

ABSTRACT

BROOME, ASHLEY IKARD. Effects of Supplementing Synthetic Amino Acids into Low Crude Protein Liquid Diets fed to Pigs from 1.5 to 5.5 kg. (Under the direction of Joan H. Eisemann.)

Liquid diets provide a useful tool for producers with low birth weight pigs, as these pigs are at a disadvantage compared to larger littermates. There is limited use of milk replacers on commercial farms due to the high cost of protein sources. A way to potentially reduce the cost of liquid diets is to replace protein sources with synthetic amino acids (AA).

Experiment 1 was designed to determine the amount of synthetic lysine (SL) that could replace lysine from protein and maintain performance similar to pigs fed a diet containing no SL. Pigs (1.62 ± 0.20 kg) were randomly allotted to diets which replaced 0 to 40% of lysine from protein with SL, while maintaining similar GE and total lysine. Diets were fed on a restricted basis ($n=4$ /diet) in order to reduce intake differences. An additional group was fed the 0% SL replacement diet ad libitum (AL, $n=5$) to determine intake level. Intake for restricted pigs was restricted to 80% of AL and adjusted on a daily basis. Pigs fed AL had greater ($P < 0.01$) ADG, ADFI, G:F, water, CP, fat, and ash accretion than restricted fed pigs. Gain, G:F, water, CP, and ash accretion decreased ($P < 0.05$) linearly from 286 g/d, 1.09, 180.6 g/d, 38.6 g/d, and 5.4 g/d for pigs fed the 0% replacement diet to 229 g/d, 0.86, 134.2 g/d, 26.3 g/d, and 3.6 g/d for pigs fed the 40% replacement diet, respectively. Crude protein accretion also showed a quadratic effect ($P < 0.05$) as the decrease occurred more rapidly at greater SL replacement. Fat accretion increased ($P < 0.05$) linearly from 16.4 g/d for pigs fed the 0% replacement diet to 28.4 g/d for pigs fed the 40%

replacement diet. Intake and PUN concentration did not differ ($P > 0.05$) among restricted fed pigs. Replacement of lysine from protein with SL did not produce performance similar to pigs on the control diet. Based on quadratic data for ADG and CP accretion, the next AA could become limiting between 15 and 19% SL replacement.

Experiment 2 was designed to determine the order of limiting AA beyond lysine (LYS). Pigs (1.71 ± 0.30 kg Rep. 1, 1.62 ± 0.11 kg Rep. 2) were allotted randomly to seven diets for a deletion assay using a positive control diet (PC) with AA concentrations and ratios to LYS at or above NRC recommendations, a negative control diet (NC) that reduced AA concentrations and ratios to LYS to 60% of ratios in the PC diet, a supplemented negative control diet (Supp. NC) with AA supplemented to provide concentrations and ratios to LYS similar to those found in the PC diet, and deletion diets which removed threonine (-THR), tryptophan (-TRP), sulfur amino acids (-SAA), or phenylalanine (-PHE) from Supp. NC. All diets contained 4.2 Mcal GE and 20.6 g LYS/kg DM, and were fed ad libitum ($n=8$ /diet). Blood samples were taken to measure PUN. Gain for pigs fed PC (346 g/d), NC (269 g/d), and Supp. NC (315 g/d) diets differed ($P < 0.05$). Gain of pigs fed deletion diets was similar. The SAA deletion diet produced less gain (291 g/d, $P < 0.05$) than the Supp. NC diet. Intake was similar in pigs fed the PC and NC diets, and greater ($P < 0.05$) than for pigs on other diets. Efficiency decreased for pigs fed the NC diet ($P < 0.05$) compared to other diets. Pigs fed the SAA deletion diet had the greatest PUN (6.96 mM, $P < 0.05$). Based on increased PUN concentration and decreased gain relative to the Supp. NC diet, it is likely that the SAA were next limiting.

Effects of Supplementing Synthetic Amino Acids into Low Crude Protein Liquid Diets
fed to Pigs from 1.5 to 5.5 kg

by
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DEDICATION

This thesis is dedicated to my entire family. I could not have done any of this without you.

BIOGRAPHY

Ashley Ikard Broome was born on August 28, 1983 in Spruce Pine, NC. She was the first child of Bruce Ikard and Carla Cook. Her family resides in Spruce Pine, NC where she attended Mitchell High School, and earned her diploma in May 2001. From August 2001 through May 2005 she attended North Carolina State University where she received her Bachelor of Science degree in Animal Science, with a minor in Nutrition. Through her undergraduate coursework she developed an interest in swine nutrition which led her to enroll in the Animal Science graduate program at NCSU in the fall of 2005.

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Chapter 1

LITERATURE REVIEW

Introduction

Approximately 20% of each litter of pigs will be born at what is considered a low birth weight (<1.15 kg; Cabrera et al., 2003). Neonatal pig death, for both normal and low birth weight pigs, occurs for many reasons, such as starvation and disease. Producer identified causes of preweaning death reported by the USDA showed that starvation accounted for 13.8% of neonatal pig death, with scours accounting for 9.3% (USDA-NAHMS, 2007). Low birth weight pigs tend to have a much lower chance of survival than their larger littermates due to competition for milk from the sow, which can lead to starvation (Lecce, 1971). Low birth weights also put these animals at a disadvantage because it is likely that they will not receive the same amount of colostrum as their littermates have received, thus putting them at a greater risk for disease (Lecce, 1971).

Liquid milk replacer diets are being produced and marketed as a supplement to sow's milk, which could serve to increase weaning weights and average daily gain (Azain et al., 1996; King et al., 1998; Dunshea et al., 1999; Wolter et al., 2002). A benefit of the use of milk replacers would be to help reduce the number of deaths caused by starvation by providing more ways for the animals to receive nourishment without having to completely rely on sow's milk. Various types of milk replacers have been used (Azain et al., 1996; King et al., 1998; Dunshea et al., 1999; Wolter et al., 2002). Sources of protein used in milk replacers include whey protein concentrate (Azain et al., 1996; Auldish et al., 1997; King et al., 1998; Kim et al.,

2001) and dried skim milk (Azain et al., 1996; Auldist et al., 1997; King et al., 1998). Milk replacer diets are also used in some situations to ease the transition from liquid to dry feeding in early weaned pigs (Zijlstra et al., 1996; Kim et al., 2001).

In the study by Azain et al. (1996) average weaning weight improved from 5.6 to 6.4 kg, and total litter weight increased from 52.2 to 60.9 kg with the addition of milk replacer. Dunshea et al. (1999) found that with the addition of supplemental milk the average weight of each pig at 20 d of age was 6.74 kg, in comparison to the average weight of 6.13 kg without supplementation. Average daily gain increased with supplementation and was 291 g/d with milk supplementation versus 223 g/d without supplementation (Dunshea et al., 1999). In the study of King et al. (1998) average daily gain increased from 239 to 277 g/d with the addition of supplemental milk from d 0 to 28. Wolter et al. (2002) saw a significant increase in average pig weight from 5.69 to 6.60 kg in week 3 of their study with the supplementation of milk replacer.

Liquid diets also have been used post-weaning to help ease the transition from liquid diets to dry. Zijlstra et al. (1996) found that feeding milk replacers to pigs weaned at 21 d increased ADG from 245 g/d to 319 g/d from d 21 to d 28 of the study when compared to conventional weaning directly to a dry starter diet. Kim et al. (2001) found that feeding liquid diets increased pig weights, as well as increased average daily gain in pigs after weaning when compared with dry diets that were nutritionally equivalent. Kim et al. (2001) found that pigs fed liquid diets were 21% heavier and had 44% greater average daily gain at 14 d after weaning than pigs that were fed the dry diets. In contrast, Armstrong and Clawson (1980) found no

difference in performance when feeding cow's milk along with dry diets versus dry diets alone in early weaned pigs. Animals fed dry diets, supplemented with cows milk gained 90 g/d, and animals fed dry diets alone gained 125 g/d (Armstrong and Clawson, 1980).

Automatic feeding systems have been used to provide milk replacers in farrowing crates (Azain et al., 1996), and in artificial rearing systems (Boyd et al., 1995; Kim et al., 2004). Automatic feeding systems can be used within an artificial rearing system to provide milk replacer diets to low birth weight animals in an attempt to increase their weight to match that of their littermates. Boyd et al. (1995) found that the growth potential was at least 400 g/d for pigs fed through artificial rearing, which is 74% greater than the value of 230 g/d that was found for sow-reared pigs (Boyd et al., 1995). These values are averages from birth to 21 d of age (Boyd et al., 1995).

The reasons behind using automatic feed systems are the ability to supply the pigs with specific nutrients through the feeding of commercial diets, as well as to help maintain or increase growth performance of the piglets (Boyd et al., 1995; Kim et al., 2004). Artificial rearing systems also can be used to provide an environment for low birth weight pigs where there is no risk of crushing.

Automatic feeding systems have been used to help reduce stress at weaning. There are many stressors related to the weaning process. The animal is removed from the sow and its littermates, commingled with animals from other litters, and immediately moved from a liquid diet to a dry diet. It is suggested that with the use of automatic feeding systems in the nursery, the animals can be gradually

transitioned from liquid to dry diets (Dunshea et al., 1999). This cannot completely ameliorate the stresses that occur at weaning, but this provides the potential to reduce a portion of the stress that can occur.

Producers have been hesitant to use manufactured milk replacer diets due to the high cost, as well as additional labor costs due to maintenance of the liquid feeding system. One reason these diets are expensive is because of the protein sources that are used in the production of the diets. Typical protein sources for milk replacers are whey protein concentrates, caseinates, and dried skim milk. In order to decrease the cost of milk replacers, alternatives to conventional protein sources have been studied.

Vegetable proteins, such as hydrolyzed vegetable protein mix and isolated soy protein, were studied as possible alternatives to using traditional protein sources, such as whey protein concentrate for liquid diets. Ebert et al. (2005) used four diets in their study, a positive control diet using whey and containing 22% crude protein as the main protein source, two diets using hydrolyzed vegetable protein mix (HVPM) to substitute for whey protein (replacing either 31% whey protein with HVPM, or 62% of whey protein with HVPM), and a negative control diet that replaced 62% of the whey with isolated soy protein. All diets were formulated to have 4.45 Mcal metabolizable energy per kg of diet. Ebert et al. (2005) found that diets using hydrolyzed vegetable protein mix or isolated soy protein produced significantly greater total weight gain and crude protein deposition than whey protein concentrate. Piglets fed the vegetable protein diets had an average weight of 8.2 kg, with animals fed the whey diet having an average weight of 6.8 kg at the end of

the 17 d study. Animals fed the whey diet also accrued 37 g/d of protein, while animals accrued 49, 55, and 54 g/d of protein on the 31% HVPM diet, the 62% HVPM diet, and the isolated soy protein diet, respectively. Based on increased average pig weight and protein accretion, vegetable proteins appear to be an adequate substitute for more traditional protein sources (Ebert et al., 2005).

Amino acid requirements for neonatal pigs

Another more widely studied alternative to using traditional protein sources is the use of crystalline amino acids to reduce the amount of protein in swine diets (Kerr et al., 1995; Figueroa et al., 2002). Amino acid requirements change as the animal increases in body weight (Coma et al., 1995). There is little information about amino acid requirements for pigs during the early stages of growth (Auldism et al., 1997). Requirements for pigs from 1 to 5 kg were found to be approximately 30% greater than the requirements for pigs from 5 to 10 kg (Kim et al., 1983). There have been many studies used to determine the amino acid requirements presented in the NRC (1998). The majority of the studies performed with young pigs have a starting weight of 5 kg (Seve et al., 1991; Chung and Baker, 1992; Owen et al., 1995), with few studies reporting requirements for pigs less than 5 kg (Leibholz and Parks, 1987; Leibholz, 1988). The NRC used a summary of the research from each weight group to determine each group's requirement for each amino acid. Given the lack of research performed using pigs less than 5 kg, the requirements for this group have been estimated from the data for heavier animals (NRC, 1998). In order to properly formulate diets to maximize growth for animals of this age, it is imperative to have more accurate estimates of amino acid requirements.

Lysine is generally considered the first limiting amino acid in typical nursery diets for swine, as well as grow/finisher diets, containing corn/soybean meal, with methionine being considered the second or third limiting amino acid for nursery pigs when fed these dietary ingredients (NRC, 1998; Gaines et al., 2005). While this is the general consensus, there have been many debates about which amino acid is the first limiting. The first limiting amino acid is defined as the amino acid that is present in the least amount in the diet in relation to the animal's requirement for that amino acid. Lysine is recognized as the first limiting amino acid in corn/soybean meal diets (Russell et al., 1983; Mavromichalis et al., 1998; Soltwedel et al., 2006) and sorghum/soybean meal diets (Brudevold and Southern, 1994).

Auld et al. (1997) used a whey protein concentrate/skim milk powder diet to determine lysine and protein requirements for young pigs between 2 and 7 kg. The diets ranged from 1.72 to 5.44 g lysine/Mcal of gross energy, with crude protein ranging from 10.9 to 34.5%. A lysine requirement of 5.10 g lysine/Mcal of gross energy was determined by Auld et al. (1997) based on a quadratic model. This level of lysine inclusion resulted in a maximum protein deposition of 39.1 g/d.

Methionine also has been considered as a first limiting amino acid in nursery diets for swine fed milk-based protein sources. Nursery diets can be limiting in methionine due to their high concentration of milk based products, such as whey, casein, and dry skim milk (Becker et al., 1955). In addition to milk based products, soy protein diets also have been found to be limiting in methionine, and due to this, Reifsnyder et al. (1984) researched methionine requirements and the use of methionine hydroxyl analogue to supplement diets low in methionine. Pigs can be

fed a number of diets throughout the nursery stage of production. In the early nursery phase, pigs are more likely to be fed milk-product-based diets, before being transitioned to grain based diets. Because of this shift in diets through the nursery phase, the first limiting amino acid during the nursery phase would likely shift from methionine to lysine with the change in diets.

Lysine is used as a reference point for amino acid requirements and amino acid ratios. Baker (1997) states that lysine is used as a reference point for amino acid requirements due to the fact that it is used almost completely for protein accretion and it is also consistently a limiting amino acid.

Ideal protein is considered the exact balance of amino acids needed to meet an animal's needs, whether they be for growth or maintenance (Firman and Boling, 1998). The concept of ideal protein gives requirements of amino acids based on their ratios with lysine. As the requirement for lysine changes based on the stage of production of the animal, the requirements for all other indispensable amino acids will change as well to maintain the ideal protein ratios for that stage of production (Firman and Boling, 1998). By using the concept of ideal protein, there should be minimal excesses or deficiencies in amino acids, as well as minimal nitrogen excretion (Firman and Boling, 1998; NRC, 1998).

Urea nitrogen as an indicator of amino acid deficiency

Nitrogen is excreted in many forms, but it is predominantly excreted in the forms of urea and ammonia in mammals as an end product of amino acid metabolism (Wright, 1995). Urea is synthesized from ammonia and bicarbonate through the urea cycle in the liver (Edmonds and Baker, 1987; Wright, 1995; Wu,

1995). Due to the toxicity of ammonia, much of the ammonia in the body is converted to urea, which is less toxic. Both urea and the ammonia that is not converted to urea are removed from the body through urine (Zervas and Zijlstra, 2002), with ammonia also being excreted in the feces (Shriver et al., 2003).

Urea nitrogen is measured in either the urine or the plasma, and these measurements are used to determine if an animal is receiving adequate protein and/or the appropriate balance of amino acids (Brown and Cline, 1974). Measuring urea nitrogen in the plasma can also give an indication of urea nitrogen levels in the urine (Zervas and Zijlstra, 2002; Shriver et al., 2003).

After prolonged periods of protein deficiency, through either starvation or decreased levels in the diet, the body will attempt to preserve protein, and this can result in decreased urea nitrogen production/excretion (Edmonds and Baker, 1987; Wykes et al., 1996). In the study by Edmonds and Baker (1987), pigs were fasted for either 4 or 8 days or fed a 3% or 30% crude protein diet for the same length of time (Edmonds and Baker, 1987). Both plasma and urinary urea nitrogen decreased with fasting and feeding of the 3% crude protein diet compared to the levels seen in animals fed the 30% crude protein diet (Edmonds and Baker, 1987). Wykes et al. (1996) fed a 3% crude protein diet for 8 weeks, as well as a 20% crude protein control diet. As in the study by Edmonds and Baker (1987), plasma urea nitrogen was decreased over the last 4 weeks of the study in animals fed the 3% crude protein diet compared to animals fed the 20% crude protein control diet. With starvation and extremely decreased levels of protein there is a decrease in urea nitrogen production.

Urinary and plasma urea nitrogen levels also can be indicators of whether an animal is receiving the proper balance of amino acids in their diets. Brown and Cline (1974) saw a linear decrease in both urinary and plasma urea nitrogen with the addition of synthetic lysine and tryptophan to a 16% crude protein corn/soybean meal diet. Both Kephart and Sherritt (1990) and Kerr and Easter (1995) saw significant decreases in plasma urea nitrogen with the feeding of low crude protein diets supplemented with synthetic amino acids. Kephart and Sherritt (1990) supplemented lysine, tryptophan, threonine, isoleucine, methionine, and valine into a low protein (10% crude protein) diet for 20 kg pigs. These amino acids were supplemented into the diet to match the amounts present in the high protein (17% crude protein) diet. Kerr and Easter (1995) used a similar design, supplementing lysine, tryptophan, and threonine into a low protein (12% crude protein) diet in order to provide similar amounts of amino acids to a high protein (16% crude protein) diet for 18 kg pigs. In both of these studies, a decrease ($P < 0.05$) in PUN was seen with the feeding of supplemented low crude protein diets compared to feeding high protein diets. It is likely that this occurred because of the reduction in nitrogen that came from decreased dietary crude protein. These studies also saw a decrease ($P < 0.05$) in PUN when feeding the supplemented low protein diet compared to feeding an un-supplemented low protein diet. This result was likely seen due to an improvement of amino acid balance with the supplementation of synthetic amino acids. This allows for protein synthesis to increase and become more efficient, and this can cause a decrease in urea production (Brown and Cline, 1974; Shriver et al., 2003).

Low crude protein diets supplemented with synthetic amino acids

There has been research done showing that high levels of protein which are common in diets for young, early-weaned pigs could potentially lead to changes in the types of bacteria in the gastrointestinal tract of the pig which can potentially cause post-weaning diarrhea (Wellock et al., 2006). Producers have used dietary antibiotics to help reduce the incidence of post-weaning diarrhea, but a growing concern of producers is the growing prevalence of antibiotic-resistant bacteria (Mathew et al., 1998). Antibiotics are used in animal feeds to enhance growth and feed efficiency, as well as to reduce illness (Nyachoti et al., 2006; Wegener, 2003). Reducing crude protein in nursery diets could potentially reduce post-weaning diarrhea by removing the source of nutrients for the bacteria, and this could in turn allow for a reduction in use of dietary antibiotics (Wellock et al., 2006). Wellock et al. (2006) measured total coliforms, as well as lactobacilli using diets that ranged from 23 to 13% crude protein. With increased crude protein, Wellock et al. (2006) saw a significant increase in coliforms in the digesta in the proximal colon and the feces which could lead to diarrhea. The diets in this study were supplemented with lysine, methionine, threonine, and tryptophan to allow for the decreased amounts of amino acids coming from protein as it was decreased in the diets. To be able to decrease crude protein concentration and maintain performance, diets have been supplemented with synthetic amino acids so that the body would still receive adequate amounts of the essential amino acids (Kerr et al., 1995; Figueroa et al., 2002). There have been concerns about feeding high amounts of synthetic amino acids in diets due to trends for increased body fatness with a reduction in dietary

crude protein (Noblet et al., 1987; Tuitoek et al., 1993; Kerr et al., 1995). Despite this trend for increased fatness, there has been maintenance of ADG and G:F with supplementation of synthetic amino acids (Kerr et al., 1995; Figueroa et al., 2002).

One study was done using a high and low crude protein series of diets covering the nursery, grower, and finisher phases (Kerr et al., 1995). Kerr et al. (1995) found that with the addition of lysine, tryptophan, and threonine to a series of low protein diets containing either 15, 11 or 10% crude protein, making the levels of amino acids equivalent to the levels in a series of high crude protein diets containing 19, 15, or 14% crude protein, produced performance similar to animals on the high crude protein diets. The pigs used in this study were 4 weeks of age, and were fed diets composed of a blend of corn and soybean meal (Kerr et al., 1995). During the starter phase, the animals on the high crude protein (19% crude protein) and the supplemented low crude protein (15% crude protein) diet had an average daily gain of 420 g/d (Kerr et al., 1995).

Figueroa et al. (2002) found that average daily gain was the same in diets supplemented with lysine, tryptophan, threonine, and methionine containing 12% crude protein and a 16% crude protein control diet. There was a significant decrease in average daily gain as crude protein decreased from 12 to 11% (Figueroa et al., 2002). The animals fed the diets containing 12 to 16% crude protein had similar average daily gains; while the animals fed the 11% crude protein diet had lower ($P < 0.05$) gains than animals on all other treatments (Figueroa et al., 2002).

Alternatively, Guay et al. (2006), Nyachoti et al. (2006), Taylor et al. (1979), and Gomez et al. (2002) all saw decreases in average daily gain as protein decreased despite the diets being supplemented with synthetic amino acids. Guay et al. (2006) used 37 kg pigs fed corn/soybean meal diets containing from 7.8 to 16.1% crude protein, supplemented with synthetic amino acids to meet the true ileal digestibility requirements presented in the NRC (1998). Guay et al. (2006) found that reducing crude protein produced a linear decrease in average daily gain.

Nyachoti et al. (2006) performed their experiment with pigs approximately 18 days of age and weighing approximately 6 kg. The diets were formulated to have differing amounts of crude protein, but were formulated to have equal amounts of lysine, isoleucine, leucine, methionine, threonine, tryptophan, and valine in each of the diets. Equal levels of these amino acids were maintained through supplementation with synthetic amino acids. The crude protein varied from 17 to 23%. A linear decrease was seen in average daily gain as crude protein decreased in the diets. There was also a quadratic effect as gain decreased more rapidly at lower crude protein levels (Nyachoti et al., 2006). The diets were a blend of corn/wheat/soybean meal and fed in a mash form (Nyachoti et al., 2006).

Taylor et al. (1979) performed their experiment with 25 kg pigs. The experimental treatments for this study were barley based, and had differing levels of crude protein, ranging from 10 to 18%. All diets were formulated to contain equal amounts of lysine. This was achieved by supplementing the diets with synthetic lysine HCl. Taylor et al. (1979) did see maintenance of average daily gain as crude

protein decreased from 18 to 15%, with the decreases in gain occurring as crude protein decreased from 15 to 10%.

Gomez et al. (2002) performed their experiment with 32 kg pigs, and also found that with the addition of lysine, threonine, tryptophan, and methionine to a 12% crude protein diet there was a decrease in performance when compared to a control diet containing no synthetic amino acids. Lysine was added into the low protein diet up to the level that was present in the control diet, while the additional amino acids were added to provide ideal ratios of the amino acids relative to lysine. Animals fed the control diet gained 1,060 g/d, while animals fed the low protein diet gained 970 g/d (Gomez et al., 2002).

In summary, there are many debates as to whether supplementation of diets with synthetic amino acids can produce performance similar to animals that are receiving diets with no synthetic amino acids. There are differing effects of supplementing synthetic amino acids into diets due to the age of the animals used in the studies, as well as the severity of the reduction of crude protein in the diets. Studying the order of limiting amino acids beyond those that are first limiting will provide the foundation for better formulation of diets and a better balance of amino acids present in low protein diets. With the use of amino acid requirements and the knowledge of the order of limiting amino acids, diets can be formulated using synthetic amino acids and potentially produce performance similar to animals being fed more traditional diets containing no synthetic amino acids. Along with the order of limiting amino acids, the requirements of all of the essential amino acids must be known for animals to be able to grow similar to those animals fed diets containing no

synthetic amino acids. Many studies have examined the use of synthetic amino acids to replace protein for weaning age animals or older. There have been no studies of this kind with neonatal pigs.

Order of limiting amino acids

While, it is generally recognized that either lysine or methionine are the first limiting amino acids in the majority of swine diets, one still has to consider the order of limiting amino acids beyond the first limiting. The addition of the first limiting amino acid without the addition of other essential amino acids in a diet can effectively make another amino acid first limiting. Knowing the order of limiting amino acids in diets will allow for better formulation of the whole amino acid profile, as well as potentially allow for the removal of additional crude protein from diets.

Taylor et al. (1981) used 25 kg pigs, and fed diets ranging from 11.9 to 14.4% crude protein. All diets contained similar levels of lysine, and were barley/soybean meal/white-fish meal based. The dietary treatments consisted of a basal diet with the addition of different combinations of amino acids. Methionine, threonine, tryptophan, and isoleucine were supplemented to the basal diet in groups of three. The researchers found that the addition of threonine was consistent with increased performance, thus leading the researchers to determine that threonine was the second limiting amino acid in barley based diets (Taylor et al., 1981).

Russell et al. (1983) used pigs that were 18 kg. The experimental diets were corn/soybean meal based. The basal diet contained 12% crude protein and the positive control diet contained 16% crude protein. The diets contained equal amounts of lysine and methionine, and the basal diet was supplemented with

tryptophan, threonine, or isoleucine. With the addition of both tryptophan and threonine, or tryptophan alone, the animals gained at a rate similar to those animals on the positive control diet. Animals on the control diet gained 510 g/d, with the animals being fed the basal diet plus tryptophan and threonine gained 543 g/d and animals fed the basal diet plus tryptophan only gained 507 g/d (Russell et al., 1983). In addition, animals fed the basal diet plus threonine only gained 424 g/d (Russell et al., 1983). This led the researchers to conclude that tryptophan was the next limiting amino acid in corn/soybean meal diets beyond lysine and methionine, but with the increase in gain with the addition of tryptophan and threonine to this diet, the researchers determined that threonine becomes limiting very quickly after tryptophan (Russell et al., 1983).

Mavromichalis et al. (1998) used pigs that were an average weight of 8.9 kg. The diets were corn/soybean meal based, and there was a low protein negative control diet that contained 13.5% crude protein supplemented with lysine and a high protein positive control diet that contained 19.2% crude protein. There was one diet in which the negative control diet was supplemented with tryptophan, threonine, methionine, and valine. By using a deletion assay, the researchers found that the deletion of threonine and valine depressed growth the most. Average daily gain for the positive control diet was 533 g/d, and the average daily gain for the supplement diet minus threonine or minus valine was 453 g/d and 402 g/d, respectively (Mavromichalis et al., 1998). The gain to feed ratio was only depressed when threonine was removed from the diet, with the positive control diet having a G:F ratio

of 575 g/kg and the diet minus threonine having a G:F ratio of 472 g/kg (Mavromichalis et al., 1998).

There are many varying results that come from determining the next limiting amino acid beyond lysine, as well as the order of limitation after lysine. Threonine has been considered to be the next limiting amino acid after lysine, and sometimes methionine, with tryptophan and valine also being potentially next limiting in animals that are nursery age or older depending on the protein source used in the diets. While these amino acids have been found to be limiting beyond lysine, these results are still specific to the diets that were studied. Based on the dietary ingredients and the concentrations of those ingredients within the diet, any of the indispensable amino acids could be limiting. Similar to studies dealing with low protein, amino acid supplemented diets, there has been little to no research on the order of limiting amino acids with neonatal pigs. These results could be predicted based on the known order of amino acid limitations in specific protein sources, as well as extrapolations from older animals, but the most accurate results will come from studies performed with young pigs.

Milk replacer diets provide a potential way to help low birth weight pigs reach their growth potential. The supplementation of these diets with synthetic amino acids can reduce the cost of the diets, and possibly make them more widely used in commercial settings. In order to study the possibilities of supplementing low crude protein milk replacer diets with synthetic amino acids, experiment 1 explored the replacement of total dietary lysine with synthetic lysine in an attempt to maintain performance similar to animals fed a diet containing no synthetic amino acids, as

well as determine the point of synthetic lysine replacement where the next limiting amino acid became limiting. Once the point of the next limiting amino acid is determined, it will be important to establish what amino acid is next limiting in the study diets. This information could be used to better supplement low protein diets. Experiment 2 attempted to determine the order of limiting amino acids in whey/dry skim milk/casein diets beyond lysine. It was hypothesized that the predicted order of limiting amino acids in the study diets would be threonine, the sulfur amino acids, phenylalanine, and tryptophan. This order was based on amino acid concentrations in the low protein diet compared to NRC recommendations.

Chapter 2

Response to partial replacement of total dietary lysine with synthetic lysine in pigs from 1.5 to 5.5 kg fed liquid diets

INTRODUCTION

Liquid diets can provide a useful tool for producers to help increase growth performance of low birth weight pigs (≤ 1.15 kg). Low birth weight pigs are at a disadvantage compared to their larger littermates. Due to their small size they will likely have problems nursing the sow due to competition with other, larger littermates (Lecce, 1971). Starvation accounts for 13.9% of neonatal pig death (USDA-NAMHS, 2007), and one way to potentially alleviate this problem is to supplement low birth weight pigs with a liquid milk replacer diet until weaning (Azain et al., 1996; King et al., 1998; Dunshea et al., 1999; Wolter et al., 2002).

Presently there is limited use of liquid milk replacer diets on commercial farms. This is due partially to the increased amounts of labor that feeding these diets entail. New technology is constantly being developed to ease the feeding of liquid diets in a commercial setting. Automatic feeding systems have been used in both farrowing crates, as well as in artificial rearing systems in which pigs are removed from the sow (Boyd et al., 1995; Zijlstra et al., 1996; Kim et al., 2004); however a more difficult problem is the cost of liquid diets.

Milk replacers are expensive due to the use of whole protein sources in the diets. A way to potentially reduce the cost of liquid diets is to supplement synthetic amino acids into low protein diets. The objective of this study was to determine the

amount of synthetic lysine that could replace lysine from protein in liquid diets and maintain performance similar to animals on a control diet containing no synthetic lysine.

MATERIALS AND METHODS

All procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee. Pigs were obtained from the North Carolina State University swine farm.

Animals and Diets

Thirty male and female pigs were weaned between 24 and 48 h after birth. Prior to being removed from the sow, pigs underwent processing that included injection with iron and needle teeth clipping. The pigs weighed 1.617 ± 0.202 kg initially, and were randomly assigned to one of 7 treatment groups. There were 6 experimental diets that ranged from 0% to 50% (increasing from 0% to 50% in 10% increments) of the total lysine being replaced by synthetic lysine. Synthetic lysine was included in increasing percentages to determine how much lysine from crude protein could be removed from the diet before other amino acids became limiting, thus limiting the growth of the animals. The 6 experimental diets were fed at a constrained intake, with 4 pigs per treatment. The 6 remaining pigs were assigned to the 0% synthetic lysine replacement diet, but were fed ad libitum, which was the seventh treatment group.

The amount of feed given to each restricted-fed pig was determined each morning based on the average metabolic body weight of all of the constrained pigs. The restricted fed pigs were targeted to gain at 80% of the ad libitum treatment group's gain.

The experimental diets were formulated to contain similar GE and lysine concentration. The level of lysine used was 4.8 g Lys/Mcal GE. This is lower than

the requirements found by Auldist et al. (1997) and Lewis et al. (2006). The lower value was used in order to ensure that lysine was first limiting in the study diets, as well as to allow for differences between the treatments to be seen more easily. The amount of lysine in each diet remained similar across treatments, but the amount of lysine from protein varied (Figure 2.1). Overall crude protein varied between treatments as well (Table 2.1).

Dry ingredients were assembled to mix the 0% synthetic lysine replacement diet and the 50% synthetic lysine replacement diet. Once mixed, 180 g of dry diet/kg of water were mixed using a Fisher Dyna-mix (Fisher Scientific; Pittsburg, PA) for 5 min. and homogenized with a T50 basic homogenizer (Ultra-Turrax; Pittsburg, PA) for 5 min. Gentamicin (100 mg/mL) was added to all diets at 0.01 mL per kg of water to reduce the incidence of scours throughout the course of the study. Proper proportions of the 0% replacement diet and the 50% inclusion diet were blended to make the intermediate diets. Liquid diets were stored at 4°C and used within 2 d after the addition of water.

Housing and Management

After weaning, pigs were moved into individual cages. The temperature in the room was maintained between 26 and 29°C for the study, and supplemental heat was also provided by heat lamps placed between the cages (one lamp for every two pigs). Pigs were trained to drink by sucking the diet through a rubber nipple that was attached to a piece of plastic tubing with one end placed into a plastic bottle hanging above each cage. Pigs were trained using the 0% replacement diet on d 0, and

were assigned to their respective diet on d 1. Weights were recorded at 0800 on d 1 for the beginning of data collection for the experiment.

Pigs were fed at 0800, 1600, and 2300 daily. Weigh backs were measured at the 0800 and the 1600 feedings to determine how much each pig was consuming of its respective diet per day. Pigs were weighed daily at the 0800 feeding. After all pigs were weighed, the amount to be fed was determined based on the average body weight of the restricted fed pigs and the feeding rate that would allow these animals to be fed at 80% of the ad libitum fed pigs. Pigs fed ad libitum were fed 20% more feed than had been eaten the day before.

Tissue Collection

Pigs were maintained on treatments until they weighed between 5.5 and 5.8 kg. Each pig was electrocuted and exsanguinated and the gastrointestinal tract was removed and emptied (including stomach, small and large intestine). The gall bladder and urinary bladder were emptied as well. All tissues were saved, including blood, and were frozen at -20°C until further processing for analysis. Before freezing, an empty body weight was recorded.

Pigs were allowed to thaw for approximately 14 h before further processing. Pigs were ground using a grinder (Tor-rey; Model M-22R1; Houston, TX). Before grinding, pigs were cut into small pieces using a reciprocating saw (DeWalt; Model DEWDW304; Hampstead, MD) so that they could fit through the guard plate on the top of the grinder. The empty body was passed twice through a large die, three times through a 1.5 cm die, once through a 0.5 cm die, and once more through the 1.5 cm die to ensure a homogenous mix. Two representative samples were taken

following the final pass through the 1.5 cm die and stored at -20°C until further processing.

Laboratory Analysis

Dry matter of the empty body was determined by freeze drying the ground samples. The samples were lyophilized for 2-3 days. The condenser of the freeze dryer was maintained at -60°C , while the silicon fluid heating the trays of the freeze dryer were maintained at 75°C . The dry weight was used to determine the dry matter content of the empty body. Dry samples then were frozen in liquid nitrogen and powdered using a blender (Waring; Model 34BL22; New Hartford, CT). Aliquots of the powdered samples were analyzed for ash, crude protein, and fat content. Crude protein was determined by Kjeldahl analysis (AOAC, 1997) and fat by the Folch procedure (Folch et al., 1957). Ash was determined as the residual sample following 20 hours in a muffle furnace at 550°C .

For each chemical analysis, sample dry matter was determined by placing 2 g samples in an oven at 101°C for 20 h. Dry matter of the liquid diet was determined by placing 3 g of milk in an oven at 101°C for 22 h.

Crude Protein Content

Approximately 0.2 g of tissue, a Kjel tab, 7.5 mL of sulfuric acid, and two glass beads were placed in a digestion tube and vortexed. After this, 1.5 mL of 30% hydrogen peroxide was added in increments of 0.5 mL, and then an additional 2 mL of hydrogen peroxide were added to the digestion tube in 1 mL increments giving a final volume of 3.5 mL of hydrogen peroxide. Once this reaction had calmed, the mixture was vortexed. The digestion tubes were then placed in a 360°C digestion

block and were heated for 6 hours. After the 6 hours of digestion, the tubes were removed from the block and allowed to cool for 1 hour before dilution. Deionized water was added to each tube as they were vortexed. Each tube was then filled up to the 75 mL mark on the digestion tube and stoppered. Each tube was then inverted several times to mix the contents, and then the tubes were allowed to cool overnight. The tubes were then readjusted back to 75 mL and mixed again by inverting. Approximately 25-30 mL from each tube was poured into a pre-labeled 30 mL plastic bottle and was refrigerated until the samples were analyzed by a Technicon Auto-analyzer (Technicon Industrial Systems; Tarrytown, NY). Nitrogen was determined based on a colorimetric method, with color produced by the reaction of ammonia, sodium salicylate, sodium nitroprusside, and sodium hypochlorite (AOAC, 1997).

Fat Content

Fat content of the empty body was determined by the Folch procedure (Folch et al., 1957). Approximately 1 g of tissue was placed in a 50 mL polypropylene homogenization tube, and then 20 mL of a chilled chloroform-methanol (2:1) solution was added to each tube. The contents of the tubes were then homogenized for 1 min using a polytron (Brinkmann Instruments; Westbury, NY). The homogenate was filtered into a second 50 mL centrifuge tube through Whatman number 1 filter paper. Four mL of 0.9% saline solution was added to each tube, and then each tube was vortexed. All tubes were then centrifuged for 10 min at 1000 x g at 4°C. After centrifuging, the lower phase was removed from each tube with disposable 22.9 cm borosilicate glass Pasteur pipets (Fisher Scientific; Pittsburgh, PA) and transferred to

dried and weighed scintillation vials. The samples were then dried for 1-2 hours using nitrogen gas, and then placed in an oven at 104°C for 2 h. The samples were placed in a dessicator for approximately 30 min to cool, and were then weighed.

Plasma Urea Nitrogen and Plasma Amino Acids

One blood sample was taken on d 11 of the study to measure plasma urea nitrogen concentration and plasma amino acid concentrations. Animals were all fed by 0900, with blood samples being taken at 1100. The blood samples were centrifuged for 20 min at 1000 x g at 4°C (Beckman J-6B; Golden Valley, MN), and the plasma was removed and stored at -20°C. Plasma urea nitrogen concentration was analyzed using a colorimetric assay based on the Berthelot procedure (Teco Diagnostics; Anaheim, CA). Each sample and test standard were analyzed in duplicate. All of the kit reagents were at room temperature before the assay was performed. The enzyme reagent (1.5 mL) was added to each tube before the addition of 10 µL of sample. Each tube was then vortexed and placed in a water bath at 37°C for 5 min. The tubes were removed from the water bath, and 1.5 mL of the color reagent was added to each tube. The tubes were vortexed again, and then placed in the water bath for an additional 5 min. Once the tubes were removed from the water bath, the absorbance was read on a spectrophotometer at 580 nm and the data were recorded. Plasma samples were sent to Ajinomoto Heartland LLC (Chicago, IL) to be analyzed for total amino acid concentrations.

Efficiency of Nitrogen Retention

The efficiency of nitrogen retention was calculated by dividing crude protein retention of the empty body by crude protein intake. Crude protein retention was

determined by subtracting an initial crude protein value from the data from Lewis et al. (unpublished) from an ending crude protein amount for each pig based on the whole body composition from the current study. Crude protein intake was determined by combining the percentage of crude protein in the diet and the feed intake for the entire study.

Statistical Analysis

The data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., version 9.1, Cary, NC). Each individual pig was the experimental unit. Performance data (ADG, ADFI, and G:F), as well as water, CP, fat, and ash accretion, PUN concentration, CP intake, days on study, and efficiency of nitrogen retention, were analyzed by ANOVA for effects of dietary treatment. Orthogonal linear combinations of the means were used to test for a linear and quadratic effect to increasing amounts of synthetic lysine used to replace dietary lysine in the diets. A contrast comparing data from pigs fed the 0% synthetic lysine inclusion diet ad libitum with data from all pigs fed the restricted diets also was included. A quadratic equation was fit to the ADG and CP accretion data using the PROC NLIN procedure of SAS. PROC NLIN was used to determine the 95% confidence interval of the predicted gain or CP accretion at 0% synthetic lysine replacement.

RESULTS

Performance Data

Due to depressed growth rate, the animals fed the 50% synthetic lysine replacement diet were removed from the study. Also, one pig originally in the 0%

replacement ad libitum fed group was removed from the study due to illness. All data are from animals fed the 0% to 40% synthetic lysine replacement diets. The source of lysine, whether from protein or synthetic lysine, significantly influenced growth performance of pigs from 1.5 to 5.5 kg. Average daily gain and G:F ratio showed a linear decrease ($P < 0.01$) as the amount of total lysine replaced with synthetic lysine increased in the restricted diets (Figures 2.2, 2.3; Table 2.2). Average daily feed intake did not differ ($P > 0.05$) among the restricted diets (Figure 2.4; Table 2.2). Crude protein intake also showed a linear decrease ($P < 0.01$) as the amount of total lysine replaced with synthetic lysine increased in the restricted diets (Table 2.2). The average number of days on study linearly increased ($P < 0.01$) as the amount of total lysine replaced with synthetic lysine increased in the restricted diets (Table 2.2). Number of days on study also showed a quadratic response ($P < 0.05$) as the number of days on study increased more rapidly as synthetic lysine replacement increased (Table 2.2). Pigs fed the 0% synthetic lysine replacement diet ad libitum had greater ($P < 0.01$) ADG, G:F, ADFI, and CP intake, and fewer ($P < 0.01$) days on study than pigs fed the restricted diets (Table 2.2).

Body Composition Data

Body composition also was affected by the source of lysine in the diets of pigs. Empty body weights were not different among treatments. Percentage composition of the empty body of the 0% replacement, restricted fed group was 75.9% water, 24.1% dry matter, 15.5% protein, 5.2% fat, and 2.9% ash (Table 2.3). With increased synthetic lysine replacement in the diets water, protein, and ash

percentages decreased ($P < 0.01$; Table 2.3), while fat and dry matter percentages increased ($P < 0.01$; Table 2.3).

Water, CP, and ash accretion decreased ($P < 0.01$) linearly as synthetic lysine increased in the restricted diets (Figures 2.5, 2.6, 2.7; Table 2.4), similar to the effect seen in percent body composition. Crude protein accretion also showed a quadratic effect ($P < 0.05$) as the accretion decreased more rapidly at greater synthetic lysine replacement (Figure 2.6; Table 2.4). In contrast, fat accretion showed a linear increase ($P < 0.01$) as synthetic lysine replaced increasing amounts of total lysine in the restricted diets (Figure 2.8; Table 2.4). Efficiency of nitrogen retention showed a quadratic response ($P < 0.05$) between the restricted treatments (Figure 2.9; Table 2.4). Pigs fed the 0% synthetic lysine replacement diet ad libitum had greater ($P < 0.01$) water, CP, ash, and fat accretion than pigs fed the restricted diets. Pigs fed the 0% synthetic lysine replacement diet ad libitum had a trend ($P = 0.06$) for different efficiency of nitrogen retention than animals fed the restricted diets.

A quadratic equation was fit to the average daily gain and crude protein accretion data. This was used to estimate the level of synthetic lysine replacement that would produce average daily gain or crude protein accretion at 95% of the rate of pigs fed the restricted 0% synthetic lysine replacement diet (Figures 2.10, 2.11). These values were 14.8% and 19.0% of total lysine replaced with synthetic lysine, for average daily gain and crude protein accretion, respectively.

Plasma Urea and Amino Acids

Plasma urea nitrogen concentration did not differ ($P > 0.05$) among pigs fed restricted diets, but there was a trend ($P = 0.079$) for a decrease in PUN concentration as synthetic lysine replacement increased. Plasma urea nitrogen was greater ($P < 0.01$) for pigs fed the 0% synthetic lysine replacement diet ad libitum than pigs fed the restricted diets (Figure 2.12; Table 2.4). Amino acid concentrations were measured in plasma samples taken from each pig on day 11 of the study. There were no linear or quadratic responses ($P > 0.05$) among treatments as the proportion of total lysine being replaced by synthetic lysine increased. Lysine, cysteine, methionine+cysteine, arginine, isoleucine, leucine, valine, histidine, alanine, glutamate, aspartate, phenylalanine, proline, and tyrosine were present in greater ($P < 0.05$) concentrations in plasma samples taken from pigs fed the 0% synthetic lysine replacement diet ad libitum when compared to values from the pigs fed the restricted diets (Table 2.5). Threonine, methionine, glycine, and serine were the only amino acids that were not present in different ($P > 0.05$) concentrations in the 0% synthetic lysine replacement diet fed ad libitum compared to the diets fed at a restricted level (Table 2.5).

DISCUSSION

In the current study, protein was removed from the diet and synthetic lysine was added to maintain a constant percentage of lysine in the diet. The current study focused on young, early weaned animals fed liquid diets. Removal of crude protein from liquid diets provides a way of potentially reducing the cost of these diets (Kim et al., 2001). Supplementation of diets with synthetic amino acids provides a method

for successfully removing additional crude protein and maintaining growth similar to animals fed more traditional diets (Kerr et al., 1995; Figueroa et al., 2002). Lysine was supplemented to the study diets based on its position as the most common first limiting amino acids in nursery pig diets (Russell et al., 1983; Mavromichalis et al., 1998; NRC, 1998; Gaines et al., 2005; Soltwedel et al., 2006), as well as its use as a reference point for other amino acid requirements. Once the total amount of dietary lysine that can be replaced by synthetic lysine is determined, additional amino acids can be supplemented into the diets, which could improve the overall balance of amino acids.

There was a linear decrease in ADG as total lysine replacement increased in the diets and dietary crude protein decreased from 24 to 14.5%. This is similar to the result seen in the study by Taylor et al. (1979) using 25 kg pigs as crude protein decreased below 15% in the diets. Growth was maintained in the Taylor et al. (1979) study as crude protein was decreased from 18 to 15% (Taylor et al., 1979). The current study showed a linear decrease in gain once lysine from protein began to be removed. The data from the current study and the data from the Taylor et al. (1979) were analyzed differently, with the data from the current study being analyzed by a linear contrast and the Taylor et al. (1979) was analyzed using intersecting regression lines. Despite the differences in the data analysis, both studies showed that once crude protein is decreased to a point, the addition of lysine alone cannot sustain ADG. It is possible that due to the stage of growth (growing pigs; 25 kg) of the pigs used in the study by Taylor et al. (1979), the pigs were able to better accommodate the decrease in crude protein, thus growth was able to be maintained

longer as crude protein was decreased. Data from additional studies also demonstrate decreased average daily gain as crude protein was removed from the diets (Gomez et al., 2002; Guay et al., 2006; Nyachoti et al., 2006), but these studies supplemented additional amino acids beyond lysine. Gomez et al. (2002) decreased crude protein from 16 to 11%, Guay et al. (2006) decreased crude protein from 16 to 8%, and Nyachoti et al. (2006) decreased crude protein from 23 to 17%. In addition to the decreases in average daily gain, these studies also showed decreases in feed efficiency (Gomez et al., 2002; Guay et al., 2006; Nyachoti et al., 2006). These results are similar to the results from the current study as feed efficiency decreased as the percentage of total lysine replaced by synthetic lysine increased in the diets.

The decreases in performance could be the result of an amino acid imbalance that occurred as protein was removed from the diet. This is a likely cause of the depressed growth seen in the current study and the study by Taylor et al. (1979) because lysine was the only amino acid supplemented. It is possible that the amounts of amino acids added in the additional studies were not adequate to maintain growth. Another potential cause of the decrease in gain is a lack of total nitrogen in the diet because of the reduced protein. The decreases in feed efficiency seen in both the current study and the aforementioned studies was not surprising due to the decreased gain seen in all of these studies while feed intake was maintained. A reduction in nitrogen could impair the body's ability to produce non-essential amino acids, which could again lead to an imbalance of amino acids. An imbalanced supply of amino acids would limit the body's ability to synthesize protein. The unused amino acids would likely be broken down and used as a source of

energy in the body (Berg et al., 2002). These explanations also provide a reason for the reduction in crude protein accretion that was seen as lysine from protein was replaced with synthetic lysine.

Other similar studies have shown the ability to feed pigs diets containing additional synthetic amino acids and maintain levels of growth equivalent to animals fed a diet containing no synthetic amino acids (Kerr et al., 1995; Figueroa et al., 2002). It is possible that these researchers found more positive results due to better diet formulation. Through better formulation, a better balance of amino acids could have been present in these diets, thus leading to better performance. These studies supplemented an array of essential amino acids, whereas our study only supplemented lysine. Because of this, it is not surprising that we did not see the same maintenance of performance as protein was removed from the diets. As previously described, the current study diets most likely became limiting in additional essential amino acids as crude protein was decreased in the diets, which would lead to a decrease in performance.

A quadratic equation was fit to the data from the current study. This was done in order to estimate an acceptable level of total lysine to be replaced with synthetic lysine. This value was based on the 95% confidence interval surrounding the values for gain and crude protein accretion for animals fed the 0% synthetic lysine replacement diet. A quadratic model was used as it relates well to physiological mechanisms (Auldist et al., 1997). With no amino acid supplementation performance is effectively at a plateau, and as synthetic lysine is increased in the diet and total lysine from protein is removed it is expected that there

will be a decrease in performance that will be well fit by a quadratic function. When using the data for average daily gain a value of 14.8% synthetic lysine replacement was produced. Crude protein accretion data produced a value of 19.0% synthetic lysine replacement. These values for average daily gain and crude protein accretion represent amounts of synthetic lysine that could be supplemented into the diet and produce growth or accretion at 95% of the values for animals fed a diet containing no synthetic lysine. There were different values for synthetic lysine replacement based on the gain data and the crude protein data. This is likely due to the fact that when accounting for CP accretion, there is only one factor to account for, but when dealing with ADG, there are multiple factors to account for, such as gaining protein, water, fat, and ash. Due to the fact that ADG accounts for accretion of many compounds, there are more factors that affect the amount of total lysine that can be replaced with synthetic lysine, which could explain the decreased level of supplementation found when looking at the 95% confidence interval for gain.

Accretion rates of water and protein decreased linearly ($P < 0.05$) as crude protein was removed from the diet. A linear increase ($P < 0.05$) in fat accretion was also seen as crude protein decreased in the diets. This is different from the results found in a similar study (Noblet et al., 1987). The low protein, high lysine diet fed in the study by Noblet et al. (1987) with 20 kg pigs maintained water, protein, and fat accretion values similar to those from animals fed a high protein, high lysine diet. Fat accretion also showed similarities between the low protein, high lysine diet and the low protein, low lysine diet. These similarities could be due to a trend for increased body fatness with supplementation of synthetic amino acids (Noblet et al.,

1987; Tuitoek et al., 1993; Kerr et al., 1995). In the current study, as crude protein decreased and other amino acids besides lysine were not supplemented in diets, it was not surprising that water and protein accretion decreased in the body. The diets fed in the Noblet et al. (1987) study were supplemented with lysine, threonine, and tryptophan, which would likely explain why a reduction in crude protein allowed for water, protein, and fat accretion values to remain similar. With lower amounts of protein in the diet, and the inevitable limitations in amino acids that will occur with a reduction in crude protein, there will tend to be less protein and more fat accrued in the body. With less protein and increased fat present in the body; there will be less water accretion as water is stored with protein and not fat.

There was a linear increase in fat accretion seen in the data from the current study. This could be due to trends seen for increased body fatness with a reduction in dietary crude protein (Noblet et al., 1987; Tuitoek et al., 1993; Kerr et al., 1995). With a reduction in dietary crude protein, there will likely be an increase in dietary fat. This occurred in the current study as the amount of total dietary lysine was replaced with synthetic lysine. To maintain similar amounts of GE in the dietary treatments, a combination of lactose and fat were added to the diets. As dietary protein decreased, dietary fat increased at a more rapid rate than lactose in the diets. A study done by Oliver et al. (2005) was designed to determine the response of piglets to feeding high fat, low lactose and low fat, high lactose diets. Animals on both treatments in the Oliver et al. (2005) study had similar ME intakes, despite the energy source. Although total body composition calculations were not performed, Oliver et al. (2005) did measure circulating non-esterified fatty acids (NEFA)

concentrations. With the feeding of the high fat, low lactose diet, Oliver et al. (2005) found increased concentrations of NEFA in the blood, which would provide dietary fatty acids for greater fat accretion than a lower fat diet. In the current study, as fat increased in the diets, fat accretion also increased. This result agrees with the increase in circulating NEFA concentrations found in the study by Oliver et al. (2005). If circulating NEFA concentrations increased in the current study this would explain the increase in fat accretion and the decrease in protein accretion seen with the increasing replacement of total dietary lysine with synthetic lysine. Another explanation for an increase in fat accretion would be the decrease in dietary protein that occurred with the supplementation of increasing amounts of synthetic lysine.

Efficiency of nitrogen retention showed a quadratic response as replacement of dietary lysine with synthetic lysine increased, with efficiency increasing from 68% with 0% synthetic lysine replacement to 78% with 20% synthetic lysine replacement, and decreasing to 72% with 40% synthetic lysine replacement. Kerr and Easter (1995) found increased efficiency of nitrogen retention with the supplementation of lysine, tryptophan, and threonine to a low protein diet. This suggested that there was an increase in the utilization of dietary amino acids for laying down body protein. Similar results were seen with supplementation of lysine, methionine, threonine, tryptophan, isoleucine, and valine to a 14% crude protein diet in a study by Shriver et al. (2003). In the current study, the supplementation of lysine into low crude protein diets increased the efficiency of nitrogen retention to a point after which the efficiency began to decrease. This quadratic response likely occurred due to limitations in additional amino acids beyond lysine. Based on the quadratic data for

CP accretion, it is possible that the next limiting amino acid becomes limiting at 19.0% synthetic lysine replacement, which is where the efficiency of nitrogen retention reaches its maximum value. Once additional amino acids become limiting in the diet, it is expected that the efficiency of nitrogen retention would begin to decrease as protein synthesis becomes less efficient.

Plasma amino acid concentrations did not differ as crude protein decreased in the diets for the current study, but there were numerical differences seen between pigs fed the 0% synthetic lysine replacement and the 40% synthetic lysine replacement diets. This is different from the results of the study by Figueroa et al. (2002), where as the amount of crude protein decreased in the diets, the essential amino acids that were not supplemented into the diets significantly decreased in the plasma. Plasma urea nitrogen concentrations also decreased as crude protein levels decreased (Figueroa et al., 2002). PUN concentrations did not differ as crude protein intake decreased during the current study, but there was a trend ($P = 0.079$) for a decrease. It would be expected that the concentrations of amino acids and urea nitrogen would decrease as the crude protein intake decreased. Based on the data from previous research showing a decrease in plasma amino acid and urea nitrogen concentrations with a decrease in protein, there is no logical explanation as to why there was no change in plasma amino acid concentrations or PUN concentrations as crude protein intake decreased in the restricted fed pigs. Because there was a trend ($P = 0.079$) for a difference, it is possible with a larger sample size differences could be seen. The only differences seen in plasma amino acids and PUN concentrations in the current study occurred between the ad libitum treatment

and the restricted treatments. Crude protein intake was greater ($P < 0.01$) in animals fed the ad libitum treatment than in animals fed the restricted treatments, which was to be expected. It is likely that the increased concentrations of plasma amino acids and PUN found in the pigs fed the 0% synthetic lysine replacement diet ad libitum was due to the additional feed intake, which led to the increased crude protein intake seen in the ad libitum fed pigs.

Based on the linear and quadratic contrast data from this study, the addition of synthetic lysine alone did not produce performance similar to animals on the control diet. It is possible, based on the quadratic ADG and CP accretion data that 15 to 19% of total dietary lysine could be replaced with synthetic lysine. The results from the linear and quadratic contrasts used to analyze study data show that it is necessary to supplement additional amino acids to low crude protein diets to potentially produce similar performance to animals fed diets containing no synthetic amino acids.

Table 2.1: Composition of experimental diets (g/kg air dry before being constituted with water).¹

Ingredients, g/kg	% Total lysine replaced with synthetic lysine					
	0%	10%	20%	30%	40%	50%
AMP 80 ²	27.0	24.3	21.6	18.9	16.2	13.5
NFDM ²	524.0	469.8	415.6	361.4	307.2	253.0
Lactose	312.3	358.3	404.2	450.2	496.1	542.1
Fat Pak 80 ²	66	77.1	84.2	93.4	102.5	111.6
Sodium Casienate	40	36	32	28	24	20
Xanthan Gum	10	10	10	10	10	10
Lysine HCl	0.0	2.6	5.2	7.8	10.4	13.1
CaCO ₃	2.7	2.9	3.1	3.4	3.6	3.8
Dicalcium Phosphate	6.8	9.8	12.7	15.7	18.6	21.6
Mineral Premix ³	5	5	5	5	5	5
Vitamin Premix ^{4,5}	1.3	1.3	1.3	1.3	1.3	1.3
NaCl	5	5	5	5	5	5
Calculated Composition						
Total Lysine, g/kg	20.6	20.6	20.6	20.6	20.6	20.6
Crude Protein, g/kg	241.6	217.4	193.3	169.1	145.0	120.9
Fat, g/kg	59.4	66.0	72.6	79.2	85.9	92.5
Lactose, g/kg	592.7	611.1	629.4	647.9	666.1	684.5
Gross Energy, Mcal/kg	4.3	4.3	4.3	4.3	4.3	4.3
Lysine, g/Mcal GE	4.8	4.8	4.8	4.8	4.8	4.8

¹ All diets were made to contain 180 g dry diet in addition to 1 kg water to give 1.18 kg liquid diet.

² AMP 80=Whey protein concentrate (American Protein Corporation, Ankeny, IA), NFDM=Non-fat dry milk (Milk Specialties, Dundee, IL), Fat Pak 80= Animal fat blend (Milk Specialties, Dundee, IL).

³ Added to contain mg/kg liquid diet: Calcium, 7.51; Phosphorous, 4.12; Sodium, 2.13; Chloride, 0.3; Iron, 15; Potassium, 15.18; Cobalt, 0.15; Copper, 1.39; Iodine, 0.3; Magnesium, 0.77; Manganese, 3.75; Selenium, 0.05; Zinc, 17.63 (Merricks, Inc, Middleton, WI).

Table 2.1 (continued)

⁴ Added to contain mg/kg liquid diet: Calcium, 31.2; Vitamin C, 50.19; Pantothenic Acid, 5.85; Niacin, 6.45; Riboflavin, 1.63; Vitamin K, 1.0; Biotin, 0.01; Vitamin B 12, 8.58; Thiamine, 0.40; Vitamin B 6, 0.78; Folic Acid, 0.54 (Merricks, Inc, Middleton, WI).

⁵ Added to contain IU/kg liquid diet: Vitamin A, 6,435; Vitamin D, 1,287; Vitamin E, 10.7 (Merricks, Inc, Middleton, WI).

Table 2.2: Effect of percent replacement of total lysine with synthetic lysine on average daily gain, feed intake, feed efficiency, crude protein intake, and days on study.¹

Variable	% Total lysine replaced with synthetic lysine						SEM
	0%	10%	20%	30%	40%	0% ad lib	
ADG, g/d ^{2,4}	286	283	268	252	229	363	6.50
ADFI, g DM/d ⁴	256	249	250	263	266	317	7.53
G:F ^{2,4}	1.12	1.14	1.07	0.97	0.86	1.15	0.04
CP Intake, g/d ^{2,4}	61.9	54.1	48.4	44.4	38.6	76.6	1.50
Days on Study ^{2,3,4}	14.3	14.0	14.8	16.0	17.5	11.4	0.42

¹ Values are least square means. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. SEM for n = 4. The 0% to 40% treatments: Diets had 0 to 40% of lysine from protein replaced with synthetic lysine. The 0% ad lib: 0% lysine replacement diet fed ad libitum.

² Linear response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.01$).

³ Quadratic response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.05$).

⁴ Values were different between restricted and ad libitum diets ($P < 0.01$).

Table 2.3: Effect of percent replacement of total lysine with synthetic lysine on empty body weight, water, dry matter, ash, fat, and protein composition in the empty body.¹

Variable	% Total lysine replaced with synthetic lysine					0% ad lib	SEM
	0%	10%	20%	30%	40%		
Empty Body Weight, kg	5.29	5.16	5.34	5.38	5.30	5.36	0.07
Water, % ²	75.88	74.90	74.07	72.41	70.54	74.06	0.42
Dry Matter, % ²	24.13	25.10	25.94	27.59	29.46	25.94	0.42
Ash, % ²	2.85	2.66	2.73	2.61	2.54	2.71	0.07
Fat, % ²	5.22	6.15	7.11	8.68	10.36	6.87	0.37
Protein, % ^{2,3,4}	15.50	15.44	14.62	14.24	13.36	15.24	0.18

¹ Values are least square means. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. SEM for n = 4. The 0% to 40% treatments: Diets had 0 to 40% of lysine from protein replaced with synthetic lysine. The 0% ad lib: 0% lysine replacement diet fed ad libitum.

² Linear response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.01$).

³ Quadratic response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.05$).

⁴ Values were different between restricted and ad libitum diets ($P < 0.01$).

Table 2.4: Effect of percent replacement of total lysine with synthetic lysine on water, protein, fat, and ash accretion, plasma urea nitrogen concentration, and efficiency of nitrogen retention in the empty body.¹

Variable	% Total lysine replaced with synthetic lysine					0% Ad Lib	SEM
	0%	10%	20%	30%	40%		
Water, g/d ^{2,4}	180.6	172.8	171.3	155.9	134.2	220.2	5.67
Protein, g/d ^{2,3,4}	38.6	38.1	35.4	32.7	26.3	48.7	1.33
Fat, g/d ^{2,4}	16.4	19.6	22.5	25.6	28.4	27.7	1.22
Ash, g/d ^{2,4}	5.4	4.6	4.9	4.5	3.6	6.2	0.39
PUN, mM ⁴	2.10	1.57	1.49	1.36	1.40	3.88	0.27
Efficiency of N Retention ^{3,5}	0.68	0.75	0.78	0.77	0.72	0.69	0.02

¹ Values are least square means. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. SEM for n = 4. The 0% to 40% treatments: Diets had 0 to 40% of lysine from protein replaced with synthetic lysine. The 0% ad lib: 0% lysine replacement diet fed ad libitum.

² Linear response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.01$).

³ Quadratic response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.05$).

⁴ Values were different between restricted and ad libitum diets ($P < 0.01$).

⁵ Efficiency of nitrogen retention was calculated by dividing crude protein retention of the empty body by crude protein intake.

Table 2.5: Effect of percent replacement of total lysine with synthetic lysine on plasma amino acid concentrations.¹

AMINO ACID	% Total lysine replaced with synthetic lysine ²					AD LIB ²	SEM ²
	0% ²	10% ²	20% ²	30% ²	40% ²		
Lysine ³	0.378	0.356	0.344	0.361	0.356	0.447	0.017
Threonine	0.261	0.247	0.248	0.256	0.257	0.271	0.015
Methionine	0.030	0.034	0.032	0.032	0.034	0.032	0.002
Cystine ³	0.164	0.159	0.148	0.148	0.156	0.186	0.007
Met + Cys ³	0.194	0.193	0.179	0.180	0.189	0.218	0.009
Arginine ³	0.305	0.298	0.277	0.294	0.293	0.354	0.013
Isoleucine ³	0.154	0.147	0.144	0.151	0.143	0.179	0.008
Leucine ³	0.429	0.397	0.384	0.401	0.387	0.488	0.017
Valine ³	0.336	0.323	0.287	0.317	0.315	0.355	0.016
Histidine ³	0.162	0.156	0.147	0.148	0.149	0.185	0.009
Alanine ³	0.263	0.241	0.233	0.244	0.233	0.293	0.011
Glutamate ³	0.653	0.605	0.575	0.605	0.596	0.749	0.030
Glycine	0.177	0.170	0.166	0.186	0.176	0.186	0.008
Aspartate ³	0.497	0.474	0.464	0.480	0.471	0.555	0.019
Phenylalanine ³	0.238	0.228	0.214	0.222	0.223	0.276	0.010
Proline ³	0.255	0.236	0.235	0.252	0.237	0.293	0.018
Serine	0.269	0.251	0.252	0.268	0.270	0.275	0.014
Tyrosine ³	0.181	0.180	0.162	0.180	0.165	0.205	0.012

¹Values are least square means. The 0% to 40% treatments: Diets had 0 to 40% of lysine from protein replaced with synthetic lysine. The 0% ad lib: 0% lysine replacement diet fed ad libitum.

² 10% treatment: n = 3; 0%, 20%, 30%, and 40% treatments: n = 4; 0% ad lib: n = 5; SEM: n = 4.

³ Concentrations were greater in ad libitum fed than in restricted fed pigs ($P < 0.05$).

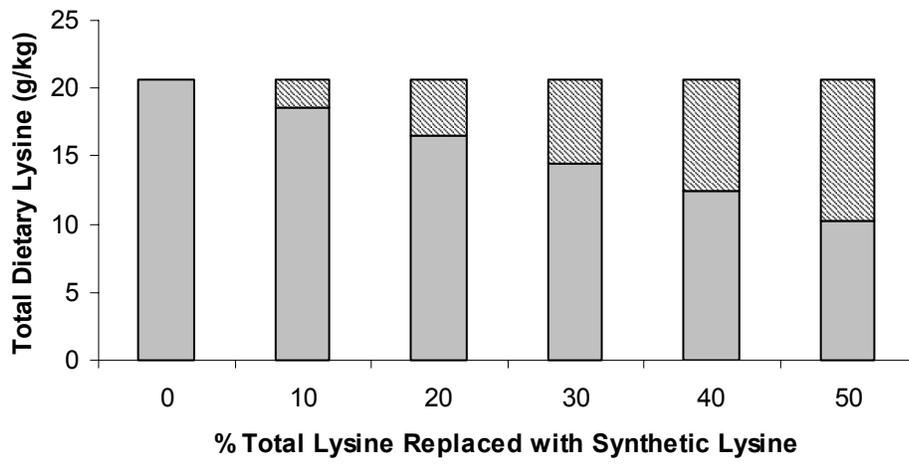


Figure 2.1: Breakdown of source of total dietary lysine. Lysine from dietary protein is represented in gray, with synthetic lysine being represented by the diagonal bars.

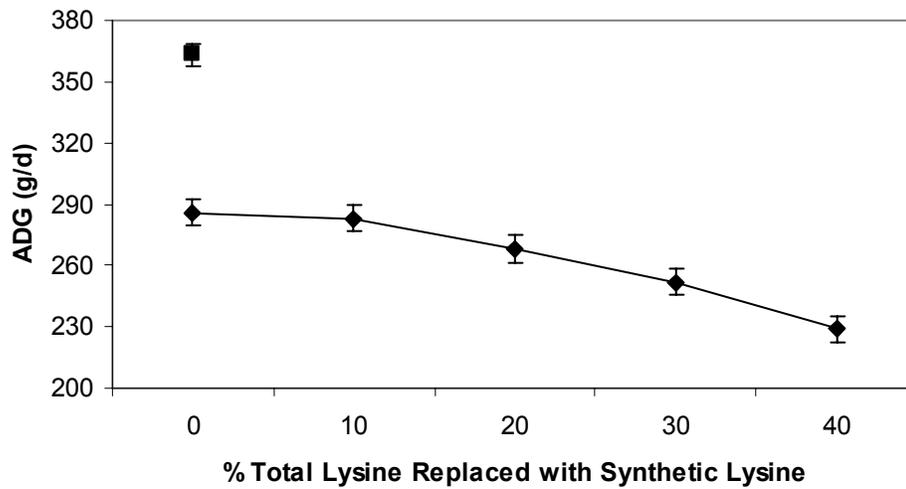


Figure 2.2: Effect of percent replacement of total lysine with synthetic lysine on average daily gain. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A linear decrease ($P < 0.01$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum gained at a greater rate ($P < 0.01$) than pigs on the restricted diets.

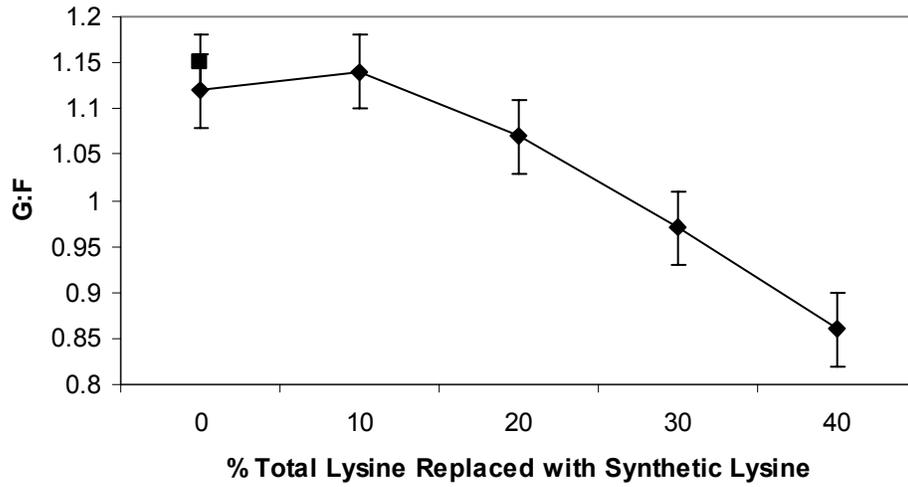


Figure 2.3: Effect of percent replacement of total lysine with synthetic lysine on feed efficiency. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A linear decrease ($P < 0.01$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum had greater feed efficiency ($P < 0.01$) than pigs on the restricted diets.

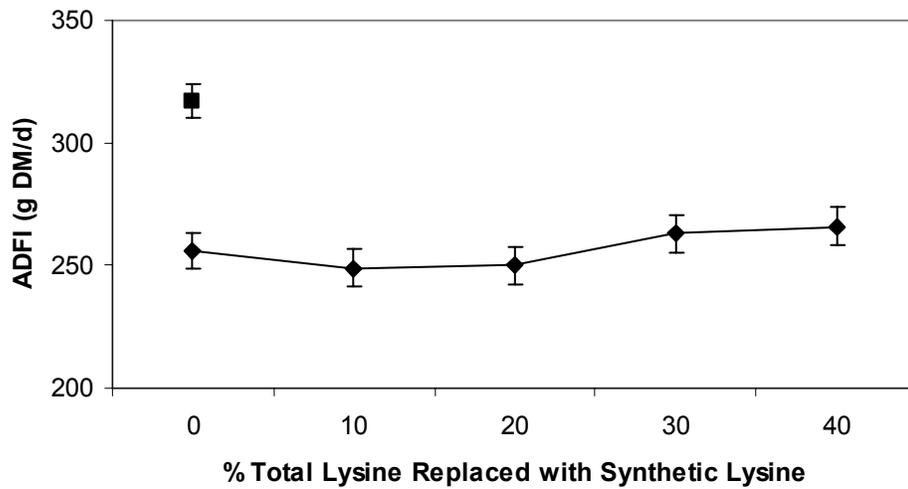


Figure 2.4: Effect of percent replacement of total lysine with synthetic lysine on feed intake. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. Pigs fed ad libitum had greater feed intake ($P < 0.01$) than pigs on the restricted diets.

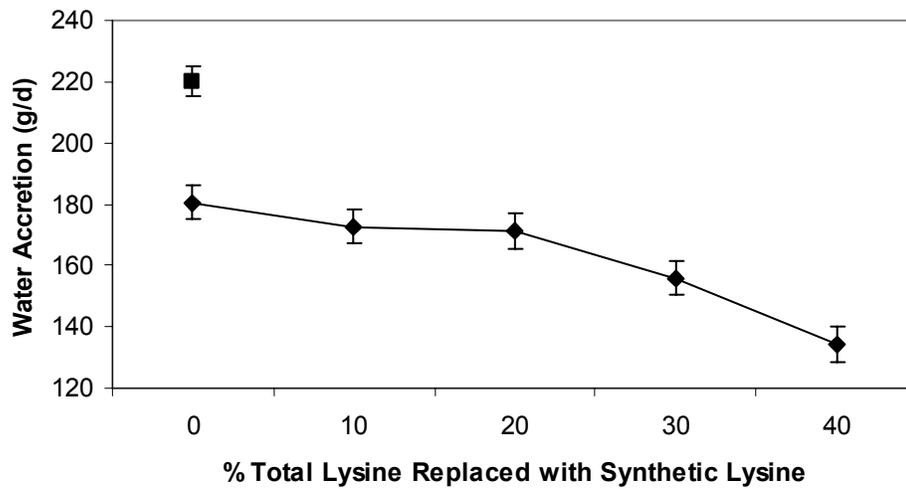


Figure 2.5: Effect of percent replacement of total lysine with synthetic lysine on water accretion. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A linear decrease ($P < 0.01$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum accrued water at a greater rate ($P < 0.01$) than pigs on the restricted diets.

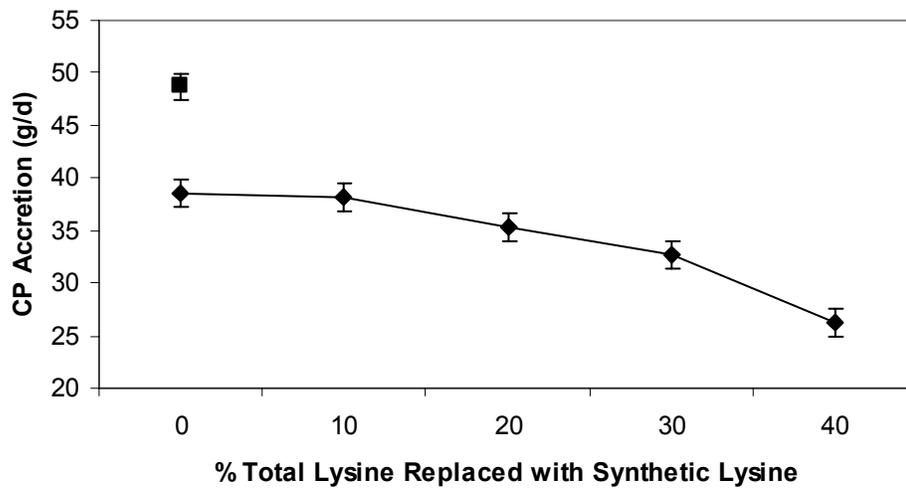


Figure 2.6: Effect of percent replacement of total lysine with synthetic lysine on crude protein accretion. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A linear decrease ($P < 0.01$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum accrued protein at a greater rate ($P < 0.01$) than pigs on the restricted diets. Crude protein also showed a quadratic response to percent replacement of total lysine with synthetic lysine within restricted diets ($P < 0.05$).

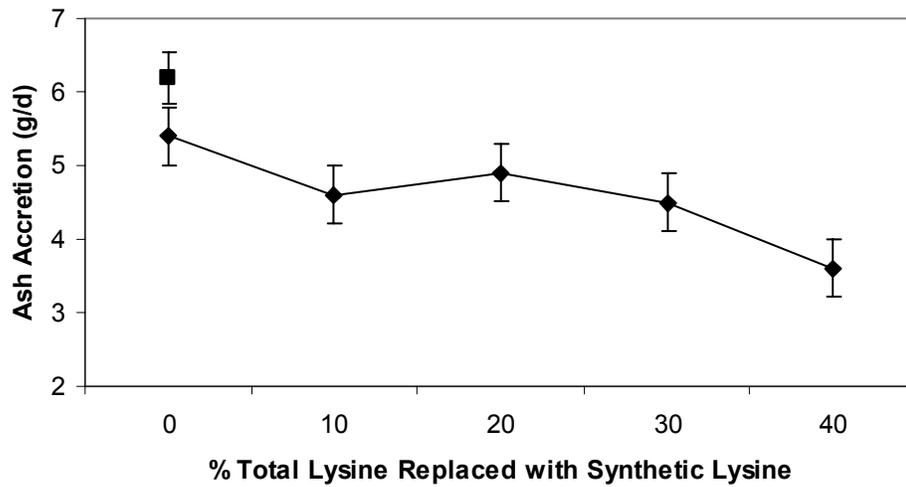


Figure 2.7: Effect of percent replacement of total lysine with synthetic lysine on ash accretion. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A linear decrease ($P < 0.01$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum accrued ash at a greater rate ($P < 0.01$) than pigs on the restricted diets.

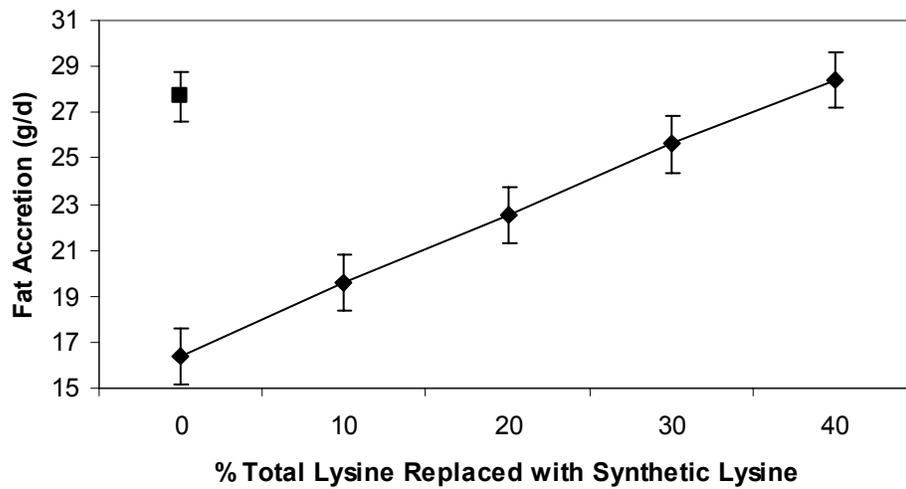


Figure 2.8: Effect of percent replacement of total lysine with synthetic lysine on fat accretion. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A linear increase ($P < 0.01$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum accrued fat at a greater rate ($P < 0.01$) than pigs on the restricted diets.

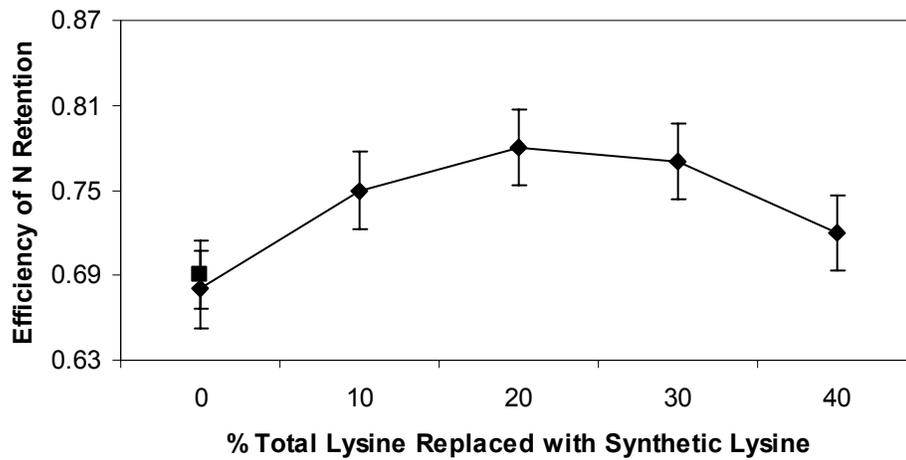


Figure 2.9: Effect of percent replacement of total lysine with synthetic lysine on efficiency of nitrogen retention (CP retention/CP intake). Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. A quadratic response ($P < 0.05$) was seen as percent replacement of total lysine with synthetic lysine within restricted diets increased. Pigs fed ad libitum had a trend for a different ($P = 0.06$) efficiency of nitrogen retention than pigs on the restricted diets.

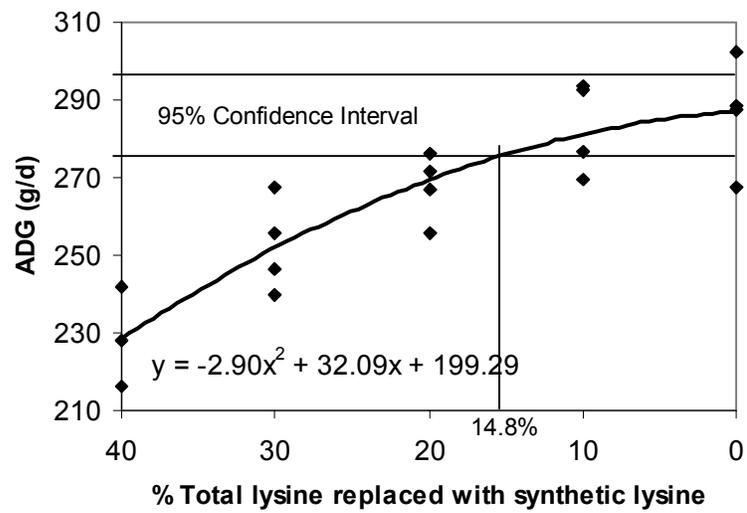


Figure 2.10: Quadratic equation fit to average daily gain data. The 95% confidence interval of the predicted gain at 0% synthetic lysine replacement is shown. The value of 14.8% represents the amount of total lysine that can be replaced with synthetic lysine and produce gain at the lower bound of the 95% confidence interval.

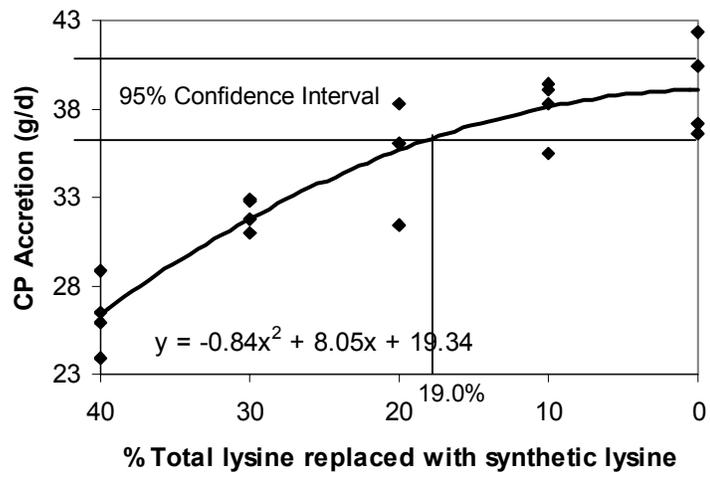


Figure 2.11: Quadratic equation fit to data. The 95% confidence interval of the predicted crude protein accretion at 0% synthetic lysine replacement is shown. The value of 19.0% represents the amount of total lysine that can be replaced with synthetic lysine and produce gain at the lower bound of the 95% confidence interval.

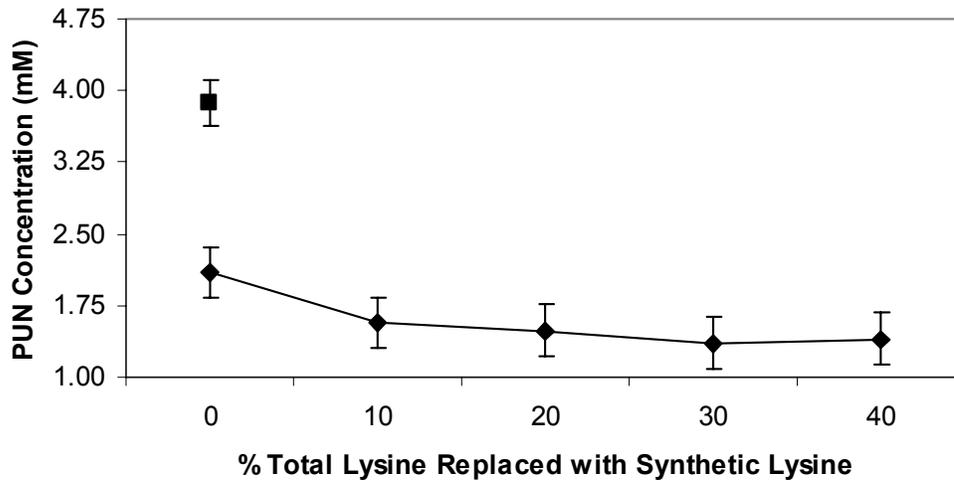


Figure 2.12: Effect of percent replacement of total lysine with synthetic lysine on plasma urea nitrogen concentration. Square symbol represents ad libitum data, and diamond symbol represents restricted data. Data points are mean values \pm SEM. There were 4 pigs per treatment, except the ad lib treatment, where there were 5 pigs. Pigs fed ad libitum had greater PUN concentrations ($P < 0.01$) than pigs on the restricted diets.

Chapter 3

Determination of the order of limiting amino acids in milk-based liquid diets for pigs from 1.5 to 5.5 kg

INTRODUCTION

Lysine is considered the first limiting amino acid in typical swine nursery diets, with methionine being considered the second or third limiting amino acid for this stage of growth (NRC, 1998; Gaines et al., 2005). Methionine is known to be limiting in milk based dietary ingredients (Becker et al., 1955), which could cause diets for young pigs to be deficient in methionine because of the high concentrations of milk based ingredients in the diets. Depending on the dietary ingredients used when formulating diets, as well as the stage of growth of the animals, there are a number of amino acids that could become limiting, as all essential amino acids have the potential to become limiting as others are supplemented into the diet. Several studies have been completed in order to determine the order of limiting amino acids beyond lysine and methionine (Taylor et al., 1981; Russell et al., 1983; Mavromichalis et al., 1998), but few of these have explicitly examined with milk-based products.

There has been successful maintenance of growth with the supplementation of synthetic amino acids into low protein diets compared to traditional high protein diets in previous research using nursery pigs (Kerr et al., 1995) and growing pigs (Figueroa et al., 2002). In order to appropriately supplement low protein diets it is necessary to know the order of limiting amino acids. Therefore, the purpose of this

study was to determine the order of limiting amino acids beyond lysine in a whey protein concentrate/dry skim milk/sodium caseinate diet for pigs from 1.5 to 5.5 kg.

MATERIALS AND METHODS

All procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Animals and diets

Fifty-six male and female pigs were weaned between 24 and 48 hours after birth. Prior to being removed from the sow, pigs underwent processing procedures described previously. This study was conducted in two replicates; with replicate 1 lasting from April 20 – May 7, 2007, and replicate 2 lasting from June 1 – June 18, 2007. The pigs weighed 1.714 ± 0.295 kg for replicate 1 and 1.618 ± 0.107 kg for replicate 2, and were randomly assigned to one of seven experimental diets (Table 3.1). A high protein (24.3%) positive control diet and a low protein (15.8%) negative control diet were utilized in this study. The positive control diet was supplemented with amino acids to ensure that all essential amino acid amounts were at least 110% of NRC recommended values (NRC, 1998). The positive control diet also was formulated to contain similar concentrations of amino acids to sow's milk. Arginine, histidine, and tryptophan were present in lower amounts in the milk replacer diet than in the sow's milk (> 10% difference). Arginine and tryptophan were supplemented into the positive control diet, while histidine was not supplemented into the positive control diet. Amino acids also were supplemented to the positive control diet in order to maintain amino acid ratios to lysine that were either at or above NRC ratios for protein accretion (NRC, 1998). The negative control diet had lower amounts of protein and synthetic amino acids to decrease amino acid ratios to lysine to 60% of the ratios present in the positive control diet. Amino acid

concentrations in the negative control diet were reduced to 60% of the positive control as well. Threonine, tryptophan, phenylalanine, cystine, and arginine were supplemented into the negative control diet in order to achieve the desired ratios. Ratios of the additional un-supplemented amino acids were reduced to 60% of the levels in the positive control through the reduction of crude protein. Lysine was added to maintain a similar concentration in the negative control and positive control diets. There was also a supplemented negative control diet, in which synthetic amino acids were added, to bring the amino acid concentrations and amino acid ratios to lysine up to the ratios present in the positive control diet. The additional four diets (deletion diets) removed specific amino acids from the supplemented negative control diet, bringing the amino acid concentration and ratio to lysine for that amino acid to 60% of the ratio found in the positive control diet and equal to the ratio in the negative control diet. The amino acids that were deleted from the supplemented negative control diet to make the deletion diets were threonine (THR), tryptophan (TRP), sulfur amino acids (methionine and cystine, SAA), and phenylalanine (PHE). The supplemented negative control diet and the deletion diets contained similar low protein levels (20.66% crude protein). The study diets were isoenergetic. Lysine was supplemented into the negative control, the supplemented negative control, and the deletion diets in order to maintain a constant ratio of lysine to gross energy. The amount of lysine in all of the study diets remained constant through the supplementation of synthetic lysine. Glutamic acid also was added to the supplemented negative control diet and the deletion diets in order to replace part of the nitrogen that was removed as a result of removing crude protein and essential

amino acids from the diets. The supplemented negative control diet and the deletion diets all contained similar amounts of crude protein (Table 3.1). It was important for the crude protein to remain constant so that any differences seen between the diets could be attributed to the deletion of individual amino acids and not a varying amount of nitrogen.

Dry ingredients were weighed and mixed for each of the seven diets. Lysine, threonine, tryptophan, arginine, histidine, isoleucine, leucine, valine, cystine, and phenylalanine were provided by Ajinomoto Heartland LLC (Chicago, IL). Methionine and glutamic acid were purchased from Dyets, Inc (Bethlehem, PA). Once combined, the dry diets were mixed with water for 10 min. The negative control diet also was homogenized using an Ultra-Turrax T50 Basic homogenizer (Pittsburg, PA) for 2.5 min. Gentamicin (100 mg/mL) was added to all diets at 0.01 mL per kg of water to reduce the incidence of scours throughout the study. Liquid diets were stored at 4°C and were fed within 2 days after the addition of water.

Housing, Management, and Sample Collection

After weaning, pigs were moved into individual cages. The temperature in the room was maintained between 26 and 29°C for the study, with supplemental heat being provided from heat lamps. Pigs were trained to drink from the gravity flow system as described previously. Pigs were again trained using the positive control diet. The following morning (d 1) each pig was assigned to their respective diet. Weights were recorded at 0800 on d 1 for the beginning of data collection for the experiment.

Pigs were fed at 0800, 1400, and 2300 daily, with weigh backs measured at each feeding. All pigs were fed ad libitum, and were fed 20% more feed than had been provided for the pig the day before. Blood samples were taken on d 5 and d 10 of the study for determination of the plasma urea nitrogen. Animals were all fed by 0900, with blood samples being taken at 1100. Pigs were removed from the study between 5.5 and 5.8 kg and euthanized by electrocution and exsanguination.

Laboratory Analysis

Dry matter of the liquid diets was determined by placing a 3 g sample of milk in an oven at 101°C for 22 h. Blood samples were centrifuged for 20 min at 1000 x g at 4°C (Beckman J-6B; Golden Valley, MN) and the plasma was removed. The plasma was frozen at -20°C until used for determination of urea nitrogen concentration using a colorimetric assay based on the Berthelot procedure (Teco Diagnostics; Anaheim, CA). A complete amino acid analysis was performed on samples of the dietary protein sources used in the current study, which were whey protein concentrate, dried skim milk, and sodium caseinate. The analysis was completed at the Experimental Station Chemical Laboratories (Columbia, MO).

Statistical Analysis

The data were analyzed as a completely randomized block design using the GLM and the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). Performance data (ADG, ADFI, and G:F), CP intake, and days on study were analyzed by ANOVA in PROC GLM for effects of dietary treatment, replicate, and the interaction. Treatment and replicate were analyzed as fixed effects. Where the main effect of treatment was significant ($P < 0.05$), treatment means were separated using the

PDIFF option in GLM to complete pairwise comparisons of the least square means for ADG, ADFI, and G:F. Plasma urea nitrogen concentration was analyzed using ANOVA in PROC Mixed for the effects of treatment, replicate, sample time (d 5 or d 10), and interactions. Treatment, replicate, and sample time were fixed effects, with sample time also being analyzed as a repeated measure. The repeated statement was used to determine if plasma urea nitrogen values were affected by sample time. Treatment showed a main effect on plasma urea nitrogen concentration ($P < 0.05$), and treatment means were separated using the PDIFF option in Mixed. Significance level was $P < 0.05$.

RESULTS

Performance Data

Replicate and the treatment x replicate interaction were not different for ADG, ADFI, G:F, CP intake, or days on study ($P > 0.05$), so these data were not presented. Treatment showed a main effect on gain, feed intake, feed efficiency, CP intake, and days on study ($P < 0.05$). Animals fed the positive control diet gained 346 g/d, while animals fed the negative control diet gained 269 g/d ($P < 0.05$). All other values fell between the ADG of pigs fed the positive and negative control diets, with the positive control diet producing greater gain and the negative control diet producing less gain than all other treatments ($P < 0.05$; Table 3.2). The supplemented negative control diet also produced greater ADG than the SAA deletion diet ($P < 0.05$) with values of 315 g/d and 291 g/d, respectively. The animals fed the additional deletion treatments (-THR, -TRP, and -PHE) produced similar average daily gain values ($P > 0.05$; Table 3.2).

Average daily feed intake was greater in pigs fed the positive control diet and the negative control diet ($P < 0.05$) than the supplemented negative control diet and the deletion diets (-THR, -TRP, -SAA, and -PHE; Table 3.2). The animals fed the positive control diet consumed 306 g/d and the animals fed the negative control diet consumed 310 g/d. The animals fed the supplemented negative control diet and the deletion diets all consumed similar amounts of feed ($P > 0.05$; Table 3.2).

Feed efficiency was decreased for the animals fed the negative control diet ($P < 0.05$) when compared to all other treatments (Table 3.2). The positive control diet (1.17) produced greater G:F ratios ($P < 0.05$) than the negative control diet, the THR deletion diet, and the SAA deletion diet, with values of 0.86, 1.09, and 1.06, respectively (Table 3.2).

Crude protein intake was greatest (73.3 g/d) for animals fed the positive control diet ($P < 0.05$), with animals fed the negative control diet having the least (49.2 g/d) CP intake ($P < 0.05$; Table 3.2). Animals fed the supplemented negative control diet and the deletion diets all had similar amounts of CP intake, with the values falling between the amounts for the positive control diet and the negative control diet (Table 3.2).

The number of days on study was the greatest ($P < 0.05$) for animals fed the negative control diet (15.1 days). Animals fed the positive control diet, the supplemented negative control diet, and the tryptophan deletion diet were on the study for the least ($P < 0.05$) number of days (11.3, 12.5, and 12.5 days, respectively). The number of days on study was similar for animals fed the supplemented negative control diet and the deletions diets.

Plasma Urea Nitrogen

There was no replicate or treatment x replicate interaction for PUN concentration ($P > 0.05$). Plasma urea nitrogen concentrations were greatest ($P < 0.05$) for animals fed the SAA deletion diet with an average value of 6.96 mM (Table 3.3). Pigs fed the THR deletion diet had similar concentrations to the animals fed the positive control diet (Table 3.3), with these treatments having lower ($P < 0.05$) PUN concentrations than animals fed the SAA deletion diet. The PUN concentration for the animals fed the negative control diet was the lowest numerically with an average value of 2.42 mM. Animals fed the negative control diet had similar PUN concentrations to animals fed the supplemented negative control diet and the PHE deletion diet (Table 3.3). The animals fed the supplemented negative control diet also had similar PUN concentrations to animals fed the TRP deletion diet (Table 3.3).

The average PUN concentration over both replicates for the sample taken on d 5 was less ($P < 0.01$) than the average value for the sample taken on d 10, with values of 3.95 mM and 4.84 mM, respectively. The positive control diet was the only diet that showed differences ($P < 0.05$) between the sample on d 5 (3.52 mM) and d 10 (7.57 mM; Figure 3.1; Table 3.3), even though there was a trend for a difference ($P = 0.079$) between the values for the SAA deletion diet with a value of 6.38 mM for the sample taken on d 5 and a value of 7.53 mM for the sample taken on d 10 (Table 3.3).

DISCUSSION

Lysine and/or methionine are recognized as the first limiting amino acids in the majority of swine diets which contain milk products in the early nursery phase, and corn/soybean meal as pigs move from the nursery phase on to the grow/finisher phases (NRC, 1998; Gaines et al., 2005). Despite the recognition of lysine and sometimes methionine as first limiting, one still has to consider the order of limiting amino acids beyond the first limiting. The current study used an amino acid deletion approach, similar to that of Mavromichalis et al. (1998) to help determine the next limiting amino acid in the whey/dry skim milk/casein diet. A high protein positive control diet and a low protein negative control diet were used. The supplemented negative control diet and the additional deletion diets contained similar amounts of crude protein in order to make sure that any differences seen between the deletion diets was due to the deletion of amino acids and not a difference in the amount of nitrogen present in the diets. Based on the concentration of amino acids in the negative control diet compared to NRC recommendations, the predicted order of limiting amino acids after lysine was threonine, the sulfur amino acids, phenylalanine, and tryptophan. The amounts of amino acids in the diet as a percentage of NRC recommendations were very similar, with threonine being at 65.0% of NRC, the sulfur amino acids at 67.1% of NRC, phenylalanine at 73.7% of NRC, and tryptophan at 77.2% of NRC. These values and the predicted order are based on dietary amino acid values, with the amino acid values for the dietary protein sources coming from a complete amino acid analysis. The predicted order of limiting amino acid was originally threonine, tryptophan, phenylalanine, and the

sulfur amino acids. This order was determined using amino acid values for the dietary protein sources coming from the companies that produced the ingredients. These values were slightly different from the analyzed values, so the order of limitation was slightly different based on the amino acid values for the protein sources. Analyzed amino acid values were used because these values were determined specifically for the protein sources used in the current study. Results from the current study showed that all treatments produced lower ($P < 0.05$) gain than the positive control diet. Numerically, the least amounts of gain were produced by animals fed the SAA deletion diet and the THR deletion diet.

Methionine is commonly limiting in nursery diets due to high concentrations of milk based products like dry skim milk and whey protein concentrate (Becker et al., 1955), which were used in the current study diets. In addition to low amounts of methionine, cysteine also is present in low concentrations in milk based products (Becker et al., 1955). Cystine is two cysteine molecules joined by a disulfide bond. The current study focused on both sulfur amino acids (Met + Cys), instead of methionine only due to the low amounts of cysteine present in milk based protein sources. Because cysteine is not a limiting amino acid, there is no specific requirement for cysteine in the diet, but there is an NRC requirement for methionine+cysteine. Although cysteine is a non-essential amino acid, a diet that is low in methionine would not be able to provide adequate amounts of substrates for the production of cysteine within the body, effectively making cysteine a limiting amino acid. Also, based on the calculated order of limitation, methionine alone was not one of the four next limiting amino acids in the study diets, but the combination of

methionine and cysteine was a part of the predicted order of limitation based on the amounts in the diet compared to the NRC requirement. This is another reason for studying both of the sulfur amino acids, instead of methionine alone. Because of this, cystine was supplemented into the study diets in addition to methionine, and both were removed from the sulfur amino acid deletion diet.

The results from the current study showed that the removal of the sulfur amino acids from the diet tended to depress growth to the largest extent. The supplemented negative control produced similar gain to all of the deletion diets, except for the SAA deletion diet, which produced lower ($P < 0.05$) gain. Because of the difference in performance between the SAA deletion diet and the supplemented negative control diet and the numerical data which showed the SAA deletion diet produced the least gain it can be hypothesized that the sulfur amino acids are next limiting in the diets for the current study, although this was not the predicted result. It is possible that the NRC recommended values for threonine and the sulfur amino acids are slightly inaccurate, so the predicted order of limitation could be incorrect. There is only a 2.1% difference between the amounts of amino acids in the diet as a percentage of NRC recommendations for threonine and the sulfur amino acids, so it is possible if there was a small inaccuracy in the recommended values for either threonine or the sulfur amino acids, the predicted order could easily be different.

Animals fed the positive control diet in the current study gained at a rate of 346 g/d. Results from a study by Kim et al. (2001) using an artificial rearing system showed average daily gain values of 397 g/d. The age of the pigs at the start of each study was different, with the current study using 1 d old pigs and the study by

Kim et al. (2001) using 11 d old pigs. There have been no studies done using pigs of a similar age to the pigs used in the current study while also using similar dietary ingredients. The dietary composition for both the current study and the study by Kim et al. (2001) were very similar, but due to the differences in starting age, it is not possible to compare the results from the Kim et al. (2001) study to the current study. It can be hypothesized that if the animals from the current study were similar in age to the animals in the study by Kim et al. (2001), there would have been more similar values for average daily gain.

It was predicted that animals fed the supplemented negative control diet would gain similar to animals fed the positive control diet due to both diets providing similar total amounts of amino acids and similar amino acid ratios. The differences between the diets were the amount of crude protein, with the positive control diet having 24.3% crude protein and the supplemented negative control diet having 20.7% crude protein, and the source of the amino acids in the diets, coming from protein or synthetics. One potential reason for the decreased gain with the feeding of the supplemented negative control diet could be the decreased amount of crude protein in the supplemented diet (20.7%) compared to the positive control diet (24.3%). For pigs from 3-5 kg, the crude protein requirement set by the NRC is 26.0% crude protein (NRC, 1998). With this decrease in crude protein compared the NRC requirements, it is possible that the supplemented negative control diet was limiting in crude protein, which could have depressed the growth rate of these pigs. Another possible reason for the depressed growth rate of the animals fed the supplemented negative control diet could be the decreased ($P < 0.05$) feed intake

compared to the animals fed the positive control diet, with the animals fed the supplemented negative control taking in 285 g DM/d and the animals fed the positive control diet taking in 306 g DM/d. With the depressed feed intake, the animals fed the supplemented negative control diet were receiving fewer nutrients present in the diets than the animals fed the positive control. Also, with the supplemented negative control diet already containing less crude protein, the depressed feed intake decreases the protein intake of these animals even more and likely depresses the growth of these animals. Animals fed the positive control diet and the supplemented negative control diet remained on the study for a similar number of days (11.3 and 12.5 days, respectively), so the decreased feed intake was not affected by the number of days the animals were on study.

Urea nitrogen values measured in plasma can be used to determine if an animal is receiving adequate protein and/or the appropriate balance of amino acids (Brown and Cline, 1974). Urea nitrogen values can be increased if there are excess amounts of protein in the diet or if there is an imbalance of amino acids. Animals fed the SAA deletion diet had the greatest ($P < 0.05$) PUN concentrations. Animals fed the positive control diet in the current study showed much higher PUN concentrations (5.55 mM) than animals fed positive control diets in other studies. Results of a study with similar age pigs fed a whey protein concentrate based diet show values of 2.36 mM at day 7 and 2.40 mM at day 14 for animals fed a 25% crude protein positive control diet (Kim et al., 2004). Baby pigs have a dietary crude protein requirement of 26.0% crude protein from 3-5 kg and a requirement of 23.7% crude protein from 5-10 kg (NRC, 1998), so crude protein values similar to the ones

seen in the current study and the study by Kim et al. (2004) are common due to the young age of the pigs. With an imbalance of amino acids, amino acids not used for protein synthesis will be oxidized and the nitrogen will be metabolized via the urea cycle, thus producing greater PUN concentrations. The positive control diet was supplemented with synthetic amino acids in order to provide amino acid ratios to lysine that were similar to NRC ratios. It is possible that the increased PUN concentrations in the current study compared to the study by Kim et al. (2004) were due to differences in amino acid concentrations in the dry milk replacer diets. The current study diets contained less lysine, leucine, isoleucine, and threonine on a dry matter basis than the milk replacer diet used by Kim et al. (2004). Although the amino acids were present in the current study diets in excess of NRC recommendations, the differences in the amounts of these amino acids were the main difference between the diets used in these studies. This could lead to questions about the accuracy of the NRC requirements for amino acids in this age pigs. The amino acid concentrations found in the milk replacer diet in the study by Kim et al. (2004) were similar to the amounts found in sow's milk analyzed by their lab. The positive control diet in the current study also was formulated to be similar to sow's milk, but the diets in the study by Kim et al. (2004) were formulated more closely to sow's milk than the current study diets. Another potential cause for the differences in PUN concentration is that the current study diets were formulated slightly below the lysine to energy requirement. The current study was formulated to provide 4.8 g lysine/Mcal GE, while the requirement presented in the study by Auldist et al. (1997) was 5.1 g lysine/Mcal GE based on ADG and the requirements

presented by Lewis et al. (2006) were 5.5 g lysine/Mcal GE based on CP accretion and 5.9 g lysine/Mcal GE based on ADG. The lysine requirements from both the study by Auld et al. (1997) and the study by Lewis et al. (2006) were determined using a quadratic model. Because of this limitation in the diets, PUN concentrations also could have been affected. Based on the differences in amino acid concentrations, it is possible that the current study diets could have been slightly limiting in some amino acids, which could have caused an imbalance of amino acids in the body, thus causing an increase in PUN concentration.

Between d 5 to d 10 there was an increase ($P < 0.05$) in PUN concentration in the animals fed the positive control diet. There was also a trend ($P = 0.079$) for an increase in PUN concentration in animals fed the SAA deletion diet. The animals fed the positive control diet likely had increased PUN at d 10 due to the changing of dietary crude protein and dietary amino acid requirements. Crude protein requirements are very high right after birth (26.0%), and the requirements decrease as the animal ages (NRC, 1998). The positive control diet contained 24.3% crude protein, which is slightly in excess of the amount of crude protein that is required for the pigs from 5-10 kg. At d 10, animals fed the positive control diet weighed an average of 4.9 kg, so this diet was in excess of the protein requirements for this weight and this could explain the increase in urea from d 5 to d 10. The trend for an increase seen in the animals fed the SAA deletion diet could have been caused by protein synthesis becoming more inefficient as the body became more deficient in the sulfur amino acids. At d 5, the body was likely already becoming deficient in the sulfur amino acids, but as the deficiency continued on to a later time in the study, the

body could become even more deficient. This could explain the trend for an increase at d 10.

Results from a study by Shriver et al. (2003) show decreases ($P < 0.05$) in plasma and urinary urea nitrogen with a reduction in crude protein from 18% in their positive control diet to 14% in their supplemented low protein diet. The supplemented low protein diet was fortified with lysine, threonine, methionine, tryptophan, isoleucine, and valine in order to achieve ideal ratios to lysine, similar to the approach used when formulating the supplemented negative control diet in the current study. The current study decreased crude protein from 24.3% in the positive control diet to 20.7% in the supplemented negative control diet and the deletion diets. With the 14% diet in the study by Shriver et al. (2003) and the supplemented negative control diet in the current study, the amino acids were supplemented into the lower crude protein diets to provide similar amino acids to the positive control diets for both studies. Although different amounts of crude protein were present in the diets for the current study and the study by Shriver et al. (2003), the results from both studies showed a similar decrease ($P < 0.05$) in PUN concentration between the positive control diets and the supplemented diets.

Animals fed the supplemented negative control diet had decreased urea concentration as compared to animals fed the SAA deletion diet and the THR deletion diet. This result is likely due to the addition of synthetic amino acids, without an amino acid deletion, which allowed for protein synthesis to become more efficient despite the lower amounts of amino acids coming from crude protein in the diets. Without the deletion of an individual amino acid to cause less efficient protein

synthesis, supplemented amino acids in the supplemented negative control will allow for more efficient use of amino acids and cause a decrease in urea production (Brown and Cline, 1974; Shriver et al., 2003). Animals fed the TRP deletion diet and the PHE deletion diet produced PUN concentrations similar to the animals fed the supplemented negative control diet. This result shows that both tryptophan and phenylalanine were not next limiting in the diets for the current study due to the decreased PUN concentration values.

The animals fed the positive control diet had greater ($P < 0.05$) PUN concentrations than animals fed the negative control diet. This is likely due to the differing amounts of crude protein intake. Animals fed the positive control diet took in 73.3 g/d of crude protein, while the animals fed the negative control diet took in 49.2 g/d crude protein. The animals fed the positive control diet were taking in an adequate amount of protein for the first few days of life, but as the pigs grew the amount of crude protein present in the diet was most likely in excess of the requirements for that age. Because of this, the animals on the positive control diet had increased PUN concentrations compared to the negative control diet. The amount of crude protein being taken in by the animals fed the negative control diet was limiting over the entire study, so it was likely that the body was attempting to conserve protein due to the deficiency. The conservation of protein could cause a decrease in urea production (Edmonds and Baker, 1987; Wykes et al., 1996).

Animals fed the negative control diet and the supplemented negative control diet had similar PUN concentrations, with the supplemented negative control diet having a numerically greater PUN concentration. This similarity could be due to

crude protein intake. The animals fed the negative control diet took in 49.2 g/d crude protein and the animals fed the supplemented negative control diet took in 59.9 g/d crude protein. It is likely that both of these diets limited crude protein intake, which could have led to protein conservation in the body (Edmonds and Baker, 1987; Wykes et al., 1996).

The PUN concentration values for both the threonine deletion diet and the sulfur amino acid deletion diet were greater ($P < 0.05$) than the values for the other deletion diets used in this study. This result coincides with the numerically decreased average daily gain and feed efficiency values in these same diets. These increased values are indicative of a greater imbalance of amino acids than was present in the other deletion diets.

It is difficult to determine the most limiting amino acid in our diets based on the performance data because of the lack of significant differences between the deletion diets, but due to the numerical differences one can speculate that the sulfur amino acids and threonine could be the next limiting amino acids. The animals fed the sulfur amino acid diet had a greater ($P < 0.05$) PUN concentration (6.96 mM) than the animals fed the threonine diet (5.70 mM). This result, in conjunction with the performance data, led to the suggestion that the sulfur amino acids were second limiting in the current study diets after lysine, with threonine being third limiting, and tryptophan/phenylalanine being fourth limiting. The PUN concentration values for the TRP deletion diet and the PHE deletion diet were similar. The predicted order of limitation based on the recommended NRC values was threonine, the sulfur amino acids, phenylalanine, and tryptophan. It is possible that the slight difference seen in

the order of limitation based on the PUN concentrations and the predicted order are due to the inadequacies of the NRC recommended values for pigs weighing 1 to 5 kg. There has been the least amount of research done on the amino acid requirements of neonatal pigs, which could mean that the requirements set forth for this age group of pigs could be less accurate than originally thought.

Based on the results from the current study, it is predicted that the sulfur amino acids are the next limiting in whey/dry skim milk/casein diets. The predicted order beyond the sulfur amino acids is threonine and then tryptophan/phenylalanine. The order of limitation after the sulfur amino acids is based solely on numerical differences, so additional research would need to be done to definitively determine the order of limiting amino acids.

Summary Discussion

Neonatal pig death for low birth weight pigs occurs for many reasons. A preventable cause of death for low birth weight pigs is starvation. Low birth weight pigs tend to have a much lower chance of survival than their larger litter mates due to competition for milk from the sow, which can lead to starvation. In order to provide these low birth weight animals with adequate amounts of milk, artificial rearing systems have been developed to provide milk replacer diets.

Supplementing synthetic lysine alone into low crude protein liquid diets did not allow pigs to maintain performance and body composition similar to animals that were fed a high protein diet that was not supplemented with synthetic amino acids. It is likely, based on ADG data, that the next limiting amino acid became limiting at 14.8% replacement of lysine from protein with synthetic lysine, and based on CP accretion data, the next limiting amino acid became limiting at 19.0% replacement of lysine from protein with synthetic lysine. Without supplementation of additional essential amino acids, beyond the first limiting, growth and protein accretion become difficult due to the imbalance of amino acids present in a low protein diet.

The addition of only the first limiting amino acid will cause the second limiting amino acid to become first limiting. Because of this, it is necessary to supplement low protein diets with other essential amino acids. In order to properly supplement low protein diets it is necessary to know the order of limitation beyond the first limiting amino acid. In our study it was difficult to determine the order of limitation of amino acids beyond lysine due to the lack of variation in performance between the deletion diets. Based on numerical gain and feed efficiency values, as well as

differences in PUN concentration, it is possible that sulfur amino acids are second limiting in our diets, with threonine being third limiting, but additional research is required to definitively determine the order of limitation beyond lysine.

A possible method for better determining the order of limiting amino acids in diets similar to the current study diets is to make the deletion diets more deficient in the amino acids in question. In the current study, the amino acids that were studied were all approximately at 70% of the NRC recommended values. It is possible that if the amino acids were at 50-60% of the NRC recommended values more differences would become apparent. A decrease of this nature was discussed when planning the experiment to determine the order of limiting amino acids, but in order to have amino acids at 50-60% of NRC recommendations calls for removing a significant amount of protein. A similar reduction of protein was seen in the 50% synthetic lysine replacement diet used in the first study that was discussed, and the pigs fed this diet did not grow. It is possible that supplementation with additional amino acids to achieve similar ratios to the NRC recommendations would allow for the pigs to grow on such a low protein diet, but this is not certain.

Table 3.1: Composition of experimental diets (g/kg air dry before being constituted with water).^{1, 2}

Ingredients, g/kg	Dietary Treatment						
	PC	NC	Supp. NC	Supp. -THR	Supp. -TRP	Supp. -SAA	Supp. -PHE
WPC 8600 ³	27.0	16.2	16.2	16.2	16.2	16.2	16.2
NFDM ³	524.0	314.4	314.4	314.4	314.4	314.4	314.4
Lactose	295.6	479.7	433.9	432.6	432.7	432.7	431.6
Fat Pak 80 ³	79.0	114.0	95.0	95.0	95.5	96.0	98.0
Sodium Casienate	40.0	24.0	24.0	24.0	24.0	24.0	24.0
Xanthan Gum	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Lysine HCl	0.0	10.2	10.3	10.3	10.3	10.3	10.3
Arginine HCl	1.0	0.5	5.2	5.2	5.2	5.2	5.2
Histidine	0.0	0.0	2.6	2.6	2.6	2.6	2.6
DL-Methionine	0.0	0.0	2.7	2.7	2.7	0.0	2.7
Threonine	1.5	0.9	6.1	0.9	6.1	6.1	6.1
Tryptophan	0.4	0.2	1.7	1.7	0.2	1.7	1.7
Isoleucine	0.0	0.0	5.4	5.4	5.4	5.4	5.4
Leucine	0.0	0.0	9.7	9.7	9.7	9.7	9.7
Valine	0.0	0.0	6.5	6.5	6.5	6.5	6.5
Phenylalanine	1.2	0.7	5.5	5.5	5.5	5.5	0.7
Cystine	1.6	1.0	2.1	2.1	2.1	1.0	2.1
Glutamic Acid	0.0	0.0	20.5	26.9	22.6	24.5	24.6
CaCO ₃	3.0	2.4	2.4	2.4	2.4	2.4	2.4
Dicalcium Phosphate	4.0	14.5	14.5	14.5	14.5	14.5	14.5
Mineral Premix ⁴	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Premix ^{5,6}	1.3	1.3	1.3	1.3	1.3	1.3	1.3
NaCl	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Calculated Composition							
Total Lysine, g/kg	20.6	20.6	20.6	20.6	20.6	20.6	20.6
Crude Protein, g/kg	243.1	158.1	206.6	206.6	206.6	206.6	206.6

Table 3.1 (continued)

Fat, g/kg	69.7	95.1	79.9	79.9	80.3	80.7	82.3
Lactose, g/kg	559.1	635.4	589.9	588.7	588.7	588.7	587.7
Gross Energy, Mcal/kg	4.2	4.2	4.2	4.2	4.2	4.2	4.2

¹ PC = positive control; NC = negative control; Supp. NC = supplemented negative control; Supp.-THR = supplemented negative control - THR; Supp.-TRP = supplemented negative control - TRP; Supp.-SAA = supplemented negative control - MET+CYS; Supp.-PHE = supplemented negative control - PHE.

²All diets were made to contain 180 g dry diet in addition to 1 kg water to give 1.18 kg liquid diet.

³WPC 8600=Whey protein concentrate (Hilmar Ingredients, Hilmar, CA), NFDM=Non-fat dry milk (Milk Specialties, Dundee, IL), Fat Pak 80=Animal fat blend (Milk Specialties, Dundee, IL).

⁴ Added to contain mg/kg liquid diet: Calcium, 101.25; Iron, 14.22; Cobalt, 0.12; Copper, 1.20; Iodine, 0.12; Magnesium, 45.75; Manganese, 2.10; Selenium, 0.04; Zinc, 14.22.

⁵ Added to contain mg/kg liquid diet: Calcium, 36.86; Vitamin C, 4.43; Pantothenic Acid, 0.77; Niacin, 0.56; Riboflavin, 0.44; Vitamin K, 0.01; Biotin, 0.001; Vitamin B 12, 0.0004; Thiamine, 0.10; Vitamin B 6, 0.13; Folic Acid, 0.02.

⁶ Added to contain IU/kg liquid diet: Vitamin A, 1,064; Vitamin D, 177; Vitamin E, 5.32.

Table 3.2: Effect of amino acid deletion on average daily gain, feed intake, feed efficiency, crude protein intake, and days on study.¹

VARIABLE	Dietary treatment ²							SEM
	PC	NC	SUPP. NC	SUPP. -THR	SUPP. -TRP	SUPP. -SAA	SUPP. -PHE	
ADG, g/d	346 ^a	269 ^b	315 ^c	298 ^{c,d}	310 ^{c,d}	291 ^d	307 ^{c,d}	7.33
ADFI, g DM/d	306 ^a	310 ^a	285 ^b	273 ^b	275 ^b	277 ^b	273 ^b	7.72
G:F	1.17 ^a	0.87 ^b	1.11 ^{a,c}	1.09 ^c	1.12 ^{a,c}	1.06 ^c	1.13 ^{a,c}	0.02
CP Intake, g/d	73.3 ^a	49.2 ^b	59.9 ^c	57.2 ^c	57.6 ^c	58.0 ^c	56.5 ^c	1.64
Days on Study	11.3 ^a	15.1 ^b	12.5 ^{a,c}	13.8 ^c	12.5 ^{a,c}	13.3 ^c	12.9 ^c	0.45

¹Values are least square means. Values with different superscripts are significantly different ($P < 0.05$). N = 8 for all treatments and SEM.

² PC = positive control; NC = negative control; Supp. NC = supplemented negative control; Supp.-THR = supplemented negative control - THR; Supp.-TRP = supplemented negative control - TRP; Supp.-SAA = supplemented negative control - MET+CYS; Supp.-PHE = supplemented negative control - PHE.

Table 3.3: Effect of amino acid deletion and sample d on plasma urea nitrogen concentration (mM).¹

SAMPLE DATE	Dietary Treatment ²							SEM
	PC ³	NC	SUPP. NC	SUPP. -THR	SUPP. -TRP	SUPP. -SAA	SUPP. -PHE	
D 5	3.52	2.26	2.98	5.18	4.12	6.38	3.22	0.45
D 10	7.57	2.59	3.57	6.23	3.60	7.53	2.78	0.47
Mean	5.55 ^a	2.42 ^b	3.27 ^{b,c}	5.70 ^a	3.86 ^c	6.96 ^d	3.00 ^{b,c}	0.33

¹Values are least square means. Values with different superscripts are significantly different ($P < 0.05$). N = 8 for all treatments and SEM.

²PC = positive control; NC = negative control; Supp. NC = supplemented negative control; Supp.-THR = supplemented negative control - THR; Supp.-TRP = supplemented negative control - TRP; Supp.-SAA = supplemented negative control - MET+CYS; Supp.-PHE = supplemented negative control - PHE.

³Values differ between d 5 and d 10 for the PC diet ($P < 0.05$).

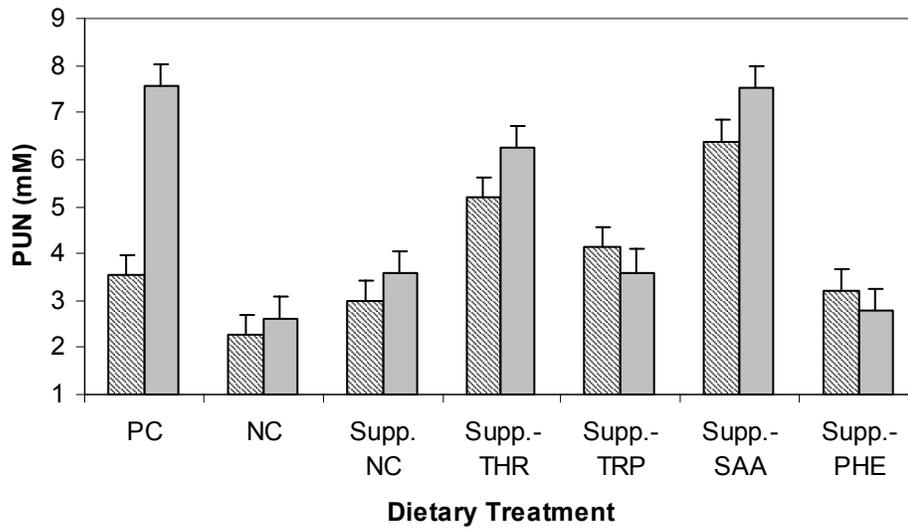


Figure 3.1: Effect of sample d on plasma urea nitrogen concentration (mM). Diagonal bars represent samples taken on d 5 and gray bars represent samples taken on d 10, with 8 animals per treatment. Values differ between d 5 and d 10 for the PC diet ($P < 0.05$). Treatments: PC = positive control; NC = negative control; Supp. NC = supplemented negative control; Supp.-THR = supplemented negative control - THR; Supp.-TRP = supplemented negative control - TRP; Supp.-SAA = supplemented negative control - MET+CYS; Supp.-PHE = supplemented negative control - PHE.

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