value of 10.0 was derived for pigs receiving 0.62% TD TSAA.

In order to try and create a response curve for TSAA, we reformulated a different basal diet with a lower concentration of TD TSAA in EXP 4. In EXP 4 (Table 3.8), the addition of TSAA increased ADG (linear,  $P = 0.01$ ; quadratic,  $P = 0.08$ ) and decreased PUN (linear and quadratic,  $P < 0.001$ ). However, pigs receiving the basal diet with varying levels of TSAA had lower ADG ( $P < 0.05$ ) and G:F ( $P < 0.001$ ) than pigs fed the PC. Break point analysis was unable to predict requirement estimates for ADG and PUN.

Our review of recent estimates of the TSAA requirement of young pigs (Table 3.2) resulted in 11 estimates from refereed journals (Chung et al., 1992a,b; Owen et al., 1995a,b; Matthews et al., 2001b) and five estimates from abstracts (De La Llata et al., 1998; Gaines et al., 2003b, 2004a,b). The same procedure was used in the TSAA



Table 3.7. Growth performance of pigs fed graded levels of total sulfur amino acids in Experiment 3<sup>a,b</sup>

		0.62%	0.68%	0.74%	0.80%	0.86%	
	Positive	TD	TD	TD	TD	TD	
Response	control	TSAA	TSAA	TSAA	TSAA	TSAA	SEM
TSAA intake, g/d	5.02	3.60	3.77	4.11	4.43	4.91	---
TSAA, mg/g ADG <sup>a</sup>	14.20	10.00	11.10	11.70	12.10	13.60	---
Initial BW, kg	6.74	6.69	6.70	6.70	6.72	6.76	0.04
Final BW, kg	11.70	11.87	11.37	11.60	11.85	11.82	0.17
Growth performance							
ADG, g	355	360	339	350	367	362	11
ADFI, g	571	581	555	556	554	571	15
G:F, g/g	0.622	0.620	0.613	0.631	0.662	0.635	0.019

<sup>a</sup>Data are means of five replicates with five pigs per pen.

<sup>b</sup>No treatment effects ( $P > 0.10$ ).

Table 3.8. Growth performance and plasma urea nitrogen concentrations of pigs fed graded levels of total sulfur amino acids in Experiment 4<sup>a,b</sup>

		0.52%	0.58%	0.64%	0.70%	0.76%	
	Positive	TD	TD	TD	TD	TD	
Response	control	TSAA	TSAA	TSAA	TSAA	TSAA	SEM
TSAA intake, g/d	5.39	3.15	3.81	4.10	4.61	4.87	---
TSAA, mg/g ADG <sup>a</sup>	12.86	8.51	10.16	10.90	12.20	12.82	---
Initial BW, kg	6.38	6.35	6.34	6.39	6.39	6.41	0.03
Final BW, kg <sup>b</sup>	11.82	10.72	11.23	11.12	11.23	11.34	0.14
Growth performance							
ADG, g <sup>b,c</sup>	419	337	375	376	378	380	10
ADFI, g	613	605	657	641	658	641	21
G:F, g/g	0.686	0.557	0.574	0.588	0.575	0.592	0.014
Plasma							
Urea N, mmol/L <sup>d,e</sup>	2.81	1.77	0.61	0.34	0.27	0.17	0.12

<sup>a</sup>Data are means of five replicates with five or six pigs per pen.

<sup>b</sup>TSAA linear (P < 0.05).

<sup>c</sup>TSAA quadratic (P < 0.05).

<sup>d</sup>TSAA linear (P < 0.001).

<sup>e</sup>TSAA quadratic (P < 0.001).

review as previously discussed for Lys. The average review estimate for TSAA was 9.3 mg TSAA/g ADG. The milligrams TSAA required per gram ADG for pigs fed 0.58% TD TSAA in EXP 4 was 10.2, which is close to the 10.0 value calculated for pigs fed 0.62% TD TSAA in EXP 3. It is also similar to the suggestion of Peak (2005) that the required value is near 10.4 for growing, finishing pigs. This value also falls within the range of estimates from the studies summarized in Table 2, although slightly higher than the average value of 9.3 mg TSAA/g ADG. As indicated previously, performance of pigs fed the incremental concentrations of TSAA diet in EXP 4 never reached the levels of pigs fed the PC. Nonetheless, we feel that performance was close to that of pigs fed the PC, and the milligrams TSAA required per gram of ADG should be nearly the same for a range of performance levels near that required for maximal performance.

When our estimates of the Lys and TSAA requirement are evaluated on the basis of mg AA/g ADG, the ratio of TSAA:Lys on a TD basis is 0.54. This estimate is consistent with our review of the literature that suggests the ratio of TSAA:Lys is 0.53. The NRC (1998) suggests a TD TSAA:Lys ratio of 0.57, however the ratio of Met:Lys is only 0.27. Chung and Baker (1992a) suggested that the maximal portion of the TSAA requirement that can be met by Cys is 50%. If the NRC (1998) Met requirement is doubled the resulting TSAA:Lys ratio is 0.54, which again agrees with our estimate.

In summary, data from these experiments suggest that the TD Lys requirement is 1.40% or 18.8 mg/g ADG for 6- to 12-kg pigs, and that the TD TSAA requirement is near 10.2 mg/g ADG resulting in a TSAA:Lys ratio that is not greater than 0.54.

## CHAPTER 4

### LYSINE AND TOTAL SULFUR AMINO ACID REQUIREMENTS OF LATE-FINISHING BARROWS

#### INTRODUCTION

Formulating low crude protein (CP) diets that will optimize growth performance for late-finishing pigs is dependant upon the order in which amino acids (AA) become limiting. It is generally accepted that the first three limiting AA in order are Lys, Thr, and Trp in late-finishing diets. The fourth and fifth limiting AA in a corn-soybean meal (C-SBM) diet are Ile and TSAA, respectively, when formulating to NRC (1998) requirements.

Because many of the essential AA requirements for finishing pigs are based on ratios to Lys (Fuller, 1994; Chung and Baker, 1992d; Baker, 1997), the importance of an accurate requirement estimate for Lys in the late-finishing period becomes crucial in diet formulation to minimize AA excesses. The NRC (1998) suggests a true ileal digestible (TD) Lys requirement of 0.52% for barrows with a lean gain of 350 g/d. However, the small number of recent estimates in the literature for barrows weighing between 80 and 120 kg range from 0.49% TD Lys (Hahn et al., 1995a) to 0.83% total Lys (Knowles et al., 1997).

Many researchers that have evaluated low CP diets for finishing pigs have added crystalline Met to the diet (Kephart et al., 1990; Knowles et al., 1998a; Le Bellego et al., 2001). Sources of Met are commercially available and depending upon the actual total sulfur AA (TSAA) requirement, may or may not need to be supplemented. The NRC (1998) suggests a TD TSAA requirement of 0.31%, which corresponds to a TSAA:Lys ratio of 0.60. Hahn and Baker (1995b) suggested that the required ratio of

TSAA:Lys increased as pigs become heavier due to the increased maintenance requirements of TSAA relative to the total AA requirement.

The Lys requirement for late-finishing barrows and whether or not supplemental Met is needed seems crucial in formulations of low CP diets for finishing barrows. Previous research has shown the use of plasma urea N (PUN) to be a quick and effective means of determining AA requirements of pigs (Coma et al., 1995; Knowles et al., 1997). Thus, we conducted two experiments (EXP) to evaluate the TD Lys and TSAA requirements of 90-kg barrows.

## **MATERIALS AND METHODS**

### General

All methods used in these experiments related to animal care were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee. Yorkshire, Yorkshire x Landrace, or Yorkshire x Landrace x Duroc pigs from the LSU Agricultural Center were used in each experiment. Pigs were housed in a curtain-sided building with 1.5- x 3.0-m pens and concrete slotted floors. Pigs and feeders were checked twice daily. Feed in mash form and water were provided on an *ad libitum* basis throughout the experiments. The pigs were allotted to dietary treatment on the basis of weight and ancestry in randomized complete block designs. Diets (Table 4.1) were formulated to meet or exceed AA requirements (with the exception of Lys or TSAA) for barrows gaining 350 g of lean gain per d (NRC, 1998). The diets were formulated to contain 0.45% Ca and 0.40% P. Amino acid, mineral, and ME values for feed ingredients were based on NRC (1998).

Table 4.1. Composition of diets for barrows, as-fed basis<sup>a</sup>

Ingredient	Experiment 1	Experiment 2
	0.30 % TD Lys	0.27% TD TSAA
Corn	91.70	85.39
Soybean meal, 47.5% CP	4.19	---
Cornstarch	0.50	8.84
L-glutamic acid	1.00	1.19
Limestone	0.85	0.99
Monocalcium phosphate	0.54	1.24
Salt	0.50	0.50
Mineral premix <sup>b</sup>	0.10	0.10
Vitamin premix <sup>c</sup>	0.38	0.38
L-lysine•HCl	---	0.48
L-threonine	0.12	0.16
L-tryptophan	0.04	0.06
DL-methionine	0.02	---
L-isoleucine	0.04	0.10
L-valine	0.02	0.08
L-phenylalanine	---	0.03
L-histidine	---	0.01
Calculated composition <sup>d</sup>		
ME, kcal/kg	3298	3305
CP, %	10.72	9.00
Lys, %	0.37	0.60
TD Lys, %	0.30	0.55
Ca, %	0.45	0.45
P, %	0.40	0.40
Analyzed composition, %		
Lys	0.38	0.63
Met	0.21	0.15
Met+Cys	0.43	0.31
Trp	0.12	0.11
Thr	0.48	0.41
Ile	0.42	0.36
Val	0.52	0.46

(Table 4.1 continued)

<sup>a</sup>TD = true ileal digestible; CP = crude protein.

<sup>b</sup>Provides the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, Se, 0.3 mg as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite, respectively with calcium carbonate as the carrier.

<sup>c</sup>Provides the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D<sub>3</sub>, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex) 6.2 mg; riboflavin, 10 mg; Ca-d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B<sub>12</sub>, 45 µg; d-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamin, 3.31 mg; and vitamin C, 83 µg.

<sup>d</sup>Amino acid values for corn and soybean meal (NRC, 1998) are on a TD basis using digestibility coefficients from NRC, 1998. Diets were formulated to meet or exceed NRC (1998) AA requirements for barrows gaining 350 grams of lean gain per day with the exception of Lys in EXP 1 and Lys and TSAA in EXP 2.

The AA composition of the basal diets were determined after acid hydrolysis (AOAC, 2000; Method 982.30 E[a]). Total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2000; Method 982.30 E[b]). Tryptophan content was determined after alkaline hydrolysis (AOAC, 2000; Method 982.30 E[c]).

#### Experiment 1

Forty barrows with an average initial and final BW of  $88.5 \pm 0.1$  and  $92.9 \pm 0.2$  kg were allotted to five treatments. There were four replications of barrows with two pigs per replicate. At the initiation of the EXP, all pigs received the same late-finishing diet for 7 d. On d 7 pigs were bled at 0900 and then weighed and put on treatment diets. Pigs remained on treatment diets for 6 d and were bled and weighed at 0900 on d 13.

Dietary treatments included a basal diet containing 0.30% TD Lys (Table 1) or the basal diet plus 0.075, 0.150, 0.225, or 0.300% Lys, resulting in treatment diets of 0.300, 0.375, 0.450, 0.525, or 0.600% TD Lys. The basal diet was formulated to meet or exceed AA ratios to 0.60% TD Lys based on NRC (1998) recommendations. Dietary amounts of L-Glu, cornstarch, sodium bicarbonate, and salt were varied to make all diets isonitrogenous and equal in dietary electrolyte balance.

#### Experiment 2

Eighty barrows with an average initial and final BW of  $90.9 \pm 0.1$  and  $94.3 \pm 0.3$  kg were allotted to five treatments. There were four replications of barrows with four pigs per replicate. At the initiation of the EXP, all pigs received the same late-finishing diet for 3 d. On d 3, pigs were bled at 0900 and then weighed and put on treatment



diets. Pigs remained on treatment diets for 7 d and were bled and weighed at 0900 on d 10.

Dietary treatments included a basal diet containing 0.27% TD TSAA (Table 1) or the basal diet plus 0.02, 0.04, 0.06, or 0.08% DL-Met, resulting in treatment diets of 0.27, 0.29, 0.31, 0.33, and 0.35% TD TSAA. The basal diet was formulated to provide 0.55% TD Lys. All other AA were added based on NRC (1998) ratios to Lys. Diets were kept isonitrogenous by altering amounts of cornstarch and L-Glu as DL-met was added to the basal diet.

#### Blood Sampling

Blood was collected via the anterior vena cava and placed into 4-ml tubes (Monoject, Sherwood Medical, St. Louis, MO) containing 10.0 mg of sodium fluoride and 8.0 mg of potassium oxalate. Samples were placed on ice before centrifugation at 1,500  $\times g$  at 4°C for 20 min. Plasma was collected after centrifugation and samples were frozen until analysis for PUN concentrations by the methods of Laborde et al. (1995).

#### Statistical Analysis

Data were analyzed by ANOVA procedures using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) as random complete block designs. The statistical model included treatment and replication for all EXP. Orthogonal contrasts were used to determine linear and quadratic effects for Lys or TSAA. The NLIN procedure of SAS (break point analysis) was used to estimate AA requirements when responses were observed (Robbins et al., 1979). Treatment differences were considered significant at  $\alpha = 0.10$ . The pen of pigs was the experimental unit for all data.

## **RESULTS AND DISCUSSION**

Experiment 1 was conducted to evaluate the Lys requirement of 90-kg barrows. There were no differences in average daily feed intake (ADFI), average daily gain (ADG), or gain:feed (G:F) (Table 4.2) due to the high variability in measuring performance over a short 6-d time period. Numerically, ADFI, ADG, and G:F were highest for pigs fed the diet containing 0.525% TD Lys. Plasma urea N was decreased linearly as Lys increased in the diet ( $P = 0.004$ ). In the absence of a quadratic effect, one-slope break point analysis estimated the TD Lys requirement at 0.57%.

The NRC (1998) TD Lys requirement of 0.52% for barrows gaining 350 g of lean gain per d agrees closely with our estimate of 0.57% based on PUN. Our estimate of the TD Lys requirement is considerably lower than estimates that were determined by Knowles et al. (1997), which suggested on a total basis that the Lys requirement was near 0.83%. It is important to remember that one of the most significant factors influencing the requirement of an AA on a percentage basis is the level of feed intake. Knowles et al. (1997) did not report feed intakes or growth performance in their studies using serum urea N as the response criteria. Furthermore, ratios of other essential AA to Lys were extremely high in the diets containing the lowest levels of Lys fed (i.e. TSAA:Lys = 2.17), which may have resulted in AA imbalances leading to an overestimation of the Lys requirement in their studies. There are few other empirical estimates of the Lys requirement for late-finishing barrows. In a study of the CP and Lys requirement of pigs weighing 51 to 105 kg, Cromwell et al. (1993) suggested that the total Lys requirement was 0.60% for barrows and 0.90% for gilts. Assuming a digestibility value of 85% for a

Table 4.2. Effects of dietary lysine concentration on growth performance and plasma urea nitrogen of barrows<sup>a</sup>

Item	True ileal digestible lysine, %					SEM
	0.300	0.375	0.450	0.525	0.600	
Lysine intake, g/d	9.99	11.48	15.44	18.11	19.68	---
Lysine, mg/g of ADG	15.54	14.99	16.32	18.91	22.34	---
Growth performance						
ADG, kg	0.643	0.766	0.946	0.958	0.881	0.141
ADFI, kg	3.33	3.06	3.43	3.45	3.28	0.22
Gain:feed g/kg	191	256	278	286	270	46
Plasma						
Initial urea N, mmol/L <sup>b</sup>	4.00	3.53	4.82	4.49	3.83	0.40
Final urea N, mmol/L <sup>c</sup>	3.37	2.67	2.65	2.35	2.10	0.30

<sup>a</sup>Data are the means of four replicates with two pigs per replicate with an average initial and final BW of 88.5 and 92.9 kg, respectively. The growth period lasted for 6 d.

<sup>b</sup>Pigs were bled on d 0 to determine initial plasma urea nitrogen.

<sup>c</sup>Pigs were bled 6 d after the initiation of treatment diets for determination of final PUN. Initial PUN was used as a covariate in the analysis of final PUN. Linear effect of Lys ( $P = 0.004$ ). One-slope break point analysis estimated the TD Lys requirement at  $0.57 \pm 0.08\%$ .

C-SBM diet, their TD Lys requirement would be 0.51% for barrows. Hahn et al. (1995a) used growth performance and carcass measurements to estimate the TD Lys requirement and concluded that the requirement for late-finishing barrows was 0.49% while the requirement for gilts was 0.52%. Loughmiller et al. (1998a) concluded that the requirement of 91- to 113-kg gilts was approximately 0.60% total Lys. In a review of the literature, using both empirical data and factorial analysis, Kerr et al. (1993) estimated total Lys requirements of 0.49, 0.60, and 0.73% for low, medium, and high lean growth genotype barrows.

Experiment 2 was conducted to determine whether the TSAA requirement was above the level supplied from a diet containing only corn as the source of TSAA. In EXP 2 (Table 4.3), there were no significant effects of increasing TD TSAA concentrations above 0.27% on ADFI, ADG, G:F, or PUN.

Hahn and Baker (1995b) reported that the ratio of Thr, Trp, and TSAA to Lys increases as pigs become heavier due to the increased maintenance requirements of TSAA relative to the total AA requirement. However, in their experiment they increased the ratios of Thr, Trp, and TSAA together, so it is impossible to determine whether improvements in performance were a result of one of these AA additions or of combinations of them. Knowles et al. (1998b) concluded that the optimum ratio of TSAA:Lys was not greater than 0.47 for growth and carcass muscling, but may be as high as 0.65 for minimizing fat deposition. Our lowest level of TSAA in EXP 2 (0.27%) would have resulted in a TSAA:Lys ratio of 0.48 when compared to the Lys requirement estimate of 0.56% from EXP 1. This also agrees with Loughmiller et al. (1998b) who concluded that the TSAA:Lys ratio for finishing gilts was not greater than 0.50.

Table 4.3. Effects of dietary total sulfur amino acid concentration on growth performance and plasma urea nitrogen of barrows<sup>a</sup>

Item <sup>b</sup>	True ileal digestible TSAA, %					SEM
	0.27	0.29	0.31	0.33	0.35	
TSAA intake, g/d	6.70	7.92	8.49	9.24	9.73	---
TSAA, mg/g of ADG	17.36	17.11	16.88	16.68	18.36	---
Growth performance						
ADG, kg	0.386	0.463	0.503	0.554	0.530	0.091
ADFI, kg	2.48	2.73	2.74	2.80	2.78	0.15
G:F, kg/kg	0.150	0.167	0.183	0.196	0.188	0.028
Plasma						
Initial urea N, mmol/L <sup>c</sup>	3.15	2.86	3.42	3.38	3.33	0.26
Final urea N, mmol/L <sup>d</sup>	1.37	1.18	1.31	1.21	1.21	0.11

<sup>a</sup>Data are the means of four replicates with four pigs per pen with an average initial and final BW of 90.9 and 94.3 kg, respectively. The growth period lasted for 7 d.

<sup>b</sup>No treatment effect ( $P > 0.10$ ).

<sup>c</sup>Pigs were bled on d 0 to determine initial plasma urea nitrogen.

<sup>d</sup>Pigs were bled 7 d after the initiation of treatment diets for determination of final PUN. Initial PUN was used as a covariate in the analysis of final PUN.

In a review of the Lys requirement, Kerr et al. (2002) calculated the milligrams of Lys required for one gram of ADG, and the resulting average value was 18.77 when both requirements for maintenance and growth are considered. Peak et al. (2005) made similar calculations in a review of the TSAA requirement and concluded that 10.4 mg of TD TSAA was needed for one gram of ADG. The resulting ratio of these estimates is 0.55. Our data from EXP 1 and 2 suggest a ratio not greater than 0.48. In summary, the TD Lys requirement of 90-kg barrows is near 0.57% and we observed no response to TD TSAA levels above 0.27% using PUN as the response criteria.

## CHAPTER 5

### ISOLEUCINE REQUIREMENT OF 80- TO 120-KILOGRAM BARROWS

#### INTRODUCTION

Crystalline Lys, Thr, and Trp have become economical for supplementation into many practical swine diets. To optimize these dietary AA additions there is a need for an accurate estimation of the fourth limiting AA, which is Ile according to NRC (1998) in corn-soybean meal (C-SBM) diets fed to late-finishing pigs. This is supported by Liu et al. (2000) who reported that a corn diet supplemented with Lys, Thr, Trp, Met, was deficient in Ile. The NRC (1998) requirement for Ile is based off ideal AA ratios (Wang and Fuller, 1989; Chung and Baker, 1992d; Baker, 1997). However, there is little empirical data in the literature estimating the actual true ileal digestible (TD) Ile requirement for late-finishing pigs. Early research did not consider the AA digestibility of ingredients and research was based on small numbers of pigs over a wide weight range (Becker et al., 1963; Brown et al., 1974). More recent reports are inconsistent. Parr et al. (2004) concluded that the TD Ile requirement for 87- to 100-kg barrows was near the NRC (1998) estimate of 0.30% TD Ile for high lean barrows. In contrast, Kendall et al. (2004b) suggested that the TD Ile requirement of 90-kg barrows was 0.36%.

The use of alternative protein sources such as red blood cells, which is deficient in Ile, has also created a need to know precisely the Ile requirement and to evaluate the interrelationships of the branched chain AA (BCAA). Recently, Kerr et al. (2004a) in nursery pigs and Parr et al. (2003, 2004) in growing and finishing pigs suggested that a corn-based diet containing 5% red blood cells was deficient in Ile, and that the deficiency could be corrected by the addition of crystalline Ile.

Therefore, six experiments were conducted to validate an Ile deficient diet, determine the TD Ile requirement for high lean 80- to 120-kg barrows utilizing growth, carcass traits, and minimal PUN as response criteria, and to compare the BCAA relationship of a C-SBM diet to a corn-blood cell (C-BC) diet.

## **MATERIALS AND METHODS**

### General

All methods used in these experiments related to animal care were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee. Yorkshire, Yorkshire x Landrace, or Yorkshire x Landrace x Duroc pigs from the LSU Agricultural Center were used in each experiment. Pigs were housed in a curtain-sided building with 1.5- x 3.0-m pens and concrete slotted floors. Pigs were allotted to dietary treatment on the basis of weight and ancestry in randomized complete block designs. Treatments were replicated five times in EXP 1 and four times in EXP 2 to 6, and all EXP had four barrows per replicate pen. Diets (Table 5.1) were formulated to meet or exceed the nutrient requirements (with the exception of Ile) of barrows gaining 350 g of lean gain per d (NRC, 1998) and formulated to contain 0.60% Ca and 0.50% P. Amino acid, mineral, and ME values for corn and SBM were based on NRC (1998). Blood cells (AP 301G, American Protein Corp., St. Louis, MO) were analyzed for total AA content (Table 5.2) and TD coefficients of 97.1, 95.4, 95.9, and 96.3% were used for Ile, Leu, Val, and Lys, respectively (Parr et al., 2003). Amino acid composition of the blood cells and basal diets were determined after acid hydrolysis (AOAC, 2000; Method 982.30 E(a)). Total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (AOAC,



Table 5.1. Composition of basal diets, as-fed basis<sup>a</sup>

Ingredients	EXP 1		EXP 2	EXP 3	EXP 4	EXP 5	EXP 6
	C-SBM 0.47% TD Ile	C-BC 0.24% TD Ile	C-BC 0.24% TD Ile	C-BC 0.28% TD Ile	C-SBM 0.26% TD Ile	C-SBM 0.24% TD Ile	C-SBM 0.26% TD Ile
Corn	81.88	90.80	91.27	91.22	94.31	94.82	93.47
Soybean meal, 47.5% CP	13.81	---	---	---	1.50	0.47	1.68
Blood cells <sup>b</sup>	---	5.00	5.00	5.00	---	---	---
Dry fat <sup>c</sup>	0.95	0.30	---	---	---	---	---
Limestone	1.04	1.01	1.01	1.01	1.04	1.04	1.04
Monocalcium phosphate	0.84	1.17	1.17	1.17	1.07	1.10	1.08
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium bentonite	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix <sup>d</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>e</sup>	0.38	0.38	0.38	0.38	0.38	0.38	0.38
L-Ile	---	---	---	0.04	---	---	---
L-Glu	---	0.24	0.07	0.08	0.08	0.09	0.55
L-Lys•HCl	---	---	---	---	0.37	0.40	0.37
L-Thr	---	---	---	---	0.09	0.11	0.09
L-Trp	---	---	---	---	0.04	0.05	0.04
DL-Met	---	---	---	---	---	0.01	---
L-Val	---	---	---	---	---	0.02	---
Cornstarch	---	---	---	---	0.02	0.41	0.20
Calculated composition <sup>f</sup>							
ME, kcal/kg	3343	3343	3335	3335	3293	3294	3278
CP, %	13.36	12.36	12.24	12.24	9.07	8.70	9.53
Lys, %	0.63 (0.62)	0.69 (0.65)	0.69 (0.65)	0.69 (0.66)	0.58 (0.61)	0.58 (0.63)	0.58 (0.60)

(Table 5.1 continued)

TSAA, %	0.49 (0.50)	0.41 (0.40)	0.42 (0.40)	0.42 (0.41)	0.36 (0.33)	0.35 (0.35)	0.36 (0.34)
Trp, %	0.14 (0.14)	0.12 (0.12)	0.12 (0.13)	0.12 (0.12)	0.11 (0.12)	0.11 (0.13)	0.11 (0.12)
Thr, %	0.49 (0.49)	0.47 (0.44)	0.47 (0.44)	0.47 (0.45)	0.39 (0.36)	0.39 (0.42)	0.39 (0.37)
Ile, %	0.53 (0.53)	0.27 (0.26)	0.27 (0.26)	0.31 (0.31)	0.30 (0.31)	0.28 (0.29)	0.30 (0.30)
Leu, %	1.32 (1.33)	1.55 (1.43)	1.55 (1.44)	1.55 (1.44)	0.99 (0.94)	0.96 (0.97)	0.99 (0.96)
Val, %	0.63 (0.64)	0.77 (0.74)	0.77 (0.73)	0.77 (0.74)	0.40 (0.41)	0.40 (0.40)	0.40 (0.42)
TD Lys, %	0.54	0.62	0.62	0.62	0.52	0.52	0.52
TD Ile, %	0.47	0.24	0.24	0.28	0.26	0.24	0.26
TD Val, %	0.55	0.71	0.71	0.71	0.35	0.35	0.35
TD Leu, %	1.20	1.45	1.45	1.45	0.91	0.88	0.91
Ca, %	0.60	0.60	0.60	0.60	0.60	0.60	0.60
P, %	0.50	0.50	0.50	0.50	0.50	0.50	0.50

<sup>a</sup>EXP = experiment; C-BC = corn blood cell; C-SBM = corn soybean meal; TD = true digestible.

<sup>b</sup>AP 301G, American Protein Corp., St. Louis, MO. The analyzed AA values on a percentage as-fed basis were: Lys, 8.47; Trp, 1.65; Thr, 3.78; Ile, 0.26; Met+Cys, 1.62; Leu, 12.55; Val, 8.25; Phe+Tyr, 9.17; His, 6.37; and Arg, 3.49.

<sup>c</sup>Fat Pak 100, Milk Specialties Co., Dundee, IL.

<sup>d</sup>Provides the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, 0.3 mg selenium as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite, respectively with calcium carbonate as the carrier.

<sup>e</sup>Provides the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D<sub>3</sub>, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex) 6.2 mg; riboflavin, 10 mg; Ca-d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B<sub>12</sub>, 45 µg; d-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamin, 3.31 mg; and vitamin C, 83 µg.

<sup>f</sup>Amino acid values for corn and soybean meal (NRC, 1998) are on a total and TD basis using digestibility coefficients from NRC, 1998. Blood cells were analyzed for total AA values and true digestibility coefficients of 97.1, 95.4, 95.9, and 96.3% were used for Ile, Leu, Val, and Lys, respectively (Parr et al., 2003). Analyzed values of diets are shown in parentheses.

2000; Method 982.30 E(b)). Tryptophan content was determined after alkaline hydrolysis (AOAC, 2000; Method 982.30 E(c)). Treatment diets in mash form and water were provided *ad libitum* throughout all EXP.

#### Experiment 1

Experiment 1 was conducted to validate a C-BC basal diet to subsequently be used in the Ile requirement studies. Sixty barrows with average initial and final body weight (BW) of  $93.3 \pm 0.1$  and  $113.9 \pm 1.3$  kg were used. The three dietary treatments (Table 5.1) were: 1) C-SBM, 2) C-BC diet containing 5% blood cells, or 3) C-BC with 0.26% supplemental Ile (C-BC+Ile).

Barrows were ultrasounded for 10<sup>th</sup> rib backfat thickness and loin muscle (LM) area at the beginning and end of the 28 d growth experiment. On d 14, pigs receiving the C-BC diet were taken off experiment due to a decrease in average daily feed intake (ADFI).

#### Experiment 2

Experiment 2 was conducted to estimate the Ile requirement using PUN as the response variable (Knowles et al., 1997). Eighty barrows with average initial and final BW of  $82.5 \pm 0.4$  kg and  $84.3 \pm 0.7$  kg were used. At the initiation of the experiment, all pigs received the same late-finishing diet for 3 d and then were bled at 0900 the following d. They were then weighed and allotted to treatment. The C-BC diet containing 0.24% TD Ile (Table 5.1) was supplemented with increments of 0.02% crystalline L-Ile to provide treatment diets containing 0.24, 0.26, 0.28, 0.30, or 0.32% TD Ile. Pigs remained on treatment diets for 7 d and were bled and weighed at 0900 on d 10.

Blood was collected via the anterior vena cava and placed in 4-mL tubes (Monoject, Sherwood Medical, St. Louis, MO) containing 10.0 mg of sodium fluoride and 8.0 mg of potassium oxalate. Samples were placed on ice before centrifugation at 1,500  $\times g$  at 4°C for 20 min. Plasma was collected after centrifugation and samples were frozen until analysis for plasma urea nitrogen (PUN) by the methods of Laborde et al. (1995).

### Experiment 3

Experiment 3 was conducted to estimate the TD Ile requirement using growth performance and carcass traits. Eighty barrows with average initial and final BW of  $85.3 \pm 0.1$  and  $117.8 \pm 1.8$  kg were used. Treatments were the C-BC diet containing 0.28% TD Ile (Table 5.1) supplemented with increments of 0.02% crystalline L-Ile to provide treatment diets containing 0.28, 0.30, 0.32, 0.34, or 0.36% TD Ile. The growth trial lasted for 32 or 60 d depending on slaughter date.

On d 32, three pigs per pen from the two heaviest replications and on d 60, three pigs per pen from the two remaining replications were randomly selected and killed by exsanguination after electrical stunning at the LSU Agricultural Center Meats Laboratory. Linear carcass measurements and values from total body electrical conductivity (TOBEC; Model MQI-27; Meat Quality Inc., Springfield, IL) were determined as described by Matthews et al. (2001a). Fat-free lean and percentage fat-free lean were determined by NPPC (2000) equations, which compensate for unequal BW.

### Experiment 4

Experiment 4 was conducted to determine if a C-SBM diet, seemingly deficient in Ile, would respond to dietary supplementation of Ile. Forty-eight

barrows with average initial and final BW of  $81.0 \pm 0.1$  and  $117.3 \pm 1.5$  kg were used. Pigs were fed a C-SBM diet containing 0.26% TD Ile (Table 5.1) supplemented with increments of 0.05% crystalline L-Ile to provide treatments of 0.26, 0.31, or 0.36% TD Ile. The growth trial lasted for 47 d. Pigs and the feeders were weighed on d 29 and d 47 for calculation of ADFI, average daily gain (ADG), and gain:feed (G:F).

#### Experiment 5

Experiment 5 was conducted to determine the TD Ile requirement in pigs fed a C-SBM diet. Eighty barrows with average initial and final BW of  $80.7 \pm 0.1$  and  $113.0 \pm 1.3$  kg were used. The basal diet was a C-SBM diet containing 0.24 TD Ile (Table 5.1) which was subsequently supplemented with increments of 0.03% crystalline L-Ile to provide treatment diets containing 0.24, 0.27, 0.30, 0.33, or 0.36% TD Ile. The growth trial lasted 42 or 49 d depending on slaughter date. Carcass data were collected as in EXP 3. Pigs were bled at 0900 via the anterior vena cava on d 42. Handling of the blood and analysis of PUN were the same as in EXP 2.

#### Experiment 6

Experiment 6 was conducted to determine the effect of Val + Leu addition to a C-SBM diet. Thirty-two barrows with average initial and final BW of  $88.3 \pm 0.2$  and  $113.5 \pm 1.4$  kg were used. Pigs were fed a C-SBM diet containing 0.26% TD Ile (Table 5.1) with or without 0.45% crystalline Leu and 0.31% crystalline Val. The addition of Leu + Val resulted in an Ile:Leu + Val that was the same as in the C-BC diet containing 0.28% TD Ile. The growth trial lasted for 38 d.

## Statistical Analysis

Data were analyzed by ANOVA procedures using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with treatments arranged in a randomized complete block design. The statistical model included treatment and replication for all six EXP. Initial PUN was used as a covariate in the analysis of d-10 PUN for EXP 2. In EXP 2, 3, 4, and 5, orthogonal contrasts were used to determine linear and quadratic effects of TD Ile. The two-slope NLIN procedure of SAS (break point analysis) was used to estimate a requirement based on G:F in EXP 3 (Robbins et al., 1979). Treatment differences were considered significant at  $\alpha = 0.10$ . The pen of pigs was the experimental unit for all data.

## **RESULTS**

In the diet validation experiment, EXP 1, pigs receiving the C-BC diet were taken off EXP on d 14 due to a severe decrease in ADFI, ADG, and G:F ( $P < 0.001$ ; Table 5.2). Gain:feed was increased ( $P = 0.10$ ) in pigs fed C-BC+Ile at d 14 compared with either pigs fed the C-SBM or C-BC diets. Feed intake, ADG, G:F, 10th rib backfat, and LM area were not different for pigs fed C-SBM or C-BC+Ile for the overall 28-d period.

In EXP 2 (Table 5.3), ADFI, ADG, ( $P < 0.02$ ) and G:F ( $P < 0.08$ ) were linearly increased as crystalline TD Ile was increased incrementally from 0.24 to 0.32%. Plasma urea N was not affected by Ile level.

In EXP 3 (Table 5.4), final BW, ADG, ADFI, and G:F were increased linearly ( $P < 0.01$ ) as dietary TD Ile was increased in the diet. There was no significant quadratic effect; however, break point analysis of G:F estimated the TD Ile requirement at 0.34%. There were no effects of TD Ile level on dressing percentage, 10th rib fat depth, average backfat, LM area, or percent muscle; however, carcass length ( $P < 0.08$ ) and

Table 5.2. Growth performance, 10th rib fat, and loin muscle area of barrows fed two levels of isoleucine in a corn-blood cell diet in Experiment 1<sup>a</sup>

Item	C-SBM <sup>b</sup>	C-BC		SEM
		0.24% TD Ile <sup>b</sup>	0.50% TD Ile <sup>b</sup>	
Initial BW, kg	93.3	93.3	93.4	0.1
Final BW, kg	112.9	na <sup>h</sup>	114.8	1.3
Day 0 to 14				
ADG, kg	0.581 <sup>c</sup>	0.154 <sup>d</sup>	0.701 <sup>c</sup>	0.050
ADFI, kg	2.45 <sup>c</sup>	1.50 <sup>d</sup>	2.37 <sup>c</sup>	0.10
G:F, kg/kg	0.236 <sup>e</sup>	0.101 <sup>f</sup>	0.293 <sup>g</sup>	0.021
Day 14 to 28				
ADG, kg	0.820	na <sup>h</sup>	0.831	0.057
ADFI, kg	2.69	na <sup>h</sup>	2.80	0.12
G:F, kg/kg	0.304	na <sup>h</sup>	0.298	0.015
Day 0 to 28				
ADG, kg	0.700	na <sup>h</sup>	0.765	0.048
ADFI, kg	2.56	na <sup>h</sup>	2.58	0.10
G:F, kg/kg	0.272	na <sup>h</sup>	0.295	0.010
10 <sup>th</sup> rib fat, cm				
Day 0	1.65	1.72	1.70	0.10
Day 28	2.08	na <sup>h</sup>	2.11	0.09
LM area, cm <sup>2</sup>				
Day 0	34.45	36.62	34.71	0.95
Day 28	42.09	na <sup>h</sup>	41.48	0.71

<sup>a</sup>Data are the means of five replicates with four pigs per replicate pen. Data for ADFI and G:F are on an as fed-basis.

<sup>b</sup>C-SBM = corn soybean meal, C-BC = corn blood cell diet; TD = true digestible.

<sup>c,d</sup>Means with different superscripts differ ( $P < 0.001$ ).

<sup>e,f,g</sup>Means with different superscripts differ ( $P < 0.10$ ).

<sup>h</sup>na= data not available. On d 14, pigs receiving the C-BC (0.24% Ile diet) were removed from the EXP due to a decrease in ADFI; thus no data are available for that treatment.

Table 5.3. Growth performance and plasma urea nitrogen values of barrows fed varying levels of isoleucine in a corn-blood cell diet in Experiment 2<sup>a</sup>

Item	True ileal digestible isoleucine					SEM
	0.24%	0.26%	0.28%	0.30%	0.32%	
Ile intake g/d	4.61	4.78	5.88	6.93	7.74	---
Ile intake mg/g BW gain	25.90	31.66	27.87	22.50	16.06	---
Initial BW, kg	81.8	82.2	82.6	83.5	82.4	0.4
Final BW, kg <sup>b</sup>	83.0	83.2	84.0	85.7	85.8	0.7
Growth performance						
ADG, kg <sup>b</sup>	0.178	0.151	0.211	0.308	0.482	0.101
ADFI, kg <sup>b</sup>	1.92	1.84	2.10	2.31	2.42	0.16
G:F, kg/kg <sup>c</sup>	0.073	0.086	0.095	0.126	0.201	0.049
Plasma						
Day 3 urea N, mmol/L	3.37	2.91	3.48	2.92	3.34	0.22
Day 10 urea N, mmol/L	2.76	2.87	2.79	2.98	2.77	0.21

<sup>a</sup>Data are the means of four replicates with four pigs per replicate pen. Growth performance was measured from d 3 to d 10. Pigs were bled on d 3 and d 10 and plasma urea nitrogen values from the initial bleed were used as a covariate in the analysis of final plasma urea nitrogen. Data for ADFI and G:F are on an as fed-basis.

<sup>b</sup>Linear effect (P < 0.02).

<sup>c</sup>Linear effect (P < 0.08).



Table 5.4. Growth performance of barrows fed varying levels of isoleucine in a corn-blood cell diet in Experiment 3<sup>a</sup>

Item	True ileal digestible isoleucine					SEM
	0.28%	0.30%	0.32%	0.34%	0.36%	
Ile intake g/d	7.42	8.34	9.28	10.23	11.52	---
Ile intake mg/g BW gain	12.51	12.30	12.78	12.90	13.68	---
Initial BW, kg	85.3	85.2	85.3	85.3	85.4	0.1
Final BW, kg <sup>b</sup>	110.5	116.6	118.0	120.6	123.2	1.8
Growth performance						
ADG, kg <sup>b, c</sup>	0.593	0.678	0.726	0.793	0.842	0.031
ADFI, kg <sup>b, c</sup>	2.65	2.78	2.90	3.01	3.20	0.09
G:F, kg/kg <sup>b, c</sup>	0.223	0.245	0.250	0.263	0.263	0.007

<sup>a</sup>Data are the means of four replicates with four pigs per replicate pen. Growth performance was measured for 32 or 60 d depending on processing date. Data for ADFI and G:F are on an as fed-basis.

<sup>b</sup>Linear effect ( $P < 0.001$ ).

<sup>c</sup>One-slope break point analysis estimated a requirement of  $0.34 \pm 0.01\%$  for G:F.

kilograms of fat-free lean ( $P < 0.002$ ) were increased linearly as TD Ile level increased (Table 5.5). Fat-free lean from the TOBEC analysis was increased linearly ( $P < 0.002$ ). There was no effect of TD Ile on TOBEC estimates of total fat, percent lean, or the ratio of lean to fat.

In EXP 4 (Table 5.6), Ile addition to the C-SBM diet containing 0.26% Ile did not affect ADFI or ADG, but G:F was increased linearly ( $P < 0.02$ ) with the response primarily to the 0.31% TD Ile level.

Increasing TD Ile in the C-SBM basal diet (0.24% TD Ile) did not affect final BW, ADFI, ADG, G:F, or PUN (EXP 5, Table 5.7). Similarly, Ile supplementation did not affect dressing percent, carcass length, 10th rib fat depth, LM area, fat-free lean or TOBEC estimates of kilograms of lean or percent lean (Table 5.8). Average backfat and TOBEC total fat and percent fat were increased ( $P < 0.10$ ) in a quadratic manner by Ile supplementation. Because fat content increased with no change in leanness, lean:fat was decreased as dietary TD Ile was increased (quadratic,  $P < 0.10$ ).

In EXP 6 (Table 5.9), Leu + Val addition to a C-SBM diet to create a ratio of Ile:Leu + Val identical to that in the 0.28% TD Ile C-BC diet did not affect final BW, ADFI or ADG. However, G:F was increased ( $P < 0.09$ ) when crystalline Leu and Val were added to the diet.

## **DISCUSSION**

The first step in evaluating an AA requirement is to develop a diet that is limiting only in the AA that is of interest. Furthermore, when surfeit amounts of an AA are supplemented, the diet should support optimal rate and efficiency of growth compared with a practical diet. Finally, in considering requirements for finishing pigs, the diet

Table 5.5. Carcass measurements of barrows fed varying levels of isoleucine in a corn-blood cell diet in Experiment 3<sup>a</sup>

Item	True ileal digestible isoleucine					SEM
	0.28%	0.30%	0.32%	0.34%	0.36%	
Final BW, kg <sup>b</sup>	110.6	118.5	120.6	120.4	123.2	2.2
Carcass lean and fat measurements						
Loin muscle area, cm <sup>2</sup>	46.88	48.01	49.17	47.93	49.80	1.33
Tenth rib $\frac{3}{4}$ backfat, cm	1.87	1.98	1.96	1.81	2.01	0.13
Average backfat, cm	2.56	2.77	2.71	2.72	2.77	0.10
Carcass length, cm <sup>c</sup>	82.76	82.87	83.82	83.61	84.56	0.71
Dressing percentage, %	74.41	74.23	74.05	73.82	74.93	0.51
Fat-free lean, % <sup>d</sup>	54.54	53.75	53.97	54.30	53.53	0.87
Fat-free lean, kg <sup>b, d</sup>	44.82	47.24	48.18	48.32	49.50	0.72
Carcass TOBEC analysis using carcass equations <sup>e</sup>						
Fat-free lean, kg <sup>b</sup>	42.14	43.38	44.77	45.63	46.68	0.74
Percentage lean, %	51.31	49.43	50.28	51.34	50.64	0.95
Total fat, kg	23.55	26.61	25.95	24.51	26.66	1.32
Percentage fat, %	28.48	30.07	28.88	27.51	28.84	0.94
Lean:fat, kg:kg	1.83	1.67	1.80	1.89	1.80	0.09

<sup>a</sup>Data are the means of four replicates with three pigs per replicate pen. TOBEC = total body electrical conductivity.

<sup>b</sup>Linear effect ( $P < 0.002$ ).

<sup>c</sup>Linear effect ( $P < 0.08$ ).

<sup>d</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>e</sup>Calculated using TOBEC analysis with equations from Higbie et al. (2002).

Table 5.6. Growth performance of barrows fed varying levels of isoleucine in a corn-soybean meal diet in Experiment 4<sup>a</sup>

Item	C-SBM	C-SBM	C-SBM	SEM
	0.26% TD Ile <sup>b</sup>	0.31% TD Ile <sup>b</sup>	0.36% TD Ile <sup>b</sup>	
Ile intake g/d	9.00	10.70	12.13	---
Ile intake mg/g BW gain	11.87	13.61	15.71	---
Initial BW, kg	81.1	81.0	81.0	0.1
Final BW, kg	116.7	117.9	117.2	1.5
Day 0 - 29				
ADG, kg	0.790	0.808	0.789	0.032
ADFI, kg	3.52	3.59	3.41	0.09
G:F, kg/kg	0.224	0.225	0.232	0.004
Day 29 - 47				
ADG, kg	0.706	0.752	0.743	0.043
ADFI, kg	3.28	3.23	3.29	0.15
G:F, kg/kg	0.215	0.233	0.225	0.006
Day 0 - 47				
ADG, kg	0.758	0.786	0.772	0.032
ADFI, kg	3.46	3.45	3.37	0.11
G:F, kg/kg <sup>c</sup>	0.219	0.228	0.229	0.002

<sup>a</sup>Data are the means of four replicates with four pigs per pen. Average weight of barrows on d 29 was 104.3 kg. Data for ADFI and G:F are on an as fed-basis.

<sup>b</sup>C-SBM = corn soybean meal; TD = true digestible.

<sup>c</sup>Linear effect of Ile level ( $P < 0.02$ ).

Table 5.7. Growth performance and plasma urea nitrogen of barrows fed varying levels of isoleucine in a corn-soybean meal diet in Experiment 5<sup>a,b</sup>

Item	True ileal digestible isoleucine					SEM
	0.24%	0.27%	0.30%	0.33%	0.36%	
Ile intake g/d	6.84	7.72	8.79	10.26	10.58	---
Ile intake mg/g BW gain	10.03	10.81	12.72	13.57	15.07	---
Initial BW, kg	80.7	80.6	80.8	80.8	80.8	0.1
Final BW, kg	111.6	113.0	112.2	115.3	112.7	1.3
Growth performance						
ADG, kg	0.682	0.714	0.691	0.756	0.702	0.026
ADFI, kg	2.85	2.86	2.93	3.11	2.94	0.10
G:F, kg/kg	0.241	0.250	0.236	0.243	0.239	0.006
Plasma						
Urea N, mmol/L	1.02	0.93	0.85	1.02	1.04	0.12

<sup>a</sup>Data are the means of four replicates with four pigs per pen. Growth performance was measured for 42 or 49 d depending on processing date. Data for ADFI and G:F are on an as fed-basis.

<sup>b</sup>No treatment effects ( $P > 0.10$ ).

Table 5.8. Carcass measurements of barrows fed varying levels of isoleucine in a corn-soybean meal diet in Experiment 5<sup>a</sup>

Item	True ileal digestible isoleucine					SEM
	0.24%	0.27%	0.30%	0.33%	0.36%	
Final BW, kg	113.3	116.0	113.9	116.1	114.3	1.4
Carcass lean and fat measurements						
Loin muscle area, cm <sup>2</sup>	43.51	42.28	40.90	43.54	41.24	0.98
Tenth rib $\frac{3}{4}$ backfat, cm	1.89	1.97	1.85	2.07	1.83	0.11
Average backfat, cm <sup>b</sup>	2.69	3.01	2.86	2.97	2.85	0.08
Carcass length, cm	83.29	83.71	83.40	83.40	83.29	0.54
Dressing percentage, %	74.62	74.24	74.28	74.88	74.59	0.34
Fat-free lean, % <sup>c</sup>	53.32	52.52	52.81	52.22	52.90	0.69
Fat-free lean, kg <sup>c</sup>	45.03	45.19	44.67	45.41	45.09	0.60
Carcass TOBEC analysis using carcass equations <sup>d</sup>						
Fat-free lean, kg	43.61	43.06	43.11	43.58	43.97	0.66
Percentage lean, %	51.71	50.02	50.97	50.15	51.56	0.77
Total fat, kg <sup>b</sup>	24.32	25.91	24.85	26.57	24.15	0.78
Percentage fat, % <sup>b</sup>	28.60	30.08	29.37	30.55	28.33	0.75
Lean:fat, kg:kg <sup>b</sup>	1.87	1.68	1.75	1.65	1.84	0.08

<sup>a</sup>Data are the means of four replicates with three pigs per pen. TOBEC = total body electrical conductivity.

<sup>b</sup>Quadratic effect ( $P < 0.10$ ).

<sup>c</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>d</sup>Calculated using TOBEC analysis with equations from Higbie et al. (2002).

Table 5.9. Growth performance of barrows fed diets with two different ratios of isoleucine:leucine + valine in Experiment 6<sup>a</sup>

Item	C-SBM	C-SBM	SEM
	0.26% TD Ile <sup>b</sup>	+Leu+Val <sup>b</sup>	
Initial BW, kg	88.5	88.0	0.2
Final BW, kg	111.7	115.2	1.4
Growth Performance			
ADG, kg	0.610	0.718	0.037
ADFI, kg	2.79	2.93	0.12
G:F, kg/kg <sup>c</sup>	0.218	0.245	0.007

<sup>a</sup>Data are means of four replicates with four pigs per pen. Data for ADFI and G:F are on an as fed-basis.

<sup>b</sup>C-SBM = corn soybean meal; TD = true digestible.

<sup>c</sup>Treatment effect ( $P < 0.09$ ).

should also result in carcass composition similar to pigs fed a typical diet. In EXP 1, pigs fed the C-BC diet formulated to be deficient in Ile had decreased ADG and G:F due to a decrease in ADFI. The addition of Ile restored feed intake and growth to a level equal to that of pigs fed the C-SBM positive control (PC). Moreover, evaluations of 10th rib fat thickness and LM area of pigs fed the C-BC diet with supplemental Ile was not different from pigs fed the PC. The validation of our experimental diet agrees with previous research that suggests a corn-based diet containing 5% red blood cells is deficient in Ile, and that it will support optimal growth and carcass composition of 80- to 120- kg barrows when surfeit Ile is supplied (Parr et al., 2004).

The use of PUN as a response variable has been shown to be a quick and effective means of evaluating an AA requirement for pigs at various stages of growth (Knowles et al., 1997; Guzik et al., 2002). The objective of EXP 2 was to estimate the Ile requirement at the beginning of the 80- to 120- kg growth period by using PUN as the response criteria. However, a linear increase in feed intake led to similar PUN values among pigs fed TD Ile levels from 0.24 to 0.32%. Parr et al. (2004) used feed intake as a covariate in the analysis of PUN and reported results that were inconclusive. Consequently, we feel that PUN is most likely not a good indicator of an AA requirement when feed intakes are not similar among treatments. Although pigs only received the treatment diets for 7 d in EXP 2, the magnitude of change in feed intake as Ile was increased seems significant in evaluation of the requirement and led us to increase our range of Ile levels for EXP 3.

The failure of a requirement estimate from PUN led us to conduct an EXP evaluating the TD Ile requirement of finishing barrows using growth and carcass



measurements as response criteria. Again, we observed linear effects of TD Ile on ADFI, and subsequently ADG and G:F were increased as TD Ile was increased from 0.28 to 0.36%. Objective requirement estimates based on G:F using two-slope break point analysis resulted in a requirement of 0.34% TD Ile. However, numerically ADFI and ADG were highest for pigs fed the 0.36% TD Ile diet. Our estimate of 0.34% TD Ile is higher than the 0.30% estimate of NRC (1998) and Parr et al. (2004), but agrees closely with the suggestion of Kendall et al. (2004b) that the TD Ile requirement is approximately 0.36%. The carcass responses of an increase in carcass length and kilograms of lean in EXP 3 are likely the result of an increase in final BW due to increased feed intake and growth. There were no significant differences among measurements of carcass composition when lean and fat were expressed as a percentage of the carcass weight.

Our higher than expected requirement estimate of 0.34% TD Ile in a C-BC diet suggested that a C-SBM diet formulated with crystalline sources of the first three limiting AA (Lys, Thr, and Trp) should respond to additions of Ile. Experiment 4 was conducted to compare the response of Ile in a C-SBM diet to the response previously observed in a C-BC diet. Gain:feed was increased when TD Ile was increased from 0.26% to 0.31% with no further improvement to the 0.36% level. Previous EXP with the C-BC diet indicated we would see a reduction in ADFI in a diet containing 0.26% TD Ile. Several researchers have observed feed intake responses to dietary supplementation of Ile (Parr et al., 2003; Kendall et al., 2004b; Kerr et al., 2004b). However in EXP 4, feed intake was numerically the highest in the C-SBM diet that contained 0.26% TD Ile with

all pigs having high feed consumptions (> 3.3 kg ADFI). The data from this EXP suggested that the TD Ile requirement was between 0.26% and 0.31% in a C-SBM diet.

Experiment 5 was conducted to more thoroughly evaluate the TD Ile requirement of late-finishing barrows fed a C-SBM diet. Data from EXP 4 indicated that G:F was improved when TD Ile was increased from 0.26% to 0.31%. In EXP 5, there were no significant differences in growth performance when levels of TD Ile ranged from 0.24 to 0.36%. A quadratic response of average backfat, and TOBEC estimates for total fat and percentage fat seem to be related to differences in the final BW of pigs within level of Ile supplementation. Measures of lean did not differ due to Ile supplementation. The results of this EXP do not agree with the results of EXP 4, which suggested a TD Ile requirement greater than 0.26% TD Ile for maximizing G:F in a C-SBM diet. The results of this EXP also do not agree with the results of EXP 3, which suggested a TD Ile requirement of at least 0.34% in pigs fed a C-BC diet.

A review of the literature on the Ile requirement of pigs (Table 5.10) resulted in eight requirement estimates from peer reviewed journal articles (Becker et al., 1963; Oestemer et al., 1973; Taylor et al., 1985; Parr et al., 2003; Kerr et al., 2004b; Parr et al., 2004), four estimates from experiment station reports (Bergstrom et al., 1996; James et al., 2001), and two estimates from abstracts (Lenis et al., 1997; Kendall et al., 2004b). Researchers used various techniques to estimate plateaus and break points in these studies. Thus, to evaluate data from these EXP more consistently, break point analysis was performed on treatment means to estimate requirements when the authors used some other statistical method. Because many of these EXP were based on total Ile rather than on TD, a digestibility coefficient of 88%

Table 5.10. Isoleucine requirement estimates of growing pigs

BW, kg			Growth performance			Isoleucine				Reference
Mean	Initial	Final	ADG, g	ADFI, g	G:F	Total, % <sup>a</sup>	TD, % <sup>b</sup>	TD, g/d <sup>c</sup>	mg/g ADG <sup>d</sup>	
7.0	5.6	8.4	236	257	0.92	0.78	0.69	1.77	7.50	James et al., 2001 <sup>e</sup>
7.0	5.6	8.4	212	252	0.84	0.70	0.62	1.56	7.36	James et al., 2001 <sup>f</sup>
8.3	6.6	9.9	255	358	0.71	0.69	0.61	2.18	8.55	Kerr et al., 2004b <sup>g</sup>
8.4	5.1	11.6	205	340	0.60	0.78	0.69	2.35	11.46	Becker et al., 1963 <sup>h</sup>
8.8	6.6	10.9	313	409	0.77	0.74	0.65	2.66	8.50	Kerr et al., 2004b <sup>i</sup>
10.8	5.8	15.7	385	623	0.62	0.48	0.43	2.68	6.96	Oestemer et al., 1973 <sup>j</sup>
17.5	11.4	23.5	435	972	0.45	0.42	0.37	3.60	8.29	Bergstrom et al., 1996 <sup>k</sup>
17.5	11.4	23.5	578	994	0.58	0.63	0.55	5.47	9.46	Bergstrom et al., 1996 <sup>l</sup>
29.0	18.0	40.0	685	1250	0.55	0.57	0.50	6.25	9.12	Lenis et al., 1997 <sup>m</sup>
34.7	27.0	42.3	727	1468	0.50	0.60	0.53	7.78	10.70	Parr et al., 2003 <sup>n</sup>
40.0	25.0	55.0	624	1596	0.39	0.43	0.38	6.06	9.71	Taylor et al., 1985 <sup>o</sup>
52.9	44.6	61.1	600	1775	0.34	0.39	0.34	6.04	10.07	Becker et al., 1963 <sup>p</sup>
93.0	87.0	99.0	736	1636	0.45	0.35	0.31	5.07	6.89	Parr et al., 2004 <sup>q</sup>
103.5	91.0	116.0	1210	3620	0.34	0.42	0.37	13.39	11.07	Kendall et al., 2004b <sup>r</sup>
									8.97	Overall mean

<sup>a</sup>When Ile values were not given on a total basis, values were calculated from true ileal digestible (TD) Ile by dividing by 0.88 or from the apparent digestible Ile by dividing by 0.80.

<sup>b</sup>When possible, digestibility values were taken from the articles. If they were not reported, a digestibility value of 88% was used to estimate TD Ile.

<sup>c</sup>Calculated grams of TD Ile consumed per d.

<sup>d</sup>Calculated milligrams of Ile required for one gram of BW gain.

<sup>e</sup>James et al. (2001). Diet contained 1.26% apparent digestible Lys. Break point analysis requirement estimate for ADG was 0.69% TD Ile.

<sup>f</sup>James et al. (2001). Diet contained 1.00% apparent digestible Lys. Break point analysis requirement estimates were 0.62, 0.62, and 0.61% TD Ile for ADG, ADFI, and G:F, respectively.

(Table 5.10 continued)

<sup>g</sup>Kerr et al. (2004b). Kerr indicated a requirement of 0.69% TD Ile and 9.9 mg TD Ile/gram of BW gain. Break point analysis estimates the requirement at 0.61% for both ADG and G:F.

<sup>h</sup>Becker et al. (1963). Experiment 1, break point analysis estimates the requirement at 0.68 and 0.70% for ADG and G:F, respectively. The average requirement is 0.69% TD Ile.

<sup>i</sup>Kerr et al. (2004b). Kerr indicated a requirement of 0.70% TD Ile and 9.1 mg TD Ile/gram of BW gain. Break point analysis estimates the requirement at 0.67 and 0.62% for ADG and G:F, respectively. The average requirement is 0.65% TD Ile.

<sup>j</sup>Oestemer et al. (1973). Break point analysis estimated the requirement at 0.44% for ADG and 0.42% TD Ile for G:F. The average requirement is 0.43% TD Ile.

<sup>k</sup>Bergstrom et al. (1996). Experiment 2, break point analysis estimates for pigs fed 0.75% TD Lys were 0.38 and 0.36% TD Ile for ADG and ADFI, respectively.

<sup>l</sup>Bergstrom et al. (1996). Experiment 2, break point analysis estimates for pigs fed 1.10% TD Lys were not reasonable. Performance was improved to a level of 0.55% TD Ile, with no subsequent further improvement for ADG or ADFI.

<sup>m</sup>Lenis et al. (1997). Data taken from Kerr et al. (2004).

<sup>n</sup>Parr et al. (2003). Experiment 2, break point analysis estimated requirements of 0.55 and 0.51% TD Ile for ADG and ADFI, respectively. The average requirement is 0.53% TD Ile.

<sup>o</sup>Taylor et al. (1985). Break point analysis estimated requirements of 0.41 and 0.34% TD Ile for ADG and G:F, respectively. The average requirement is 0.38% TD Ile.

<sup>p</sup>Becker et al. (1963). Experiment 4, break point analysis estimated requirements of 0.35 and 0.33% TD Ile for ADG and G:F, respectively. Data based on only four pigs per treatment.

<sup>q</sup>Parr et al. (2004). Experiment 2, break point analysis estimated the requirement at 0.31% TD Ile for ADG.

<sup>r</sup>Kendall et al. (2004b). Break point analysis estimated requirements of 0.36, 0.37, and 0.39% TD Ile for ADG, ADFI, and G:F, respectively. The average requirement was 0.37% TD Ile.

was used to calculate a TD Ile value when none was reported or when it was impossible to calculate the value from dietary ingredients. Growth performance values of pigs at or above the estimated requirement were used to calculate Ile intake and the milligrams of Ile required for 1 g of ADG. The average estimate from Table 5.10 is 8.97 mg Ile/g ADG. Kerr et al. (2004b) recently reported a similar review of the Ile requirement using some of the same data and had a slightly lower estimate of 8.69 mg Ile/g ADG.

When calculations were made to compare the milligrams of Ile needed per gram of BW gain in our C-SBM diet, the lowest value was 10.03 mg/g ADG for the diet containing 0.24% TD Ile, and pigs fed this diet had growth performance similar to pigs fed higher levels of Ile supplementation, suggesting that the 10.03 mg/g was above the requirement. Based on our review of the literature, it does not seem likely that Ile would become limiting in a C-SBM diet for late-finishing barrows unless feed intake is restricted or genetic growth potential is extremely high. An estimate of 8.97 mg Ile/g ADG from our review seems reasonable based on a lack of response to levels above that in our studies with C-SBM diets and estimates from the existing literature (Table 5.10). Kerr et al. (2002) recently reviewed the Lys requirement and estimated that 17.89 mg of Lys/g ADG was needed for optimal performance. With the use of this estimate for the Lys requirement and our review estimate for Ile, the Ile:Lys ratio is 0.50. Based on the feed intake and growth performance data of pigs in EXP 5, their TD Lys requirement would be 0.43% with a TD Ile requirement of 0.22%. Without creating a purified diet, there is no known combination of typical feed ingredients, other than red blood cells, that would allow for a response to Ile. In EXP 3 in the C-BC diet, 12.90 mg Ile/g ADG was needed to obtain optimum performance, which is much higher than was needed in

pigs fed the C-SBM diet. Based on the performance of these pigs and the Lys requirement estimate of Kerr et al. (2002), they would have required a diet containing 0.47% TD Lys. Thus, the required ratio of Ile:Lys in a diet containing 5% red blood cells would be 0.72, which seems to be an unreasonably high estimate to apply to diets that do not contain red blood cells.

Our relatively high estimate of the TD Ile requirement of late-finishing barrows when using the C-BC diet may be a result of the BCAA balance. Red blood cells are extremely high in Leu (12.55%) and Val (8.25%), but comparatively low in Ile (0.26%). Numerous researchers have suggested an antagonism amongst the BCAA (Tannous et al., 1966; Allen and Baker, 1972; Edmonds and Baker, 1987). Papet et al. (1988) observed that excess Leu increases the activities of branched-chain aminotransferase (BCAT) in the liver and jejunum and the branched-chain keto-acid deaminase (BCKAD) in the jejunum in preruminant lambs. Other researchers have suggested that an increased metabolic oxidation of Ile might occur when dietary levels of Leu are high (Tannous et al., 1966; Calvert et al., 1982; Edmonds and Baker, 1987). Edmonds and Baker (1987) demonstrated that plasma AA concentrations of Ile and Val were reduced when Leu was supplied in excess of 1 to 6%. It may be that the use of a C-BC diet overestimates the Ile requirement because of these metabolic conditions.

The calculated composition of our 0.26% TD Ile C-BC diet contained 0.54% more TD Leu than the C-SBM diet used in EXP 4 and 5. This resulted in Leu:Ile ratios of 5.58 and 3.51 for the 0.26% TD Ile C-BC and C-SBM diets (Table 5.11), respectively. Barbour et al. (1992) reported no increase in the Ile requirement of broiler chicks when Leu and Val were increased in the diet. However, their proposed imbalanced diet had a

Table 5.11. Branched chain amino acid comparison of corn-soybean meal and corn-blood cell diets<sup>a,b</sup>

Item	C-BC	C-BC	C-BC	C-SBM	C-SBM
	0.26% TD	0.36% TD	0.50% TD	0.26% TD	0.36% TD
	Ile	Ile	Ile	Ile	Ile
Ile:Lys <sup>c</sup>	0.42	0.58	0.81	0.50	0.69
Leu:Ile	5.58	4.03	2.90	3.51	2.52
Val:Ile	2.73	1.96	1.41	1.35	0.97
Leu+Val:Ile	8.31	5.99	4.31	4.86	3.49
Ile:Leu+Val	0.12	0.17	0.23	0.21	0.29

<sup>a</sup>C-BC = corn blood cell; C-SBM = corn soybean meal; TD = true digestible.

<sup>b</sup>Lysine and branched chain AA ratios are calculated from true ileal digestible values.

<sup>c</sup>True ileal digestible lysine values were 0.62% and 0.52% for the C-BC and C-SBM diets, respectively.

Leu:Ile ratio of 3.6, which is only slightly higher than in the C-SBM diets we used in EXP 4 and 5. The ratio of Leu:Ile in our 0.36% TD Ile C-BC diet was 4.03, which is approaching the range calculated for our C-SBM diets. The Leu:Ile ratio where performance starts to become impaired and the requirement for Ile increases in the pig is not known, and may vary depending on pig weight, genotype, and environment. Thus, it is difficult to determine whether or not our C-BC diet was responding to a deficiency of Ile or responding to a correction of the BCAA balance.

Experiment 6 was conducted to determine the effect of simulating the BCAA balance of the C-BC diet containing 0.28% TD Ile in a C-SBM diet containing 0.26% TD Ile. The addition of 0.45 and 0.31% crystalline Leu and Val numerically increased feed intake and significantly improved G:F compared with pigs fed the control diet. These results suggest that the ratios of BCAA in previous EXP were not the cause of the decreased ADFI and ADG in pigs fed the C-BC diet. However, there are still significant differences in the composition of the C-SBM diet compared with the C-BC diet. The C-SBM diet is approximately 2.7% lower in CP than the C-BC diet. Torres et al. (1998) reported the CP concentration in diets for rats affected the regulation of BCAT. The low CP value of the C-SBM diet in EXP 4, 5, and 6 may limit the activation of BCAT, and thus the pig is not as sensitive to excess levels of Leu and Val. Furthermore, the possibility exists that the crystalline forms of Leu and Val in our C-SBM diet were absorbed at different rates than Leu and Val from intact protein (Yen et al., 2004). Although the efficiency of utilization of crystalline AA in pigs that are fed *ad libitum* is usually considered equal to intact protein sources (Yen et al., 2004), the differences in absorptive rates may play a role in altering the rate of BCAA oxidation.



In summary, the TD Ile requirement for 80- to 120-kg barrows for maximizing feed intake, growth, feed efficiency, and kilograms of lean is not less than 0.34% or 12.90 mg Ile/g ADG in a corn diet containing five percent blood cells. In contrast, the requirement may not be greater than 0.24% in finishing barrows fed a C-SBM diet. Plasma urea nitrogen is not a good measure of the Ile requirement due to effects of Ile on feed intake. Our data would suggest that Ile is not a limiting nutrient in formulation of low CP C-SBM diets for late-finishing barrows. Further research is needed to evaluate the optimal BCAA balance and the maximum level of red blood cells that can be fed to pigs.

## CHAPTER 6

### EFFECTS OF LOW CRUDE PROTEIN DIETS ON LATE-FINISHING BARROWS

#### INTRODUCTION

Increasing environmental concerns related to N concentrations of swine manure has generated interest in the use of crystalline amino acids (AA) to lower the crude protein (CP) of swine diets. The period of growth from 80 kg to market weight is the most inefficient period of growth a pig experiences during its development for slaughter (NRC, 1998). Feed intake is near its peak, while growth rate and protein accretion is declining as the pig reaches the top of their growth curve. The use of crystalline AA to lower CP during this growth phase should significantly impact N excretion when these high feed intakes are considered.

There are several reports in the literature evaluating the effects of low CP diets for swine that have been reviewed previously (Figueroa et al., 2002; Gomez et al., 2002). There is general agreement that reducing CP by more than three or four percent will lead to a reduction in rate and efficiency of growth. Furthermore, reductions in CP often lead to increased fat deposition. Some reports in the literature suggest that formulating diets on a NE basis can prevent an increase in carcass fat when CP is lowered (Le Bellego et al., 2001, 2002). However, Knowles et al. (1998a) concluded that reduction of NE in low CP diets was not an effective means of reducing carcass fat. There may be unknown regulators of growth involved when CP is reduced.

The result of adding crystalline AA to a typical corn-soybean meal (C-SBM) diet is a reduction in the concentration of SBM. There are reports in the literature that suggest SBM contains unidentified growth promotants (Carlson et al., 1964; Griffith et

al., 1966). However, more recent knowledge concerning nutrient requirements may explain results that observed growth responses to additions of SBM to a purified diet.

We conducted two experiments (EXP) to determine whether or not the reduced amount of SBM is the reason for decreased performance when CP is lowered and to evaluate the use of a corn-crystalline AA diet during the late-finisher period.

### **MATERIALS AND METHODS**

The Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee approved all methods used in these EXP related to animal care. Yorkshire, Yorkshire x Landrace, or Yorkshire x Landrace x Duroc pigs from the LSU Agricultural Center were used in each experiment. Pigs were housed in a curtain-sided building with 1.5- x 3.0-m pens and concrete slotted floors. Pigs and feeders were checked twice daily. Feed in pelleted form for EXP 1 and mash form for EXP 2 and water were provided on an *ad libitum* basis throughout the EXP. The pigs were allotted to dietary treatment on the basis of weight and ancestry in randomized complete block designs. A previous Lys requirement study with barrows at our research unit using the plasma urea N method indicated a true ileal digestible (TD) Lys requirement of 0.57% at 93 kg BW. The NRC (1998) Growth Model indicates that the requirement for TD Lys is from 0.58 to 0.48% for barrows weighing between 82 and 114 kg and gaining 350 g of lean gain per d. In order to insure excess protein was minimized we formulated diets to 0.54% TD Lys and ratios to Lys were used for all other AA according to NRC (1998). The diets were formulated to contain 0.50% Ca and 0.45% P. Amino acid, mineral, and ME values for feed ingredients other than soy protein isolate (SPI) were based on NRC (1998). Amino

acid values for SPI are on a TD basis using analyzed AA values and digestibility coefficients for soy protein concentrate (SPC) from NRC (1998).

The AA composition of the basal diets was determined after acid hydrolysis (AOAC, 2000; Method 982.30 E[a]). Total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2000; Method 982.30 E[b]). Tryptophan content was determined after alkaline hydrolysis (AOAC, 2000; Method 982.30 E[c]).

#### Experiment 1

**General.** Eighty barrows with an average initial and final BW of  $85.5 \pm 0.1$  and  $117.5 \pm 1.0$  kg were allotted to five treatments. There were five replications of barrows with four pigs per replicate. Three pigs per pen were slaughtered for carcass data collection. Body weights and feeder weights were taken initially and at the end of the trial. Dietary treatments (Table 6.1) included: 1) C-SBM formulated to 0.54% TD Lys; 2) Diet 1 with crystalline Lys, Thr, and Trp to lower CP (LCP C-SBM); 3) corn-soy protein isolate diet (C-SPI) equal in Lys to Diet 1; 4) Diet 3 with crystalline Lys, Thr, Trp to lower CP (LCP C-SPI).

**Carcass Evaluation.** On d 32, three pigs per pen from the two heaviest replications and on d 39, three pigs per pen from the two remaining replications were randomly selected and killed by exsanguination after electrical stunning at the LSU Agricultural Center Meats Laboratory. Linear carcass measurements and values from total body electrical conductivity (TOBEC; Model MQI-27: Meat Quality Inc., Springfield, IL) were determined as described by Matthews et al. (2001a). Fat-free lean (kilograms)

Table 6.1. Composition of diets for barrows in Experiment 1, as-fed basis<sup>a</sup>

Ingredients	C-SBM	C-SBM LCP	C-SPI	C-SPI LCP
Corn	83.43	93.10	89.74	94.87
Soybean meal, 47.5% CP	13.63	3.29	--	--
Soy protein isolate, 82% CP	--	--	7.16	1.46
Limestone	0.88	0.88	0.94	0.89
Monocalcium phosphate	0.58	0.79	0.68	0.82
Salt	0.50	0.50	0.50	0.50
Sodium bentonite	0.50	0.50	0.50	0.50
Mineral premix <sup>b</sup>	0.10	0.10	0.10	0.10
Vitamin premix <sup>c</sup>	0.38	0.38	0.38	0.38
L-Lys•HCl	--	0.34	--	0.35
L-Thr	--	0.09	--	0.09
L-Trp	--	0.03	--	0.04

Calculated composition, %<sup>d</sup>

	3314	3295	3324	3296
ME, kcal/kg				
CP	13.40	9.70	13.32	9.50
TD Lys	0.54	0.54	0.54	0.54
TD Met	0.21	0.16	0.21	0.16
TD TSAA	0.43	0.34	0.43	0.33
TD Trp	0.12	0.10	0.12	0.10
TD Thr	0.42	0.36	0.43	0.36
TD Ile	0.47	0.29	0.51	0.29
Ca	0.50	0.50	0.50	0.50
P	0.45	0.45	0.45	0.45

<sup>a</sup>C-SBM = corn-soybean meal; C-SBM LCP = corn-soybean meal low CP; C-SPI = corn-soy protein isolate; C-SPI LCP = corn-soy protein isolate low CP; TD = true digestible.

<sup>b</sup>Provides the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, 0.3 mg Se as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite, respectively with calcium carbonate as the carrier.

<sup>c</sup>Provides the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D<sub>3</sub>, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex) 6.2 mg; riboflavin, 10 mg; Ca-d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B<sub>12</sub>, 45 µg; d-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamin, 3.31 mg; and vitamin C, 83 µg.

<sup>d</sup>Amino acid values for corn and soybean meal (NRC, 1998) are on a true ileal digestibility basis using digestibility coefficients from NRC, 1998. Amino acid values for soy protein isolate are on a true ileal digestibility basis using analyzed AA values and digestibility coefficients for soy protein concentrate from NRC (1998).

and percent fat-free lean were determined by NPPC (2000) equations, which compensate for unequal body weight (BW).

Pork Quality. Pork quality measurements were taken from the left side of the carcass after a 20-h chill at 2°C and determined as described by Matthews et al. (2001a) with the following exception. Drip loss was determined as described by Payne et al. (2001b).

## Experiment 2

General. Forty-eight barrows with average initial and final BW of  $85.3 \pm 0.2$  and  $112.9 \pm 2.1$  kg were allotted to three treatments. There were four replications of barrows with four pigs per replicate. Three pigs per pen were slaughtered for carcass data collection. Body weights and feeder weights were taken initially and at the end of the EXP. The two lightest replications were on trial for 47 d and the two heaviest replications were on trial for 32 d. Dietary treatments (Table 6.2) included: 1) positive control (PC); C-SBM diet formulated to 0.54% TD Lys; 2) corn based diet with crystalline Lys, Thr, Trp, and Met (C+AA); 3) negative control (NC); Diet 2 without the crystalline AA.

Carcass Evaluation. Pigs were killed by captive bolt at a commercial slaughter facility. Linear carcass measurements and fat-free lean and percent fat-free lean were taken as in EXP 1. Hams were returned to LSU and scanned with the TOBEC (Higbie et al., 1997, 2002).

## Statistical Analysis

Data were analyzed by ANOVA procedures using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) as random complete block designs. The statistical model

Table 6.2. Composition of diets for barrows in Experiment 2, as-fed basis<sup>a</sup>

Ingredients	C-SBM		Corn + sand
	positive control	Corn + AA	negative control
Corn	83.43	96.14	96.14
Soybean meal, 47.5% CP	13.63	---	---
Sand	---	---	0.64
Limestone	0.88	0.88	0.88
Monocalcium phosphate	0.58	0.86	0.86
Salt	0.50	0.50	0.50
Sodium bentonite	0.50	0.50	0.50
Mineral premix <sup>b</sup>	0.10	0.10	0.10
Vitamin premix <sup>c</sup>	0.38	0.38	0.38
L-Lys•HCl	--	0.44	--
L-Thr	--	0.12	--
L-Trp	--	0.06	--
DL-Met	--	0.02	--
Calculated composition, % <sup>d</sup>			
ME, kcal/kg	3,314	3,309	3,288
CP	13.40	8.54	7.98
Lys	0.63 (0.67)	0.60 (0.59)	0.25 (0.27)
Met	0.23 (0.25)	0.18 (0.18)	0.16 (0.16)
TSAA	0.49 (0.50)	0.37 (0.35)	0.35 (0.33)
Trp	0.14 (0.18)	0.11 (0.12)	0.06 (0.10)
Thr	0.49 (0.49)	0.40 (0.38)	0.28 (0.27)
Ile	0.53 (0.54)	0.27 (0.27)	0.27 (0.26)
Val	0.64 (0.66)	0.38 (0.37)	0.38 (0.36)
TD Lys	0.54	0.54	0.20
TD Met	0.21	0.17	0.15
TD TSAA	0.43	0.32	0.30
TD Trp	0.12	0.10	0.05
TD Thr	0.42	0.35	0.23
TD Ile	0.47	0.23	0.23
TD Val	0.56	0.33	0.33
Ca	0.50	0.50	0.50
P	0.45	0.45	0.45

<sup>a</sup>C-SBM = corn-soybean meal; TD = true digestible.

(Table 6.2 continued)

<sup>b</sup>Provides the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, 0.3 mg Se as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, sodium selenite, respectively with calcium carbonate as the carrier.

<sup>c</sup>Provides the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D<sub>3</sub>, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex) 6.2 mg; riboflavin, 10 mg; Ca-d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B<sub>12</sub>, 45 µg; d-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamin, 3.31 mg; and vitamin C, 83 µg.

<sup>d</sup>Amino acid values for corn and soybean meal (NRC, 1998) are on a TD basis using digestibility coefficients from NRC, 1998. Analyzed values for AA are in parentheses.



included treatment and replication for all EXP. Contrast statements were used to evaluate effects of soy source, CP, and their interaction for EXP 1. Contrast statements were used to compare PC to the NC, the PC to C+AA, and to compare the C+AA to the NC in EXP 2. Treatment differences were considered significant at alpha = 0.10. The pen of pigs was the experimental unit for all data.

## **RESULTS**

### Experiment 1

In EXP 1 (Table 6.3), pigs fed SPI diets had lower final BW ( $P < 0.08$ ) than pigs fed SBM diets, which was primarily due to a lower final BW of pigs fed the high CP C-SPI diet. As CP was decreased average daily feed intake (ADFI) tended to increase ( $P = 0.13$ ). Average daily gain (ADG) and final BW increased when CP was reduced in the SPI diet, but were numerically lower in the SBM diets resulting in a soy source x CP interaction ( $P < 0.02$ ). Gain:feed (G:F) was decreased with the decrease in CP in the C-SBM diet but was unchanged in the C-SPI diets (soy source x CP;  $P < 0.10$ ).

Pigs fed SPI had less average backfat, 10<sup>th</sup> rib  $\frac{3}{4}$  fat but higher percent of fat-free lean ( $P < 0.07$ , Table 6.4) than pigs fed SBM. Pigs fed SPI tended to have higher lean:fat ratios and less total fat based on the TOBEC analysis with the ham equations ( $P < 0.12$ ). Ham weight and fat-free ham lean were higher for pigs fed SPI diets ( $P < 0.10$ ). The reduction in CP increased average backfat ( $P < 0.01$ ), 10<sup>th</sup> rib  $\frac{3}{4}$  fat ( $P < 0.07$ ), total fat ( $P < 0.08$ ), ham fat ( $P < 0.01$ ), and percent ham fat ( $P < 0.10$ ), resulting in lower NPPC fat-free lean percents ( $P < 0.08$ ), TOBEC fat-free ham lean ( $P < 0.08$ ), TOBEC percent ham lean ( $P < 0.01$ ), and TOBEC lean:fat ratio using ham equations ( $P < 0.05$ ). Decreasing dietary CP in the C-SBM diet had a greater effect on increasing

Table 6.3. Effects of low crude protein diets on growth performance in Experiment 1<sup>a</sup>

Item	Treatments				Contrasts P <			PSEM
	C-SBM				Soy			
	C-SBM	LCP	C-SPI	C-SPI LCP	Source	CP	Source*Protein	
Initial BW, kg	85.7	85.4	85.5	85.5	---	---	---	0.1
Final BW, kg	119.4	117.5	114.3	118.6	0.080	---	0.010	1.0
Growth performance								
ADG, kg/d	1.00	0.95	0.92	0.98	---	---	0.023	0.02
ADFI, kg/d	3.21	3.22	3.04	3.23	---	0.125	---	0.06
G:F, kg:kg	0.314	0.295	0.301	0.304	---	---	0.094	0.006

<sup>a</sup> C-SBM = corn-soybean meal; C-SBM LCP = corn-soybean meal low CP; C-SPI = corn-soy protein isolate; C-SPI LCP = corn-soy protein isolate low CP. Data are means of five replicates of four barrows per replicate. The growth trial lasted 31 or 38 d, depending on processing date.

Table 6.4. Effects of low crude protein diets on carcass traits in Experiment 1<sup>a</sup>.

Item	Treatments				Contrasts P <			PSEM
	C-SBM		C-SPI		Soy	Source *		
	C-SBM	LCP	C-SPI	LCP	Source	CP	Protein	
Carcass lean and fat measurements								
Final BW, kg	121.39	119.33	118.16	120.60	---	---	0.018	0.83
Loin muscle area, cm <sup>2</sup>	43.10	42.88	45.29	43.70	---	---	---	1.26
10 <sup>th</sup> rib ¼ backfat, cm	2.41	2.66	2.17	2.42	0.081	0.070	---	0.12
Average backfat, cm	2.79	3.03	2.66	2.89	0.073	0.005	---	0.07
Carcass length, cm	83.74	82.21	82.30	82.63	---	---	0.070	0.47
Dressing percent, %	73.49	75.15	73.74	74.90	---	0.115	---	0.83
Fat-free lean, % <sup>b</sup>	50.53	49.40	52.30	50.60	0.068	0.080	---	0.74
Fat-free lean, kg <sup>b</sup>	45.08	44.27	45.46	45.69	---	---	---	0.84
Carcass TOBEC analysis using carcass equations <sup>c</sup>								
Fat-free lean, kg	47.13	46.18	47.08	47.56	---	---	---	0.47
Percent lean, %	52.87	51.55	54.60	52.66	---	---	---	1.08
Total fat, kg	25.17	26.80	24.48	25.50	---	0.078	---	0.69
Percent fat, %	28.19	29.81	28.19	28.20	---	---	---	0.72
Lean:fat, kg:kg	1.90	1.75	1.95	1.89	0.147	0.119	---	0.06
Carcass TOBEC analysis using ham equations <sup>d</sup>								
Fat-free lean, kg	44.99	43.04	44.86	44.62	---	0.056	0.125	0.52
Total fat, kg	25.07	27.03	23.86	25.76	0.119	0.023	---	0.74
Lean:fat, kg:kg	1.82	1.61	2.05	1.75	0.119	0.041	---	0.11
Ham lean and fat measurements <sup>d</sup>								
Ham weight, kg	10.76	10.73	10.97	10.92	0.059	---	---	0.10
Fat-free ham lean, kg	7.07	6.81	7.11	7.05	0.100	0.078	---	0.08
Percent ham lean, %	65.65	63.51	64.75	64.59	---	0.004	0.010	0.32

(Table 6.4 continued)

Total ham fat, kg	2.05	2.28	2.16	2.17	---	0.008	0.012	0.04
Percent ham fat, %	19.11	21.18	19.77	19.90	---	0.010	0.019	0.36
Butt fat thickness, cm	1.56	1.76	1.57	1.58	---	---	---	0.07

<sup>a</sup>C-SBM = corn-soybean meal; C-SBM LCP = corn-soybean meal low CP; C-SPI = corn-soy protein isolate; C-SPI LCP = corn-soy protein isolate low CP. Data are means of five replicates of three barrows per replicate. Average initial and final BW were 85.5 and 119.9 kg, respectively. The growth trial lasted 31 or 38 d, depending on processing date.

<sup>b</sup>Calculated using the equation described by the NPPC (2000), which compensates for unequal BW.

<sup>c</sup>Calculated using TOBEC analysis using carcass equations from Higbie et al. (2002).

<sup>d</sup>Calculated using TOBEC analysis using ham equations from Higbie (1997).

ham fat, and percent ham fat and reducing percent ham lean than did decreasing CP in the C-SPI diet (soy source x CP,  $P < 0.02$ ).

Pigs fed SPI had higher 45-min carcass temperature ( $P < 0.07$ , Table 6.5), 45-minute pH ( $P < 0.10$ ), and pork NPPC color scores ( $P < 0.09$ ). Decreasing CP in diets increased 45-min pH ( $P < 0.05$ ), Minolta  $a^*$  ( $P < 0.02$ ), Minolta  $b^*$  ( $P < 0.04$ ), and NPPC marbling scores ( $P < 0.02$ ). Decreasing CP of the C-SBM diet decreased drip loss percent, but drip loss was numerically higher in the LCP C-SPI diet compared to the high CP C-SPI diet (soy source\*CP,  $P < 0.06$ ).

## Experiment 2

Pigs fed the NC had lower ADG ( $P < 0.01$ , Table 6.6), G:F ( $P < 0.001$ ), and final BW ( $P < 0.08$ ) compared with pigs fed the C+AA or PC. Pigs fed C+AA had lower ADG ( $P < 0.01$ ), G:F ( $P < 0.02$ ), and final BW ( $P < 0.03$ ) compared with pigs fed PC.

Pigs fed the NC had decreased loin muscle area ( $P < 0.04$ , Table 6.7), ham weight ( $P < 0.001$ ), kilograms of ham lean ( $P < 0.006$ ), percent ham lean ( $P < 0.08$ ), and kilograms of carcass fat-free lean ( $P < 0.005$ ) compared with pigs fed either the C+AA or the PC diet. Pigs fed the PC had increased loin muscle area ( $P < 0.08$ ), dressing percent ( $P < 0.04$ ), fat-free lean ( $P < 0.001$ ), ham weight ( $P < 0.001$ ), ham lean ( $P < 0.001$ ), and percent ham lean ( $P < 0.03$ ) with lower percent ham fat ( $P < 0.05$ ) than pigs fed C+AA.

## **DISCUSSION**

Experiment 1 was conducted to evaluate whether components in SBM may be able to help explain reductions in growth performance and/or carcass composition of pigs fed low CP diets. Soy protein isolate is made by treatment of SBM with hexane

Table 6.5. Effects of low crude protein diets on pork quality in Experiment 1<sup>a</sup>

Item	Treatments				Contrasts P <			PSEM
	C-SBM	C-SBM LCP	C-SPI	C-SPI LCP	Soy Source	CP	Soy* Protein	
Temperature and pH <sup>b</sup>								
45 min temperature, C	31.04	30.91	31.28	31.40	0.062	---	---	0.18
45 min pH	6.01	6.09	6.07	6.15	0.095	0.047	---	0.04
24 h temperature, C	2.03	1.97	2.03	2.07	---	---	---	0.16
24 h pH	5.61	5.60	5.58	5.58	---	---	---	0.01
Minolta color score <sup>c</sup>								
L*	55.55	56.02	54.64	56.36	---	---	---	1.05
a*	4.61	5.76	4.78	5.60	---	0.018	---	0.36
b*	11.37	12.72	11.32	12.34	---	0.038	---	0.51
NPPC color and marbling <sup>c</sup>								
Color	2.03	2.13	2.30	2.20	0.082	---	---	0.09
Marbling	1.93	2.37	1.93	2.27	---	0.013	---	0.13
Drip loss, %	5.87	3.66	3.90	4.16	---	0.098	0.057	0.41

<sup>a</sup>C-SBM = corn-soybean meal; C-SBM LCP = corn-soybean meal low CP; C-SPI = corn-soy protein isolate; C-SPI LCP = corn-soy protein isolate low CP. Data are means of five replicates of three barrows per replicate. Average initial and final body weight were 85.5 and 119.9 kg, respectively. The growth trial lasted 31 or 38 d, depending on processing date.

<sup>b</sup>The temperature and pH measurements were taken in the longissimus muscle between the 10th and 11th ribs.

<sup>c</sup>Color scores (L\*,a\*,b\* using Minolta colorimeter) and NPPC quality scores were taken on the longissimus muscle at the 10th rib interface. The 10th rib chop was then removed and drip loss was determined by the suspension method.

Table 6.6. Effects of low crude protein diets on growth performance in Experiment 2<sup>a</sup>

Item	Treatments			Contrasts P <			PSEM
	PC	C+AA	NC	PC vs C+AA	PC vs NC	C+AA vs NC	
Initial BW, kg	85.3	85.4	85.3	--	--	--	0.2
Final BW, kg	120.6	112.1	105.9	0.030	0.002	0.080	2.1
Growth performance							
ADG, kg/d	0.90	0.68	0.49	0.008	0.001	0.01	0.038
ADFI, kg/d	3.41	3.06	3.31	--	--	--	0.153
G:F, kg:kg	0.263	0.223	0.146	0.020	0.001	0.001	0.009

<sup>a</sup>PC = positive control diet; C+AA = corn diet with crystalline AA; NC = negative control. Data are means of four replicates of four barrows per replicate. The growth trial lasted 32 or 47 d, depending on processing date.

Table 6.7. Effects of low crude protein diets on carcass traits in Experiment 2<sup>a</sup>

Item	Treatments			Contrasts P <			PSEM
	PC	C+AA	NC	PC vs C+AA	PC vs NC	C+AA vs NC	
Carcass lean and fat measurements							
Final body weight, kg	123.6	113.8	107.0	0.022	0.002	0.076	2.2
Loin muscle area, cm <sup>2</sup>	56.34	50.21	42.34	0.076	0.003	0.033	2.02
Tenth rib $\frac{3}{4}$ backfat, cm	1.86	1.85	2.11	--	--	--	0.21
Average backfat, cm	2.70	2.62	2.55	--	--	--	0.07
Carcass length, cm	85.83	84.56	84.24	0.124	0.067	--	0.50
Dressing percent, %	73.19	70.25	70.22	0.035	0.034	--	0.77
Fat-free lean, % <sup>b</sup>	56.00	55.83	52.83	--	--	--	1.48
Fat-free lean, kg <sup>b</sup>	50.54	44.45	39.56	0.001	0.001	0.003	0.68
Carcass TOBEC analysis using ham equations <sup>c</sup>							
Fat-free lean, kg	47.49	39.52	33.84	0.001	0.001	0.005	0.92
Percent lean, %	52.57	49.72	45.26	--	0.022	0.110	1.68
Total fat, kg	20.87	19.80	20.34	--	--	--	1.60
Percent fat, %	23.06	24.53	26.92	--	0.103	--	1.42
Lean:fat, kg:kg	2.47	2.12	1.74	--	0.048	--	0.21
Ham lean and fat measurements <sup>d</sup>							
Ham, kg	10.48	9.52	8.64	0.001	0.001	0.001	0.11
Fat-free ham lean, kg	7.34	6.08	5.15	0.001	0.001	0.006	0.16
Percent ham lean, %	69.97	63.91	59.59	0.024	0.002	0.076	1.42
Total ham fat, kg	1.63	1.93	2.02	--	0.087	--	0.14
Percent ham fat, %	15.58	20.24	23.46	0.044	0.005	0.129	1.29
Butt fat thickness, cm	1.26	1.30	1.28	--	--	--	0.11

<sup>a</sup>PC = positive control diet; C+AA = corn diet with crystalline AA; NC = negative control. Data are means of four replicates of three barrows per replicate. Average initial and final BW were 85.3 and 114.8 kg, respectively. The growth trial lasted 32 or 47 d, depending on processing date.

<sup>b</sup>Calculated using the equation described by the NPPC (2000), which compensates for unequal BW.

<sup>c</sup>Calculated from TOBEC analysis using ham equations from Higbie et al. (2002).

<sup>d</sup>Calculated using TOBEC analysis using ham equations from Higbie (1997).



followed by an alcohol or water wash, which results in the removal of oligosaccharides and produces SPC (Beery, 1989). Soy protein concentrate is then further processed with alkaline extraction to remove cotyledons followed by acid precipitation and the removal of soy whey (Johnson and Kikuchi, 1989). The resulting SPI has a very similar AA profile, as a percent of CP, to SBM. Therefore, SPI should simulate closely AA concentrations of a C-SBM diet when included in a diet with corn. Previous studies have evaluated different protein sources relative to SBM, but in some instances these EXP confound the effects of AA concentrations (diet AA profiles) with source of protein.

The results from EXP 1 suggest significant interactions of soy source and CP for rate and efficiency of growth. These interactions seem to be primarily a result of the numerically reduced ADFI in pigs fed a high CP C-SPI diet compared with either level of CP in a C-SBM diet or the LCP C-SPI diet. There are no reports in the literature that have evaluated the use of SPI in diets for finishing pigs. Sohn et al. (1994) fed SPI to early weaned pigs and observed no differences in feed intake compared with pigs fed a diet containing SBM for the first 14 d of the trial; however, G:F was higher for pigs fed SPI. Payne et al. (2001a) concluded that broiler chicks fed a C-SPC diet had reduced ADFI, ADG, and G:F compared with chicks fed a C-SBM diet. However, Payne et al. (2001b) reported no differences in growth performance in pigs fed a C-SPC diet compared with pigs fed a C-SBM diet, although percent lean was decreased. In barrows, addition of soy isoflavones corrected the compositional changes of feeding SPC. Shelton et al. (2003) conducted a series of EXP comparing SPI to SBM in diets for broiler chicks and reported that ADFI was consistently reduced in broilers fed the C-SPI diets. Attempts to equalize feed physical properties such as bulk density did not fully

correct the reduction in ADFI. This response suggests that there is a nutritional effect of SPI that reduces feed consumption. It is difficult to fully explain the differences in performance between our SBM and SPI diets, but they may be related to palatability issues of SPI.

The reductions of CP in EXP 1 were approximately four percent for both diets containing SBM and SPI. Our results suggest that reducing the CP had no effect on growth performance of barrows. In fact, ADG was increased in pigs fed the LCP C-SPI diet due to increased ADFI. Overall, measurements of fat were increased when CP was decreased with little effect on carcass muscling. However, muscling as a percent of carcass weight was reduced due to the increased fat. Several others have reported this increase in carcass fat when CP is decreased with the use of crystalline AA (Tuitoek et al., 1997a,b; Knowles et al., 1998a; Figueroa et al., 2002; Gomez et al., 2002a,b). Knowles et al. (1998a) suggested that pigs fed low CP crystalline AA diets should have a lower energy need for deamination of excess AA and lower pancreatic activity and therefore the NE of the diet increases, which leads to fatter carcasses. However, Knowles et al. (1998a) concluded from their research that reduction of NE in low CP AA-supplemented diets was not an effective means of reducing fat in finishing pigs. Other reports suggest that formulating with the NE system will prevent fatter carcasses (Le Bellego et al., 2001, 2002). The use of NE for comparison of energy values of low CP diets is a complex issue. First, NE values of feedstuffs are difficult to obtain and therefore some are based on prediction equations and the chemical composition of the ingredient. Second, the NE value of feedstuffs is most likely not constant (Lewis and Southern, 2001). For example, when crystalline AA are added to a C-SBM diet, the NE

of SBM should increase because the diet is more closely providing the ideal AA profile the pig requires; thus, less energy will be used in the metabolic process of deamination of excess AA. Furthermore, the NE system was established with diets having higher CP levels than those currently used for growing pigs (Le Bellego et al., 2001). Third, NE values of crystalline AA have not been determined. Our calculated values of NE in Table 1 ignore any contribution of energy from crystalline AA. Calculated values of NE are lowest for the high CP C-SBM diet and highest for the LCP C-SPI diet, and the NE value of the LCP C-SPI diet is most likely underestimated, because of the aforementioned reasons. However, growth and carcass measurements were very similar when comparing these two treatments. Our results do not suggest that NE is the cause of increased carcass fat.

The results of pork quality measurements in EXP 1, indicate that reducing CP may increase Minolta a\* and b\* values. Subjective marbling scores increased when CP was reduced and this would agree with the carcass composition data suggesting fat accretion is greater when CP is reduced. The relatively high drip loss percent in pigs fed the high CP C-SBM diet is difficult to explain and the results from pigs fed SPI do not suggest it is an effect of CP.

Experiment 2 was designed to determine whether a corn diet fortified with vitamins, minerals, and the commercially available AA (Lys, Thr, Trp, and Met) would support optimal performance and carcass composition of late-finishing barrows. Feeding a corn diet with no supplemental AA decreased rate and efficiency of growth. When crystalline AA were added, pigs performed better, but not equal to pigs fed a C-SBM diet. Pigs fed the NC had increased fat and decreased carcass muscling.

Interestingly, most measures of carcass fat were not different when pigs fed the C-SBM diet were compared to pigs fed C+AA. However, final BW and carcass muscling were reduced resulting in a lower lean percent.

Liu et al. (2001) reported that a 9.55% CP corn diet fortified with crystalline Lys, Thr, Trp, Met, Ile, and Val supported growth and carcass composition equal to gilts fed a 15.17% CP C-SBM diet. These authors also reported that deletion of crystalline Ile or Val reduced growth performance of gilts. Shelton et al. (2001) evaluated nine different protein sources for grow-finish pigs. Diets for their experiment were formulated to meet all NRC (1998) AA requirements and they maintained an equivalent Lys:calorie ratio. They reported that feeding a corn diet with crystalline AA reduced performance during the grower and early-finishing periods, but not during the late-finishing period. Nonetheless, carcass muscling was reduced and carcass fat was increased among pigs fed the corn-AA diet compared with pigs fed a diet containing SBM.

The limiting order of AA in a corn diet is as follows: Lys, Trp, Thr, Ile, Val, and Met (NRC, 1998). In our EXP, we only supplemented the commercially available AA and are aware that according to NRC (1998) our diets were deficient in Ile and Val. However, previous unpublished research in our lab has indicated no response to Ile levels above those fed in EXP 2 in a diet comprised primarily of corn that was fortified with crystalline Val. Additionally, based on NRC (1998) requirements for 80- to 120-kg barrows, the Val concentration of our corn diet should be very close to the requirement. Analyzed values for AA in our diets were close to calculated values. Based on our results and the results of Liu et al. (2001) and Shelton et al. (2001) it seems that a

deficiency of Val or Ile or both in our diet may be responsible for the reduction in performance of pigs fed a corn diet.

Eliminating SBM in diets for late-finishing barrows results in close to a five percent reduction in CP. It is possible that AA other than Ile and Val are limiting in a corn diet. Kendall et al. (2004) concluded that adding Gln and Gly together or Glu improved performance of nursery pigs fed low CP diets. Kephart and Sherritt (1990) concluded that supplementing urea, Glu, or potassium to low CP diets had no benefit in 20-kg pigs. Russell et al. (1987) concluded that addition of Glu to a diet reduced in CP by 5% had no benefit on performance. Further research evaluating individual nonessential AA is needed to determine whether or not they are limiting when CP is reduced by more than four percent.

In summary, our results suggest that reductions in the fractions of SBM other than protein are not the cause of increased carcass fat in pigs fed low CP diets. Furthermore, the fortification of a corn diet with the commercially available AA will not support optimal growth or carcass composition in late-finishing barrows. Further research needs to be conducted to evaluate limiting nonessential AA in low CP diets for pigs.

## CHAPTER 7

### EVALUATION OF LIMITING AMINO ACIDS IN DIETS FORMULATED TO BE LOW IN CRUDE PROTEIN FOR BROILERS

#### INTRODUCTION

The use of crystalline amino acids (AA) to lower the crude protein (CP) of diets for broilers has been shown to effectively reduce N excretion (Ferguson et al., 1998; Bregendahl et al., 2002; Si et al., 2004a,b). However, rate and efficiency of growth is typically lower in broilers fed diets where CP has been lowered by more than three percent, even when all known nutrient requirements are met (Fancher and Jensen et al., 1989a,b; Pinchasov et al., 1990; Aletor et al., 2000; Waldroup, 2000; Bregendahl et al., 2002).

Researchers have evaluated many potential reasons for the reduction in growth performance. These include potassium concentrations and dietary electrolyte balance (Fancher and Jensen, 1989a,b; Waldroup, 2000), concentrations of essential AA (Fancher and Jensen, 1989a,b; Kidd and Kerr, 2000; Waldroup et al., 2000), ratio of essential AA to CP level (Pinchasov et al., 1990; Cabel et al., 1991), addition of nonessential AA (Fancher and Jensen, 1989b; Pinchasov et al., 1990; Bregendahl et al., 2002), the ratio of Trp to other large neutral AA (Waldroup, 2000), and the addition of Cys to reduce Met concentrations (Waldroup, 2000). Dietary manipulations related to these proposed problems have been unsuccessful in restoring growth performance of broilers to a level equal to that of chicks fed a positive control (PC) diet. One exception is that Han et al. (1992) demonstrated that addition of essential AA and Glu to a 19%

CP diet supported equal growth performance of both slow and fast growing chicks compared with a 23% CP diet.

When crystalline AA, other than Met, are included in the formulation of diets for broilers, there is a reduction in the concentration of soybean meal (SBM) in the diet. There has been no research conducted with broilers and low CP diets that has simulated the AA profile of a normal corn-soybean meal (C-SBM) diet in the low CP diets. Therefore, we conducted five experiments (EXP) to evaluate the level of CP where performance becomes impaired and then to evaluate simulating the AA profile of both essential and nonessential AA of a high CP diet using crystalline AA in a low protein diet.

### **MATERIALS AND METHODS**

Five EXP were conducted with Ross x Ross broilers to evaluate the effects of altering AA levels of low CP diets for broilers. The methods used in all EXP were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. On d 0 posthatching, the broilers were weighed, wingbanded, and randomly allotted to dietary treatment. They were housed in environmentally-controlled brooder batteries with raised wire floors and continuous fluorescent lighting. Feed in mash form and water were provided *ad libitum* throughout each of the EXP Diets (Tables 7.1 and 7.2) were formulated to 1.12% true digestible (TD) Lys and to meet the AA ratios suggested by Baker et al. (1997). All diets were formulated to contain 3,200 kcal/kg of ME, 0.90% Ca, 0.46% nonphytate P, and to meet the requirements of all other nutrients as suggested by the NRC (1994) for broilers from 0 to 3 wk of age. A mixture of 66.67%

Table 7.1. Composition of diets for chicks fed graded levels of crude protein in Experiment 1, as-fed basis

Ingredient	16.18% CP	17.68% CP	19.18% CP	20.68% CP	22.18% CP
Corn	76.95	70.17	64.18	58.77	53.93
Soybean meal, 47.5% CP	14.29	21.24	27.14	32.33	36.77
Corn oil	1.78	2.96	3.91	4.74	5.43
Monocalcium phosphate	1.75	1.70	1.66	1.63	1.60
Limestone	1.35	1.32	1.30	1.28	1.26
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin premix <sup>a</sup>	0.05	0.05	0.05	0.05	0.05
Mineral premix <sup>b</sup>	0.25	0.25	0.25	0.25	0.25
L-Lys•HCl	0.72	0.50	0.31	0.14	---
L-Arg•HCl	0.55	0.31	0.10	---	---
DL-Met	0.40	0.33	0.28	0.23	0.19
L-Thr	0.34	0.24	0.16	0.08	0.02
L-Val	0.31	0.19	0.09	---	---
L-Ile	0.28	0.16	0.06	---	---
L-Phe	0.18	---	---	---	---
L-His	0.07	0.01	---	---	---
L-Gly	0.07	---	---	---	---
L-Trp	0.06	0.03	---	---	---
L-Leu	0.03	---	---	---	---
Choline chloride	0.08	0.06	0.03	0.01	---
Nutrient composition, % <sup>c</sup>					
ME, kcal/kg	3200	3200	3200	3200	3200
CP	16.18	17.68	19.18	20.68	22.18
CP, analyzed	15.74	17.19	19.48	21.04	21.60
Ca	0.90	0.90	0.90	0.90	0.90
P	0.67	0.69	0.70	0.71	0.72
Available P	0.46	0.46	0.46	0.46	0.46
TD Lys	1.12	1.12	1.12	1.12	1.12
TD TSAA	0.81	0.81	0.81	0.81	0.81
TD Thr	0.75	0.75	0.75	0.75	0.75
TD Trp	0.18	0.18	0.19	0.21	0.24
Lys	1.19 (1.13)	1.20 (1.19)	1.21(1.18)	1.22 (1.24)	1.23 (1.23)
Met	0.63 (0.48)	0.60 (0.50)	0.57 (0.49)	0.55 (0.52)	0.53 (0.49)



(Table 7.1 continued)

TSAA	0.87 (0.70)	0.88 (0.75)	0.88 (0.78)	0.89 (0.85)	0.89 (0.85)
Thr	0.82 (0.74)	0.84 (0.77)	0.85 (0.77)	0.86 (0.80)	0.86 (0.80)
Trp	0.21 (0.14)	0.22 (0.17)	0.24 (0.17)	0.27 (0.11)	0.30 (0.20)
Ile	0.80 (0.81)	0.81 (0.76)	0.82 (0.82)	0.86 (0.91)	0.94 (0.97)
Val	0.93 (0.90)	0.94 (0.90)	0.94 (0.96)	0.95 (1.00)	1.03 (1.06)
Leu	1.33 (1.29)	1.50 (1.34)	1.66 (1.59)	1.80 (1.78)	1.92 (1.85)
Phe + Tyr	1.14 (1.29)	1.39 (1.27)	1.60 (1.49)	1.79 (1.70)	1.94 (1.79)
Arg	1.25 (1.21)	1.26 (1.21)	1.27 (1.30)	1.35 (1.37)	1.49 (1.44)
His	0.43 (0.43)	0.44 (0.45)	0.50 (0.49)	0.55 (0.57)	0.60 (0.60)
Gly + Ser	1.25 (1.16)	1.45 (1.28)	1.68 (1.45)	1.88 (1.64)	2.04 (1.72)
Choline, mg/kg	1300	1300	1300	1300	1300

<sup>a</sup>Provides the following per kilogram of diet: copper (cupric sulfate pentahydrate), 7.00 mg; iodine (calcium iodate), 1.00 mg; iron (ferrous sulfate monohydrate), 50.00 mg; manganese (manganese sulfate monohydrate), 100.00 mg; selenium (sodium selenite), 0.15 mg; and zinc (zinc sulfate monohydrate), 75 mg.

<sup>b</sup>Provides the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; menadione 1.5 mg; vitamin B<sub>12</sub>, .02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyrodoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

<sup>c</sup>TD = true ileal digestible. Analyzed values for amino acids are in parentheses.

Table 7.2. Composition of basal diets for chicks in Experiment 2, 3, 4, and 5, as-fed basis

Ingredient	Positive control	Low crude protein
Corn	54.61	64.26
Soybean meal, 47.5% CP	36.72	17.25
Corn oil	4.78	5.35
Monocalcium phosphate	1.60	1.77
Limestone	1.26	1.33
Salt	0.50	0.50
Sand	---	5.00
Vitamin premix <sup>a</sup>	0.05	0.05
Mineral premix <sup>b</sup>	0.25	0.25
Na & K bicarbonate mix <sup>c</sup>	0.02	1.44
Choline chloride	---	0.08
L-Lys•HCl	---	0.65
L-Arg•HCl	---	0.49
DL-Met	0.19	0.40
L-Thr	0.02	0.32
L-Val	---	0.29
L-Ile	---	0.25
L-Phe	---	0.13
L-His	---	0.07
L-Trp	---	0.05
L-Leu	---	0.04
L-Gly	---	0.02
Nutrient composition, % <sup>d</sup>		
ME, kcal/kg	3200	3200
CP	22.21	16.21
Ca	0.90	0.90
P	0.72	0.66
Available P	0.46	0.46
dEB, mEq/kg	250	250
Choline, mg/kg	1341	1300
TD Lys	1.12	1.12
TD TSAA	0.81	0.81
TD Met	0.50	0.60

(Table 7.2 continued)

TD Thr	0.75	0.75
TD Trp	0.24	0.18
TD Val	0.94	0.86
TD Ile	0.86	0.75
TD Leu	1.74	1.22
TD Phe + Tyr	1.75	1.18
TD Arg	1.37	1.18
TD His	0.53	0.39
Lys	1.23 (1.30)	1.19 (1.19)
TSAA	0.89 (0.89)	0.87 (0.81)
Met	0.53 (0.53)	0.63 (0.60)
Thr	0.86 (0.84)	0.82 (0.76)
Trp	0.31 (0.28)	0.22 (0.23)
Val	1.03 (1.09)	0.93 (0.89)
Ile	0.94 (0.98)	0.80 (0.75)
Leu	1.92 (1.89)	1.33 (1.20)
Phe + Tyr	1.95 (1.84)	1.18 (1.21)
Arg	1.49 (1.52)	1.25 (1.20)
His	0.60 (0.60)	0.43 (0.41)
Gly	0.93 (0.92)	0.57 (0.56)
Gly + Ser	2.05 (1.82)	1.25 (1.13)
Asp	2.36 (2.30)	1.40 (1.23)
Pro	1.25 (1.28)	0.87 (0.81)
Ala	1.05 (1.08)	0.72 (0.71)
Glu	4.18 (3.75)	2.65 (2.22)

<sup>a</sup>Provides the following per kilogram of diet: copper (cupric sulfate pentahydrate), 7.00 mg; iodine (calcium iodate), 1.00 mg; iron (ferrous sulfate monohydrate), 50.00 mg; manganese (manganese sulfate monohydrate), 100.00 mg; selenium (sodium selenite), 0.15 mg; and zinc (zinc sulfate monohydrate), 75 mg.

<sup>b</sup>Provides the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 25 IU; menadione 1.5 mg; vitamin B<sub>12</sub>, .02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

<sup>c</sup>Contains 66.67% sodium bicarbonate and 33.33% potassium bicarbonate.

<sup>d</sup>dEB = dietary electrolyte balance; TD = true ileal digestible. Analyzed values for amino acids are in parentheses.

sodium bicarbonate and 33.33% potassium bicarbonate was used to keep the dietary electrolyte balance constant at 250 mEq/kg in diets for EXP 2 through 5.

All of the EXP contained PC and negative control (NC) diets. The PC diet usually contained approximately 22% CP and it was supplemented only with crystalline DL-Met and L-Thr. The PC diet was a typical broiler starter diet. The NC diet usually contained approximately 16% CP and it was supplemented with crystalline AA to meet the AA ratios suggested by Baker et al. (1997). Both the PC and NC diets met all known nutrient requirements of broilers 0 to 3 wk of age.

In EXP 1, 180 male broilers (40 and 507 g initial and final BW) were used to evaluate the effects of lowering CP in diets for broilers from 0- to 17-d posthatching. Diets (Table 7.1) were formulated to provide 16.18, 17.68, 19.18, 20.68, and 22.18% CP by altering the concentrations of corn and soybean meal. All diets contained 1.12% true digestible Lys and equal concentrations of all the essential AA by adding crystalline AA to meet the AA ratios suggested by Baker et al. (1997). Each treatment was replicated with 6 pens of 6 broilers each.

In EXP 2, 180 female broilers (36 and 528 g initial and final BW) were used in a factorial arrangement of treatments to evaluate growth performance of broilers fed a low CP diet supplemented with crystalline essential or nonessential AA. Each treatment was replicated with 6 pens of 6 broilers each. The trial lasted 18 d. The treatments for EXP 2 were: 1) PC formulated to 22.21% CP (Table 7.2); 2) NC formulated to 16.21% CP (Table 7.2); 3) Diet 2 with crystalline essential AA to equal the AA in PC; 4) Diet 2 with crystalline nonessential AA to equal the AA in the PC; 5) Diet 2 with both crystalline essential and nonessential AA to equal the AA in the PC.

In EXP 3, 288 female broilers (38 and 515 g initial and final BW) were used to evaluate growth performance of broilers fed a low CP diet supplemented with crystalline nonessential AA. Each treatment was replicated with 6 pens of 6 broilers each. The trial lasted 18 d. The treatments for EXP 3 were: 1) PC with 22.21% CP (Table 7.2); 2) NC formulated to 16.21% CP (Table 7.2); 3 to 7) Diet 2 with crystalline Gly, Glu, Ala, Asp, or Pro, respectively to equal each AA in PC; 8) Diet 2 with crystalline Gly, Glu, Ala, Asp, and Pro to equal PC.

In EXP 4, 300 female broilers (38 and 450 g initial and final BW) were used to evaluate growth performance of broilers fed graded dietary concentrations of Gly. Each treatment was replicated with 6 pens of 5 broilers each. The trial lasted 17 d. Chicks were fed either a PC diet (Table 7.2) or a 16.21% CP NC diet (Table 2) with Gly + Ser concentrations of 1.23, 1.35, 1.47, 1.59, 1.71, 1.83, 1.95, or 2.07%. A 10<sup>th</sup> diet was fed that was formulated to 1.23% Gly + Ser with supplemental Glu to equal the N concentration of the 2.07% Gly + Ser diet and with all essential AA to meet the ratio of Baker et al. (1997).

In EXP 5, 300 female broilers (41 and 531 g initial and final BW) were used to evaluate growth performance of broilers fed graded levels of Gly. Each treatment was replicated with 6 pens of 5 broilers. The trial lasted 17 d. Chicks were fed a PC diet (Table 7.2) with additions of 0, 0.15, or 0.30% Gly or a 16.21% CP NC diet (Table 2) formulated to contain 1.60, 1.72, 1.84, 1.96, 2.08, 2.20, or 2.32% Gly + Ser.

#### Statistical Analysis

Data were analyzed by ANOVA procedures using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) as completely randomized designs. The statistical model

included treatment for all EXP. Pen of broilers served as the experimental unit. In EXP 1, 4, and 5 orthogonal contrasts were used to determine linear and quadratic effects of lowering CP or of supplementing Gly. In EXP 2, contrast statements were used to determine effects of nonessential AA, essential AA, and their interaction and to compare each diet with the PC diet. In EXP 3, contrast statements were used to compare additions of individual nonessential AA to the PC. Also, in EXP 4, contrast statements were used to compare the PC to the low CP diet with either supplemental Glu or 2.07% Gly + Ser and also to compare the Glu diet to the 2.07% Gly + Ser diet. In EXP 5, a contrast statement was used to compare the PC diet to the 2.32% Gly + Ser diet.

## **RESULTS**

In EXP 1 (Table 7.3), lowering dietary CP linearly reduced final BW, average daily gain (ADG), and gain:feed (G:F) ( $P < 0.005$ ). Feed intake of chicks was unaffected by dietary CP.

In EXP 2 (Table 7.4), the data were analyzed as a 2 x 2 factorial arrangement that included the diets NC, NC + essential AA, NC + nonessential AA, and NC + essential and nonessential AA. In this analysis, the addition of nonessential AA increased final BW, ADG, and G:F ( $P < 0.07$ ). The addition of essential AA above their requirement had no effect on growth performance, and there was no interaction between the addition of supplemental nonessential and essential AA. The data also were analyzed by comparing each diet to the PC diet. In this analysis, ADG, final BW, and G:F were decreased ( $P < 0.05$ ) in chicks fed the PC compared with those fed the NC ( $P < 0.05$ ) or of chicks fed the NC + essential AA ( $P < 0.05$ ) diet. Similarly ADG, final BW, and daily feed intake (ADFI) were decreased ( $P < 0.05$ ) in chicks fed the NC +

Table 7.3. Growth performance of chicks fed graded levels of crude protein in Experiment 1<sup>a</sup>

Response	16.18 % CP	17.68 % CP	19.18 % CP	20.68 % CP	22.18 % CP	SEM
Final BW, g <sup>b</sup>	460	500	516	510	549	19.1
Daily gain, g <sup>b</sup>	24.69	27.10	28.04	27.68	29.97	1.12
Daily feed intake, g	35.75	37.33	36.94	34.93	36.69	1.46
Gain:feed, g/g <sup>b</sup>	0.692	0.727	0.759	0.792	0.817	0.008

<sup>a</sup>Data are means of 6 replicates of 6 broilers per replicate. The growth trial began on d 0 posthatching and lasted for 17 d. Average initial BW was 40 g.

<sup>b</sup>Linear effect of CP ( $P < 0.005$ ).

Table 7.4. Growth performance of chicks fed supplemental essential (EAA) or nonessential amino acids (NEAA) in Experiment 2<sup>a</sup>

Response	Low CP					SEM
	Positive control	Low CP	Low CP + NEAA	Low CP + EAA	Low CP + NEAA + EAA	
Final BW, g <sup>b</sup>	563	496 <sup>d</sup>	546	514 <sup>d</sup>	521 <sup>d</sup>	14.4
Daily gain, g <sup>b</sup>	29.27	25.55 <sup>d</sup>	28.32	26.57 <sup>d</sup>	26.95 <sup>d</sup>	0.80
Daily feed intake, g	36.79	34.99	35.08	35.98	33.18 <sup>d</sup>	0.91
Gain:feed, g/g <sup>c</sup>	0.797	0.730 <sup>d</sup>	0.807	0.739 <sup>d</sup>	0.812	0.011

<sup>a</sup>Data are means of 6 replicates of 6 broilers per replicate. The growth trial began on d 0 posthatching and lasted for 18 d. Average initial BW was 36 g.

<sup>b</sup>NEAA effect (P < 0.07).

<sup>c</sup>NEAA effect (P < 0.001).

<sup>d</sup>Means with a superscript differ (P < 0.05) from the positive control.



essential AA + nonessential AA diets. However, there was no difference ( $P > 0.10$ ) between growth performance of chicks fed PC and the NC + nonessential AA. In EXP 3 (Table 7.5), ADG, final BW, ADFI, and G:F were decreased ( $P < 0.05$ ) in chicks fed the NC compared with those fed the PC. The addition of Gly and the combination of Gly, Glu, Asp, Ala, and Pro to the NC diet increased G:F compared with chicks fed PC, and ADG and final BW were not different. However, chicks fed the diet containing all five nonessential AA had reduced ADFI ( $P < 0.05$ ) compared with chicks fed PC. Chicks fed diets with supplemental Glu, Ala, Asp, or Pro had reduced ADFI, ADG, and final BW ( $P < 0.05$ ) compared with those fed the PC. Gain:feed was decreased when Asp or Pro were supplemented ( $P < 0.10$ ) but there was no difference in G:F when diets were supplemented with Glu or Ala compared with chicks fed PC.

In EXP 4 (Table 7.6), final BW, ADG, and gain:feed were increased linearly ( $P < 0.001$ ) in chicks as the concentration of Gly was increased in the diet. Although the increase in ADG was linear, there was essentially no change in ADG until 1.83% Gly + Ser was fed. Chicks fed the low CP diet with 2.07% Gly + Ser had growth performance that was not different from growth performance of chicks fed the PC. Chicks fed the NC with Glu to equal the N concentration of the diet with 2.07% Gly + Ser had decreased final BW, ADG, and G:F ( $P < 0.001$ ) compared with chicks fed the PC diet or the NC diet with 2.07% Gly + Ser.

In EXP 5 (Table 7.7), there was no effect of adding Gly to the PC diet on final BW, ADFI, ADG, or G:F. Adding Gly to the NC diet increased G:F linearly ( $P < 0.001$ ), but it had no effect on ADFI, ADG, or final BW. Chicks fed the low CP diet with 2.32%

Table 7.5. Growth performance of chicks fed diets with supplemental levels of nonessential amino acids in Experiment 3<sup>a</sup>

Response	PC	LCP	LCP + Gly	LCP + Glu	LCP + Ala	LCP + Asp	LCP + Pro	LCP + NEAA	SEM
Final BW, g	555	499 <sup>c</sup>	551	509 <sup>c</sup>	474 <sup>b</sup>	499 <sup>c</sup>	492 <sup>b</sup>	538	11.2
Daily gain, g	28.70	24.88 <sup>b</sup>	28.50	26.15 <sup>c</sup>	24.23 <sup>b</sup>	25.61 <sup>c</sup>	25.22 <sup>b</sup>	27.79	0.65
Daily feed intake, g	37.46	34.95 <sup>c</sup>	35.61	35.08 <sup>c</sup>	32.16 <sup>b</sup>	34.47 <sup>c</sup>	34.03 <sup>c</sup>	34.49 <sup>c</sup>	0.85
Gain:feed, g/g	0.766	0.712 <sup>b</sup>	0.801 <sup>c</sup>	0.746	0.754	0.743 <sup>d</sup>	0.741 <sup>d</sup>	0.806 <sup>c</sup>	0.009

<sup>a</sup>Data are means of 6 replicates of 6 broilers per replicate. The growth trial began on d 0 posthatching and lasted for 18 d.

Average initial BW was 38 g. PC = positive control; LCP = low CP; NEAA = Gly + Glu + Ala + Asp + Pro.

<sup>b</sup>Means with a superscript differ ( $P < 0.001$ ) from the PC.

<sup>c</sup>Means with a superscript differ ( $P < 0.05$ ) from the PC.

<sup>d</sup>Means with a superscript differ ( $P < 0.10$ ) from the PC.

Table 7.6. Growth performance of chicks fed graded levels of Gly + Ser in Experiment 4<sup>a</sup>

Response	PC	Gly + Ser, %								+ Glu	SEM
		1.23	1.35	1.47	1.59	1.71	1.83	1.95	2.07		
Gly + Ser intake, g/d	0.67	0.40	0.43	0.46	0.52	0.55	0.56	0.67	0.68	0.38	---
Gly + Ser, mg/g ADG	25.3	17.1	18.5	20.1	21.6	22.5	23.7	25.1	26.2	16.9	---
Final BW, g <sup>b,c,d</sup>	487	434	433	426	447	446	438	491	477	423	11.1
Daily gain, g <sup>b,c,d</sup>	26.50	23.36	23.28	22.87	24.12	24.44	23.59	26.72	25.91	22.69	0.65
Daily feed intake, g	32.83	32.35	32.13	31.57	32.58	31.96	30.68	34.33	32.74	31.10	0.95
Gain:feed, g/g <sup>b,c,d</sup>	0.808	0.722	0.725	0.729	0.742	0.765	0.769	0.779	0.791	0.730	0.009

<sup>a</sup>Data are means of 6 replicates of 5 broilers per replicate. The growth trial began on d 0 posthatching and lasted for 17 d. PC = positive control; + Glu = Low CP diet with supplemental Glu to equal nitrogen in the low CP diet containing 2.07% Gly + Ser. Average initial BW was 37 g.

<sup>b</sup>Linear effect of Gly (P < 0.001).

<sup>c</sup>Positive control differs (P < 0.001) from + Glu.

<sup>d</sup>2.07% Gly + Ser differs (P < 0.001) from + Glu.

Table 7.7. Growth performance of chicks fed graded levels of Gly + Ser in Experiment 5<sup>a</sup>

Response	Positive control,			Low crude protein diet, Gly + Ser, %							SEM
	Gly + Ser, %										
	2.05	2.20	2.35	1.60	1.72	1.84	1.96	2.08	2.20	2.32	
Gly + Ser intake, g/d	0.74	0.82	0.86	0.59	0.66	0.67	0.70	0.75	0.82	0.86	---
Gly + Ser, mg/g ADG	25.7	27.4	29.3	21.1	22.38	23.9	25.4	26.4	28.2	29.1	---
Final BW, g	533	548	541	513	543	520	512	523	535	544	10.6
Daily gain, g	28.98	29.87	29.44	27.79	29.51	28.11	27.76	28.41	29.07	29.60	0.62
Daily feed intake, g	36.28	37.21	36.70	36.63	38.39	36.47	35.91	36.19	37.26	37.12	0.81
Gain:feed, g/g <sup>b</sup>	0.800	0.803	0.802	0.759	0.769	0.771	0.773	0.783	0.786	0.797	0.005

<sup>a</sup>Data are means of 6 replicates of 5 broilers per replicate. The growth trial began on d 0 posthatching and lasted for 17 d. Average initial BW was 41 g.

<sup>b</sup>Linear effect of Gly + Ser in low CP diet (P < 0.001).

Gly + Ser had growth performance that was not different from growth performance of chicks fed the PC.

## **DISCUSSION**

The objective of EXP 1 was to evaluate growth performance of chicks fed graded levels of CP in diets where all known nutrient requirements were met. Reports in the literature dealing with low CP diets have shown that keeping AA concentrations at a constant ratio to CP will not support optimal growth of broilers (Pinchasov et al., 1990; Cabel et al., 1991). Therefore, digestibility coefficients for ingredients were utilized to formulate diets to meet minimum digestible AA requirements on a percentage basis in all diets according to the ideal protein concept (Baker, 1997). Analysis of the diets for AA concentrations matched closely with calculated values with few exceptions. Diets were also formulated to be isocaloric on a metabolizable energy basis.

The results of EXP 1 show a linear decrease in ADG, final BW, and G:F when CP was reduced from 22 to 16%, even though the diet met all AA requirements of broilers. Si et al. (2004a,b) reported similar results with reduced BW and increased feed:gain ratio when CP levels were reduced below 22%. Other researchers have reported that growth performance was not reduced until the CP level was decreased below 20% (Waldroup, 2000; Sterling et al., 2005). In our study, digestible concentrations of the first three limiting AA (TSAA, Thr, and Lys) were formulated to be identical for all levels of CP. Concentrations of Arg, Ile, Val, Trp, His, Leu, and Phe + Tyr were reduced to their minimum requirement as CP was reduced in the diet.

Waldroup (2000) evaluated the effect of the ratio of Trp:large neutral AA and concluded that this ratio was not the cause of depressed growth performance of broilers

fed low CP diets. In our study, this ratio was relatively constant among levels of CP. The percent of CP provided from essential versus nonessential AA has also been suggested as a potential problem with low CP diets. However, studies evaluating additions of nonessential AA to low CP diets have been unable to show equal growth performance of broilers fed control diets (Fancher and Jensen, 1989b; Pinchasov et al., 1990; Bregendahl et al., 2002). In our study, the percent of CP provided by essential AA increased from 50.47 to 53.76% with reductions in nonessential AA. These percentages fall well within suggested tolerances for growing broilers (Bedford and Summers, 1985).

Previous research suggested that the reduced dietary electrolyte balance of low CP diets may contribute to a portion of the reduction in growth performance (Fancher and Jensen, 1989a). Other researchers that have evaluated the effect of potassium levels and electrolyte balance have been unsuccessful in optimizing growth performance of chicks fed a low CP diet (Fancher and Jensen, 1989b; Han et al., 1992; Waldroup, 2000). Nonetheless, we utilized a mixture of 66.67% sodium bicarbonate and 33.33% potassium bicarbonate to keep the dietary electrolyte balance constant at 250 mEq/kg in diets for EXP 2 through 5.

After thoroughly evaluating the literature and the concentrations of both essential and nonessential AA in our diets from EXP 1, it seemed logical to try and simulate the AA profile of our PC as closely as possible in a low CP diet by adding crystalline AA. If an AA deficiency was the cause of reduced feed utilization, performance should be optimized with the addition of these AA. Adding excess essential AA to a level equal to that found in our PC diet did not improve performance of chicks fed the low CP diet. When additions of Gly, Asp, Pro, Ala, and Glu were supplemented to the low CP diet,

ADG, final BW, and G:F were equal to that of chicks fed the PC. The addition of both essential and nonessential AA optimized G:F, but due to a reduction in feed intake, ADG, and final BW were reduced compared with chicks fed the PC.

Reports in the literature show that additions of nonessential AA to meet a minimum N requirement are not sufficient to optimize performance of broilers fed low CP diets (Fancher and Jensen, 1989b; Pinchasov et al., 1990; Bregendahl et al., 2002). Because addition of the nonessential AA resulted in growth performance equal to that of chicks fed the PC diet, the obvious next step was to determine if all AA were necessary or if one AA caused the increase in growth performance. Therefore, EXP 3 was conducted.

In EXP 3, addition of Asp, Pro, Ala, and Glu individually did not improve growth performance of chicks fed the NC diet to the level equal to that of chicks fed PC. However, the addition of Gly and the combination of Gly, Glu, Asp, Ala, and Pro increased G:F and supported similar ADG and final BW compared with chicks fed the PC. These results suggest that levels of Gly are not adequate in low CP diets to support maximal feed utilization and growth. Parr and Summers (1991) observed that supplying Gly as a source of nonessential AA increased growth of broilers fed a diet containing approximately 20% CP. Furthermore, they reported that when Glu, Ala, or Asp were provided, growth performance of chicks was not different from chicks fed the NC.

Experiment 4 was conducted to determine a requirement for Gly + Ser using the low CP NC diet from the previous EXP. Feed efficiency was improved linearly as Gly was added to the diet to provide calculated Gly + Ser concentrations from 1.23 to 2.07%. The growth performance of broilers fed the diet containing 2.07% Gly + Ser was not different from chicks fed the PC. However, there was no obvious plateau in the

growth performance data among chicks fed the increasing concentrations of Gly + Ser. Our highest Gly + Ser level was calculated to be similar to the concentration of Gly + Ser in our PC, which we had assumed was at or above the requirement.

Therefore, we conducted EXP 5 to evaluate responses of supplementing Gly to our PC diet and to create a response curve with higher levels of Gly + Ser in our low CP diet. The addition of Gly to our PC diet had no effect on performance of broilers. Once again, we observed linear effects of Gly supplementation on growth performance of chicks fed the low CP diet. Gain:feed was only optimized when 2.32% Gly + Ser was provided.

There are numerous functions of Gly in metabolic pathways, but the requirement for Gly has been thought to be primarily due to Gly involvement in the formation of uric acid and excretion of N (Coon et al., 1975; Corzo et al., 2004). The absence of a plateau in growth performance when supplemental Gly was provided to our low CP diet suggests that the requirement for Gly is higher in low CP diets than in high CP diets. Our results do not agree with Heger and Pack (1996), who concluded that the Gly + Ser requirement was higher as CP of the diet increases. However, Waterhouse and Scott (1961) stated that the Gly requirement of chicks varied between 2 and 4% depending upon the protein source fed, and chicks fed diets with reduced CP had higher Gly requirements than chicks fed high CP diets.

Our basal low CP diet in EXP 3 was formulated to 1.25% total Gly + Ser to meet the NRC (1994) minimum requirement and contained 0.02% crystalline Gly. It is generally accepted that Gly and Ser are equally effective in meeting the Gly requirement when they are considered on an equimolar basis (Baker et al., 1968;



Sugahara and Kandatsu, 1976). Analyzed values of Gly and Ser for our PC and low NC diets were slightly lower than expected. The PC was calculated to contain 2.05% Gly + Ser and had an analyzed value of 1.82%. Our NC diet with 0.02% crystalline Gly was calculated to contain 1.25% Gly + Ser and had an analyzed value of 1.13%.

The suggested requirement for Gly + Ser in the NRC (1994) is based on five references. These studies were mostly conducted with slow growing chicks and purified diets resulting in requirement estimates from 0.30 to 1.80%. More recent research evaluating the Gly + Ser requirement indicates that the requirement is above the level recommended by NRC (1994). Corzo et al. (2004) concluded that the Gly + Ser requirement to maximize feed efficiency was 1.80% for broilers from 7 to 20 d of age. They also stated that the response to Gly in an AA fortified low CP diet indicates a Gly requirement for growth and not just for maintenance associated functions. Jiang et al. (2001) also observed responses to Gly in a low CP diet with concentrations above NRC (1994) recommendations but were unable to determine a requirement.

It is possible that varying requirement estimates in the literature are due to the number of compounds that can potentially be converted to Gly. There is evidence that Thr can be converted to Gly (Baker et al., 1972). However, Jiang et al. (2001) showed improvements in BW of chicks fed a low CP diet when Gly was supplemented but not Thr. Baker and Sugahara (1970) concluded that Ser, sarcosine, glycolic acid, choline, betaine, and aminoethanol are viable precursors for Gly. Featherstone (1979) demonstrated that folic acid is closely associated with Gly and Ser utilization in that the folic acid containing enzyme, Ser hydroxymethyl transferase, is responsible for the interconversion of these two AA. The use of different protein sources and

concentrations of compounds involved in Gly metabolism among experimental diets may influence the requirement. Digestible concentrations of Thr in our diets were held constant and therefore should not have had any effect on our evaluation of a Gly response. Choline was calculated to be only slightly higher in our PC diet than in the low CP diet. However, concentrations of other Gly precursors in corn and SBM are unknown.

The efficiency of converting Ser to Gly is 71.43% when their differences in molecular weight are considered. The proportions of Gly and Ser concentrations in a particular diet may become important in evaluating a requirement. Lower than expected Ser concentrations of both our PC and NC diets led to lower analyzed values of Gly + Ser. Furthermore, when the efficiency of Ser conversion to Gly is considered, the Gly equivalent decreases to 1.56% for our PC diet and 0.97% for our basal low CP NC diet. In EXP 5, the performance of chicks was maximized at 1.56% Gly equivalent in our high CP diet and 2.06% in the low CP diet.

Previous research suggested that the Gly + Ser requirement of broilers during a period of growth may be lower when high levels of Gly + Ser were fed during a pre-test period (Coon et al., 1974; Ngo and Coon, 1976). These reports may indicate a higher requirement of Gly during early growth. Our EXP were conducted with broilers immediately posthatching. This may explain our higher requirement estimate for Gly + Ser compared with the recent reports of Corzo et al. (2004) in which broilers were pre-tested on a typical starter diet for 7 d.

Calculations were made to determine the Gly + Ser intake per d for EXP 4 and 5 (Tables 6 and 7). In EXP 4, Gly + Ser intake increased from 0.40 to 0.68 g/d as Gly was

supplemented to the diet. In EXP 5, Gly + Ser intake increased from 0.59 to 0.86 g/d. When the milligrams of Gly + Ser required for each gram of ADG was calculated it seems clear that this value is not greater than 25.7 in the PC diet. However, this value may be as high as 29.1 mg Gly + Ser/g ADG in order to maximize feed efficiency in a low CP diet.

In summary, the addition of Gly to an AA supplemented, 16% CP diet will support growth and feed efficiency equal to broilers fed a typical 22% CP diet. The requirement of Gly seems to be higher in low CP diets than in high CP diets. This requirement seems to be not less than 2.14% Gly + Ser for broilers from 0 to 17 d posthatching based on analyzed values of our diets.

## CHAPTER 8

### SUMMARY AND CONCLUSIONS

This research was conducted to determine requirements of amino acids and to evaluate the use of crystalline amino acid supplemented, low crude protein diets for swine and poultry.

Four experiments were conducted to determine the Lys and sulfur amino acid requirements for 5- to 10-kg pigs. These experiments suggest pigs in this weight range require a diet containing 1.40% true digestible (TD) Lys and the ratio of sulfur amino acids to Lys is not greater than 0.54.

Two experiments were conducted to determine the requirement of Lys and sulfur amino acids in 90-kg barrows using plasma urea nitrogen as the response. These experiments indicate a TD Lys requirement of 0.57% and there was no response to TD sulfur amino acid concentrations above 0.27%. The absence of a response to sulfur amino acid additions indicates that supplemental Met is not needed in a corn diet for late-finishing barrows.

Six experiments were conducted to evaluate the Ile requirement of 80- to 120-kg barrows. These experiments indicate a requirement of 0.34% TD Ile in a corn-blood cell diet; however, the requirement may not be greater than 0.24% in barrows fed a corn-soybean meal diet. These data indicate the use of red blood cells in swine diets may reduce feed intake and performance possibly due to the ratios of branched chain amino acids.

Two experiments were conducted to evaluate the effects of soybean meal and lowering crude protein in diets for late-finishing barrows. Results suggest that reductions

in soybean meal are not the cause of increased carcass fat in pigs fed low crude protein diets. Diets containing soy protein isolate may not support optimal growth when compared to diets containing soybean meal. Furthermore, the fortification of a corn diet with crystalline Lys, Trp, and Thr will not support optimal growth or carcass composition.

Five experiments were conducted to determine the effects of lowering crude protein in diets for broilers and to evaluate limiting essential and nonessential amino acids. Results indicate that low crude protein diets can support optimal growth of broilers when surfeit Gly is supplied in the diet. The requirement of Gly + Ser seem to be not less than 2.14% from d 0 to 17 posthatching.

In conclusion, problems that have been associated with lowering crude protein in diets for broilers are due to a deficiency of Gly and it seems that the requirement for Gly is higher when crude protein is reduced. Although information was obtained for amino acid requirements in swine, further research is needed to evaluate requirements of other essential amino acids and nonessential amino acids and to determine whether or not a deficiency of an amino acid is the cause of reduced performance and/or carcass composition when crude protein is reduced.

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## APPENDIX

### LIST OF ABBREVIATIONS

Item	Abbreviation
Amino acids	AA
Average daily feed intake	ADFI
Average daily gain	ADG
Blood cells	BC
Body weight	BW
Branched-chain amino acids	BCAA
Branched-chain aminotransferase	BCAT
Branched-chain keto-acid deaminase	BKAD
Corn	C
Crude protein	CP
Essential amino acids	EAA
Experiment	EXP
Gain:feed	G:F
Loin muscle	LM
Low crude protein	LCP
Metabolizable energy	ME
Negative control	NC
Net energy	NE
Nitrogen	N
Nonessential amino acids	NEAA
Plasma urea nitrogen	PUN
Positive control	PC
Soybean meal	SBM
Soy protein concentrate	SPC
Soy protein isolate	SPI
Total body electrical conductivity	TOBEC
Total sulfur amino acids	TSAA
True ileal digestible	TD

## VITA

Dustin W. Dean was born July 15, 1976, in Springfield, Missouri. Dustin grew up near Ash Grove, Missouri, until the age of 13 and then moved to near Dadeville, Missouri. He graduated from Dadeville High School in May, 1994. Dustin began his undergraduate education at Fort Scott Community College, where he graduated with an associates of science degree in May, 1996. He then attained his bachelor of science degree, with a major in animal science, at Kansas State University in May, 1998. Dustin began working towards his master of science degree at Kansas State University in June, 1998 and graduated in May, 2000. Dustin moved to Baton Rouge, Louisiana, in June, 2000, and began work as an instructor and coach of the livestock judging team, while working towards the doctor of philosophy degree in animal science at Louisiana State University. Currently, Dustin is a candidate for his doctoral degree in animal science.