EFFECTS OF FIBER ON THE OPTIMUM THREONINE:LYSINE RATIO FOR 25 TO 50 KG GROWING GILTS

BY

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THESIS

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ABSTRACT

Five experiments were conducted to determine the ideal Thr:Lys ratio for 25 to 50 kg growing gilts and the effects of fiber on said ratio. In Exp. 1, the objectives were to determine the standardized ileal digestible (**SID**) Lys requirement for gilts from 25 to 50 kg BW. Seventy gilts (initial BW: 24.54 ± 3.28 kg) were used in a growth assay with 2 pigs per pen and 7 pens per treatment. Diets were formulated using corn and soybean meal as the sole sources of AA. Under the assumption that Lys is the first limiting AA in corn-soybean meal diets, soybean meal concentration was increased at the expense of corn to increase SID Lys in the diets. Results indicated that 1.08% SID Lys was needed to maximize ADG and 1.10% SID Lys was needed to maximize G:F. In Exp. 2, the objectives were to determine the standardized ileal digestibility of AA in corn, soybean meal, field peas, fish meal, and soybean hulls. These ingredients were chosen because of their unique AA profiles, which facilitate their use diets that are deficient in only Lys and Thr. Six ileal-cannulated gilts (initial BW: 26.5 ± 0.74 kg) were allotted to a 6 × 6 Latin square design with 6 diets and 6 periods. The results indicated that the standardized ileal digestibility of most indispensable AA was not different among field peas, fish meal, and soybean meal, whereas the standardized ileal digestibility of some indispensable AA was less in soybean hulls than in other ingredients. In Exp. 3, the objectives were to confirm that diets deficient in only Thr and Lys can be formulated. One hundred twenty gilts (initial BW: 24.84 ± 3.39 kg) were allotted to either low fiber or high fiber diets. Within each level of fiber, the 5 different diets were formulated by changing the proportion of synthetic Thr and Lys in the diets. Resulting differences in ADG, ADFI, and G:F between diets indicated that the diets were marginally deficient in Lys and Thr. In Exp. 4, the objectives were to determine the optimal SID Thr:Lys ratio for gilts from 25 to 50 kg BW, and to determine the effects of fiber on that ratio.

One hundred ninety-two gilts were used in a growth assay with 2 pigs per pen and 8 pens per treatment. Low-fiber, as well as high-fiber, diets with SID Thr:Lys ratios at 45:100, 54:100, 63:100, 72:100, 81:100, and 90:100 were used. In both types of diets ADG and G:F increased quadratically ($P < 0.05$), as the concentration of Thr increased in the diets. For pigs fed the low-fiber diets, combined broken-line and quadratic analyses estimated the optimum SID Thr:Lys ratio at 0.66 and 0.63 for ADG and G:F, respectively. For the pigs fed high fiber diets, combined broken-line quadratic analyses estimated the optimum SID Thr:Lys requirement at 0.71 and 0.63 for ADG and G:F, respectively. In Exp. 5, the objective was to confirm the results of Exp. 4 by determining N balance in pigs fed either low-fiber or high-fiber diets that were deficient or adequate in Thr. Thirty-six growing gilts (initial BW: 29.0 ± 0.74 kg) were housed in metabolism cages and there were 9 replicates per diet. Output of N in feces was greater ($P < 0.05$) from pigs fed high-fiber diets, but output of N in urine was greater ($P < 0.05$) from pigs fed low-fiber diets. The ATTD of N was greater ($P < 0.05$) from pigs fed low-fiber diets than in pigs fed high-fiber diets, and retention of N was greater ($P < 0.05$) in pigs fed low-fiber diets than in pigs fed high-fiber diets. There was greater ($P < 0.05$) N retention in pigs fed high-Thr diets compared with pigs fed low-fiber diets. There was also an interaction ($P < 0.05$) between fiber level and Thr for output of N in feces with N output increasing ($P < 0.05$) as Thr in the high-fiber diet increased, whereas this was not the case for the low-fiber diet. Results indicate that higher fiber diets may require greater concentrations of Thr. In conclusion, results of these experiments indicate that increased fiber levels in diets fed to growing gilts may increase the requirement for Thr and diets with higher fiber levels should include a greater concentration of Thr.

Key words: amino acid digestibility, fiber, ideal protein, lysine titration, pigs, threonine, requirement

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CHAPTER 1

INTRODUCTION

Use of corn in diets for pigs often results in deficiencies of indispensable AA. This issue is usually overcome by inclusion of protein sources in the diet or the addition of AA in crystalline form. Like any AA, Thr can be included on a crystalline basis; however, the exact amount of Thr needed in diets fed to pigs is not completely established.

It is the accepted practice to express requirements for indispensable AA as a ratio relative to the Lys in the diet, but to date there is no agreement about the correct Thr:Lys ratio. The NRC (2012) indicates a standardized ileal digestible (**SID**) Thr to Lys ratio of 60:100 for 11-25 kg weanling pigs and also for 25-50 kg growing pigs (NRC, 2012). This value is in agreement with ARC (1981). However, ratios of 67:100 and 72:100 have also been suggested for the ideal Thr to Lys ratios (Wang and Fuller, 1989; Baker, 1997).

There is also the question of how the level of Thr needed in the diet will change as ingredients change. In particular, it is not clear how fiber affects the requirement for Thr by pigs. Fiber is not traditionally included in large quantities in swine diets, but as by-products from the grain processing industries are becoming become more commonly used, the effects of fiber are becoming a growing concern. An increase in dietary fiber will increase endogenous losses of nutrients including AA (Dilger et al., 2004; Hansen et al., 2006; Cervantes-Pahm et al., 2014); however, the effect on Thr is of special interest because Thr is abundant in mucin (de Lange et al., 1989; Easter, 1994). Thus, there is a considerable amount of Thr in the lining of the intestinal tracts. The concentration of Thr is also greater in endogenous protein than the concentration of any other indispensable AA (Stein et al., 1999). Increased concentration of fiber in the diet can

increase the endogenous losses of protein, and therefore also the endogenous losses of Thr (de Lange et al., 1989; Zhu et al., 2005), but it is not known if this will result in an increase in the ideal ratio of Thr:Lys in the diet.

It was the objective of this research to determine the effect of fiber in the diet on the optimal Thr:Lys ratios for 25 to 50 kg growing gilts.

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CHAPTER 2

THE EFFECTS OF FIBER ON THE OPTIMUM THREONINE:LYSINE RATIO: REVIEW OF LITERATURE

Maximizing growth in pigs is a top priority in pig production. However, the ultimate priority in swine production is maximize profits from swine production (Noblet and van Milgen, 2013). Managing the cost of feed is critical to maintaining profitable production systems, as feed is the most expensive component of swine production. Therefore, when formulating diets, it is economical to supply both energy and nutrients as close as possible to, but not in excess of, requirements (Noblet and van Milgen, 2013).

For several decades, the common practice in North America has been to formulate diets for swine using primarily corn and soybean meal (**SBM**; Baker, 1997). However, the rising cost of corn and SBM, in addition to other factors, has accelerated the demand for alternative feed ingredients. Corn-soybean meal diets were typically formulated with the intention of providing enough CP to support high levels of lean growth in pigs (Baker, 1997). However, advances in swine nutrition over the last 30 years have changed the way diets are formulated, specifically in terms of how protein is formulated into diets. In particular, protein in diets is now viewed not only on a CP basis, but also on an AA composition basis (Table 2.1; Baker, 1997). The availability of synthetic AA has made it possible to reduce the inclusion of SBM in the diets and instead add specific AA. However, to be successful with this approach, the exact requirement for each indispensable AA needs to be known.

There are 20 AA in most proteins, 10 of which are considered indispensable, because they cannot be synthesized by the animal, and therefore, these have to be included in the diet (Whittemore, 1993). The 10 indispensable AA for pigs are Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. The remaining 10 AA are considered dispensable and do not require specific supplementation in diets as they can be synthesized by the animal provided all precursors are available (NRC, 2012). The indispensable AA are included in diets as either an intrinsic part of an ingredient or in the synthetic, crystalline form. However, because supplementation of the indispensable AA is expensive, knowledge about the exact requirement of each AA is needed.

THREONINE

Threonine was discovered in 1935 by Dr. William Rose at the University of Illinois (Easter, 1994). Being the final indispensable AA to be discovered, its discovery was particularly important in that it enabled researchers to formulate completely synthetic diets (Easter, 1994). As with any AA, Thr is an organic molecule composed of an amino group and a carboxylic group that are bound to a carbon skeleton (Easter, 1994). Threonine molecules exhibit optical isomerism (Easter, 1994). The alpha carbon of the Thr molecule is asymmetrical, which results in 4 possible configurations (Lewis, 2001). These 4 forms are dextrorotatory threonine (**D-Thr**; Figure 2.1), levorotatory threonine (**L-Thr**; Figure 2.1), dextrorotatory-allothreonine and levorotatory-allothreonine. The terms dextrorotatory and levorotatory are indicative of the direction in which a beam of light will rotate when passing through an aqueous solution of the isomer, with D-Thr causing a rightward rotation and L-Thr causing a leftward rotation (Easter, 1994). The ability to utilize D-Thr is dependent on the ability of an animal to enzymatically

convert D-Thr to an alpha-keto acid, because Thr does not undergo any direct transamination (Easter, 1994; Lewis, 2001). The alpha-keto acid that is a result of de-amination of the D-Thr is then transaminated into L-Thr, which can then be reaminated for use in protein synthesis. Although data are scarce on the utilization of D-Thr in swine, it has been shown that chickens, rats, and humans are unable to utilize D-Thr (Easter, 1994). Therefore, it is assumed that swine are unable to utilize D-Thr, however, further research is needed to confirm this (Easter, 1994).

Although Thr is utilized for synthesis of proteins that are deposited in skeletal muscle and used for synthesis of energy, it has several other critical functions. Threonine is a major component of several gastrointestinal epithelial cells, digestive enzymes, and mucosal secretions (Easter, 1994). There is also a high concentration of Thr in immunoglobulins, and even a slight deficiency of Thr can have dramatic inhibitory effects on immune globulin synthesis (Easter, 1994). Threonine is also a precursor for the *in vivo* synthesis of glycine (Easter, 1994).

Amino acid catabolism can be classified in 2, non-mutually exclusive, ways that are dependent on the products of AA degradation (Tymoczko et al., 2013). These 2 classifications are as ketogenic AA and glucogenic AA and, as their names imply, they refer to the ability of the products to generate ketone bodies and fatty acids or glucose, respectively (Tymoczko et al., 2013). However, AA are not directly degraded into any of these products, but rather as precursors of those components. All of the 20 AA are initially hydrolyzed into one of the following: pyruvate, acetyl CoA, acetoacetyl CoA, alpha-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate (Tymoczko et al., 2013). Acetyl CoA and acetoacetyl CoA are the only 2 of these compounds that are considered ketogenic, while the remaining 5 intermediaries are all glucogenic (Tymoczko et al., 2013). Threonine is considered both ketogenic and glucogenic

(Tymoczko et al., 2013) because it can be a precursor of glucose via either succinyl CoA or pyruvate, or a precursor of fatty acids and ketone bodies via acetyl CoA (Tymoczko et al., 2013).

Threonine catabolism is initiated using one of 3 different enzymes. These are threonine aldolase, threonine dehydrogenase, and threonine dehydratase (Figure 2.2; Easter, 1994). Threonine dehydratase catalyzes the reaction converting threonine into α-ketobutyrate (Easter, 1994). Alpha-ketobutyrate is then converted via pyruvate dehydrogenase or the branched-chain keto acid dehydrogenase into propionate, which enters the citric acid cycle as propionyl CoA after conversion by Coenzyme A (Brosnan and Brosnan, 2013). Threonine is often also utilized as a precursor of glycine. In avians, threonine aldolase is the primary enzyme of Thr catabolism (Easter, 1994). This enzyme converts Thr into acetaldehyde and glycine (Rodwell, 1993). Threonine aldolase is also a primary enzyme for oxidation in plants, bacteria and fungi (Brosnan and Brosnan, 2013). Swine primarily utilize threonine dehydrogenase in the catabolism of Thr into glycine (Easter, 1994), in which case Thr is hydrolyzed into 2-amino-3-ketobutyrate (Easter, 1994), which is then converted to either acetyl CoA and glycine or aminoacetone, another precursor of acetyl CoA (Easter, 1994). It is estimated that 80% of Thr oxidation in swine is through threonine dehydrogenase and that this increases as Thr intake increases (Brosnan and Brosnan, 2013). In humans, threonine dehydrogenase activity is low and is not subject to fluctuation in response to increased Thr intake or hormonal activity (Brosnan and Brosnan, 2013). However, in humans, threonine dehydratase is responsive to Thr intake and responsible for an estimated 90% of Thr oxidation (Brosnan and Brosnan, 2013).

IDEAL PROTEIN

Historically, requirements for AA were described as CP requirements, and it was common to describe diets on the basis of CP level. The CP in a diet or ingredient is calculated by multiplying the quantity of N by 6.25 (NRC, 2012). Using a multiplier based on N is possible because N is mainly present in proteins. The multiplier of 6.25 is used because AA on average contain 16% nitrogen (NRC, 2012). Because Lys is the first limiting AA in a corn-SBM diet for pigs, formulating corn-SBM diets to meet the Lys requirement will also provide adequate amounts of all other indispensable AA (Easter, 1994; Baker, 1997). However, using this concept may not always provide sufficient quantities of all indispensable AA if other ingredients are included in the diet. Likewise, if synthetic Lys and possibly other synthetic AA are used in the diet, other AA may become first limiting. In addition, if diets are formulated without using synthetic AA, some AA are usually included in excess of the requirement, which will result in excretion of large amounts of N in the urine. As a consequence, determination of the exact amount of AA required for optimal growth is necessary, because that will allow for formulation of diets that meet the requirements of all AA without excessive quantities of other AA. However, determining the requirements of all indispensable AA for all groups of pigs is tedious and expensive, and may not always be practical.

Instead, the concept of using an ideal protein in diet formulation was introduced in 1981 by the Agricultural Research Council (ARC, 1981). The theory behind ideal protein was to develop a perfect profile of AA required for growth of the pig (Easter, 1994). The underlying principle behind an ideal protein is that in providing a pig all AA, in neither excess nor deficiency, maximum performance will be attained (Easter, 1994). To maximize performance, an ideal protein needs to not only meet AA requirements for maintenance, but also for live growth.

However, considering variation among pigs in terms of genetics, gender, environment, etc., it is difficult to create a single uniform requirement of AA, but by using an ideal protein, diets can be formulated to contain correct quantities of AA at any level of requirement. The ideal protein was designed as a ratio of AA relative to Lys (Easter, 1994). Lysine was chosen because it is the first limiting AA in most diets and there is more information about the Lys requirement than about the requirement for any of the other AA (NRC, 2012). The use of a ratio eliminates the need for extensive testing to determine the requirements of dietary concentrations of all AA for optimal growth. Instead, if the Lys requirement is determined, the requirement for all remaining AA can be calculated using the ideal ratio. It is, however, necessary to calculate a ratio for the varying growing stages of any particular group of pigs, to accommodate for increasing maintenance requirements in pigs of different sizes (Baker, 1997; NRC, 2012).

The first ideal protein that was determined by the Agricultural Research Council in 1981 was based on compilations of growth performance data and tissue analysis (ARC, 1981). It was assumed that tissue samples analyzed for AA collected from pigs with low levels of N excretion in the urine would allow for an accurate prediction of AA requirements (ARC, 1981). The data from tissue composition was used to complement and fill gaps left by growth performance data at the time (ARC, 1981; Easter, 1994). However, the balance of AA may not be completely accurate because it was compiled from experiments that were not specifically designed to determine AA ratios. In the following years, several experiments with a more direct approach were conducted. In 1989 and in 1992, ideal proteins based on digestible AA were published (Wang and Fuller, 1989; Chung and Baker, 1992). Baker furthered the research and published data for pigs at 3 different weight ranges (Table 2.2) in an effort to accommodate for changing growth rates and patterns throughout the animal's lifetime (Easter, 1994). However, unless all

AA are absorbed, utilized, and deposited at the same rate, the levels of AA in tissues will not represent actual requirements (Whittemore, 1993). One definition of availability is "the proportion of the total AA that is digested and absorbed in a form suitable for protein synthesis", highlighting the difference between availability and digestibility (Batterham, 1992). Additionally, ileal digestibility of AA in ingredients do not always correlate with availability of AA, with ileal digestibility typically overestimating availability in ingredients with low availability of AA (Batterham, 1992). Several experiments measuring growth performance of pigs fed diets based on SBM, cottonseed, and meat and bone meal, indicated that ileal digestibility of AA is a better indicator of AA availability when the quality of the protein source is high, and that as the quality of a protein source is degraded, particularly by heat damage, ileal digestibility decreasingly represents availability, particularly of Lys, Thr, and Met (Batterham et al., 1990; Batterham, 1992).

THE OPTIMAL THR TO LYS RATIO

Several experiments have been conducted to determine the optimum Thr:Lys ratio in growing and finishing pigs (Table 2.3). Diets used to determine the Thr:Lys ratio are formulated to be deficient in Lys and Thr as described by Van Cauwenberghe and Relandeau (2000). Most Thr requirement studies utilize growth performance, ADFI, and plasma urea N (**PUN**) as response parameters to assess responses to varying levels of Thr in diets (Chang et al., 2000; Pedersen et al., 2003; Zhang et al., 2013; Xie at al., 2013). The levels of Thr that maximize G:F, ADG, and minimize PUN are reported as estimates of the requirement (Chang et al., 2000; Pedersen et al., 2003; Zhang et al., 2013; Xie at al., 2013).

One of the earliest experiments conducted to determine Thr requirement in pigs was performed at the University of Illinois using N-balance as a response parameter (Mitchell et al., 1968). Semi-synthetic diets composed of primarily corn starch, lactose, casein and some synthetic AA were fed to 10 kg pigs and N retention was measured over graded levels of Thr resulting in an estimated requirement of 0.60% of the diet, or 67% of the Lys requirement. At the time, this value was significantly different from what had been published by other groups, with other experiments recommending values as low as 0.40% and as high as 0.92% (Mitchell et al., 1968). In 2 experiments using growth and plasma free Thr as response parameters to graded Thr levels in semi-synthetic diets fed to 15 kg and 20 kg pigs, respectively, a group at the University of Minnesota estimated the Thr:Lys ratio as 49% and 46% (Sowers and Meade, 1972). However, the Agricultural Research Council (1981) ignored these values in their estimate of the optimum Thr:Lys ratio, calling the low CP value (10.4%) of the basal diet into question. The Agricultural Research Council (1981) recommended a Thr:Lys ratio between 56 and 60%, a decrease from the 61% recommended by the Agricultural Research Council in 1967. Their recommendation was derived from averaging several estimates for the optimal Thr:Lys ratio published before 1981. Using rapeseed meal and cottonseed meal based diets supplemented with synthetic AA, 2 groups from China Agricultural University and Seoul National University utilizing growth performance as the response parameter, determined the ideal Thr:Lys ratio as 67.5% for 22 kg pigs (Li et al., 1998). That same year, a value of 60% was reported by NRC (1998) to maximize protein accretion.

Utilizing growth performance as the response parameter, Chang et al. (2000) determined that although barrows had greater ADG than gilts, it was a result of increased ADFI, and there was no interaction between sex and dietary Thr:Lys ratio. Although the experiment did not show

clear responses in growth performance, the recommendation was for a Thr:Lys ratio of 60 and 70%, for gilts and barrows, respectively. A comprehensive experiment to determine the optimal Thr:Lys ratio in finishing pigs utilized growth performance, PUN, and N-balance as response parameters to wheat, barley, and SBM diets supplemented with increasing levels of Thr (Pedersen et al., 2003). Plasma urea N, ADG, and N-balance results indicated optimal Thr:Lys ratios of 66, 62, and 64%, respectively (Pedersen et al., 2003). In 2009, a N-balance study using diets with wheat, barley, SBM, and field peas was conducted with pigs at 30, 50, 70, 90, and 110 kg BW. The optimal Thr:Lys ratios were estimated at 64, 60, 61, 59, 63, and 61, respectively, with the average estimate being 61% (Wecke and Lieber, 2009). The NRC (2012), compiling new data, still maintained the same ratio as in NRC (1988) at 60%.

In 2013, the optimal Thr:Lys ratio was determined in low CP diets supplemented with synthetic AA fed to growing pigs (Zhang et al., 2013). Utilizing ADG, G:F, and PUN as response parameters, the proposed Thr:Lys ratios were 68%, 67%, and 62%, respectively, if broken-line analysis was used to determine the ratio. Using curvilinear plateau analysis, the ratios were 73%, 70% and 65%, respectively. Low CP diets supplemented with synthetic AA were used to determine the Thr:Lys ratio in finishing barrows using ADG, GF, and PUN as response criteria (Xie et al., 2013). The optimal Thr:Lys ratio was estimated at 75%, using both quadratic and broken-line analysis.

Despite the many experiments conducted to determine the optimum Thr:Lys ratio in growing pigs, there is still considerable variation among estimates. Genetics, health status, growing stage, and diets are some of the potential variables that may affect the optimal Thr:Lys ratio. Additionally, the role fiber may play in influencing the optimal Thr:Lys ratio in growing

pigs has not been investigated, but this is an important consideration because of the varied ingredients that have been used in diets in the different studies.

FIBER

Definitions of Fiber

Fiber has had several definitions within the past 60 years. The first definition of "dietary fiber" is commonly attributed to Eben Hipsley in 1953 as the sum of "lignin, cellulose, and the hemicelluloses" (Hipsley, 1953). The definition of fiber has since had several iterations. The difficulty in defining fiber comes from the difficulty in reconciling the nutritional and physiological characteristics of fiber with the analytical methods used to isolate it (AACC, 2001). Hipsley's definition was unique in that it made an attempt to specify crude fiber into components (DeVries et al., 1999). Trowell et al. (1972) redefined dietary fiber as the "remnants of plant components that are resistant to hydrolysis by human alimentary enzymes". By 1976, the definition of dietary fiber was expanded to include all indigestible polysaccharides with a focus on physiological characteristics of edibility and resistance to digestion (Trowell et al., 1976). For several years this definition was widely accepted, however, as the demand to quantify specific portions of fiber increased, there was a need for a consensus on not only the content of fiber, but also the analytical methods used to identify fiber (DeVries et al., 1999). In 1985, the AOAC developed a working definition of total dietary fiber as the results of the Enzymatic-Gravimetric Method based on the Trowell definition of 1976 (AOAC method 985.29; DeVries et al., 1999). In 1998, the American Association of Cereal Chemists (**AACC**) appointed a review committee with updating the definition of dietary fiber (AACC, 2001). The following year, at the AACC

Annual Meeting the results of the committee were manifested in a new definition of dietary fiber: "Dietary fiber is the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber exhibits one or more of the following characteristics: laxation (fecal bulking and softening; increased frequency; and/or regularity), blood cholesterol attenuation, and/or blood glucose attenuation" (AACC, 2001). Two years later, the Institute of Medicine developed a new definition of dietary fiber as: "consist[ing] of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Total fiber is defined as the sum of Dietary Fiber and Functional Fiber (Paeschke and Aimutis, 2011). In 2009, the Codex Alimentarius proposed the following definition: "Dietary fiber means carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic, or chemical means, and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorites; synthetic carbohydrate polymers, which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities" (Paeschke and Aimutis, 2011).

Although the current definition of fiber is sure to evolve further, the core principle behind the concept of fiber, and all proposed definitions, is that it is a sum of certain non-digestible

carbohydrates. The non-digestible carbohydrates are generally classified as certain sugars, certain oligosaccharides and non-starch polysaccharides, and resistant starches (NRC, 2012); however, dietary fiber can be classified into 4 empirically-determined components: crude fiber, neutral detergent fiber, acid detergent fiber, and non-starch polysaccharides (Souffrant, 2001). Non-starch polysaccharides (**NSP**), the broadest categorization, are composed of cell wall components and non-cell wall components (NRC, 2012). Cell wall components are cellulose and hemicellulose primarily, but also can include arabinoxylans, xyloglucans, arabinogalactans, galactans, and β-glucans (NRC, 2012). Non-cell wall components of NSP are pectins, gums, and resistant starches (NRC, 2012). Crude fiber, the oldest measurement, is determined through the use of the acid-gravimetric procedure and is composed of cellulose, lignin, and some hemicellulose (Grieshop et al., 2001; Souffrant, 2001). Acid detergent fiber is composed of cellulose and lignin (Grieshop et al., 2001). Neutral detergent fiber is composed of cellulose, hemicellulose, and lignin (Grieshop et al., 2001). Because these classifications are not mutually exclusive the difference between neutral detergent fiber and acid detergent fiber is an estimate of hemicellulose (Grieshop et al., 2001).

Solubility of Fiber

Dietary fiber is often discussed in general terms of solubility and insolubility based on chemical properties of the fiber sources (Dikeman and Fahey, 2006). Soluble dietary fiber includes β-glucans, pectins, gums, and, in some models, resistant starches (Slavin and Feirtag, 2011; NRC, 2012). Insoluble fiber includes some hemicelluloses, celluloses, lignins and phenolic compounds (NRC, 2012).

Insoluble fiber has various effects on the gastro-intestinal tract of animals and on nutrient utilization (Schedle et al., 2008). Insoluble fiber is known for its fermentability in the hind gut of animals, and the possible prebiotic effect it can have on the intestinal microbiota, and thus the entire digestive process, despite being generally less fermentable than soluble fibers (Brodribb and Groves, 1978; Wenk et al., 2001). Insoluble fiber may promote gastro-intestinal health by reducing transit time of chyme or by increasing water holding capacity, and thus reduce the time the tract is exposed to potentially toxic fecal compounds (Schedle et al., 2008). Insoluble fibers can have a significant effect on gut health by increasing fecal bulk and reducing transit time (Rose and Hamaker, 2011). Insoluble fibers with low fermentability have a greater effect on increasing fecal bulk and reducing fecal transit times than soluble fibers with greater fermentability (Cummings et al., 1992). These traits are important because slower intestinal transit times may have negative effects on the animal (Lewis and Heaton, 1999). Slower transit rates result in greater absorption of short-chain fatty acids in the proximal colon, which reduces the amount of short-chain fatty acids available for the distal colon (Lewis and Heaton, 1999). Additionally, longer transit times can result in constipation and lower fecal volume (Stephen et al., 1987).

Soluble fibers are primarily associated with viscosity, while insoluble fibers are associated with fermentability, and it has been proposed that viscosity and fermentability reflect the characteristics of these fibers better than the terms solubility and insolubility (Dikeman and Fahey, 2006). Viscosity is defined as the proportional relationship between the flow of a liquid and the direction of a force on that liquid (Dikeman and Fahey, 2006). Although measuring viscosity can be difficult because of individual variation involving various factors such as pH, non-Newtonian dilatant flow properties, temperature, moisture content, and processing, viscosity

does have some general effects on digestive processes and nutrient utilization (Dikeman and Fahey, 2006). Increased viscosity due to the consumption of soluble fibers has been associated with reduced glucose absorption and reduced insulin response (Wood et al., 2000). These responses have been theorized to be the result of water-soluble fibers inhibiting nutrient absorption due to the increase in luminal viscosity and reduced rate of gastric emptying in combination with other unknown factors (Edwards et al., 1987; NRC, 2006). Soluble fiber also alters blood lipid levels through increased viscosity (Dikeman and Fahey, 2006), and soluble fiber components, particularly β-glucans, may reduce plasma concentration of lipids (Nicklas et al., 2012). Soluble fiber may also reduce plasma triacylglycerol levels and fatty acid synthesis (Topping et al., 1988). Mechanisms responsible for the altered blood lipid levels may include: decreasing rates of diffusion and cholesterol absorption, changing bile acid metabolism, changing hepatic cholesterol synthesis, reduced rates of emulsification of lipids, changing SCFA production, and altering cholesterol excretion (Dikeman and Fahey, 2006).

Effects of Fiber on Energy, N, and AA Utilization

Both soluble fibers and insoluble fibers, however, have effects on protein, OM, and energy metabolism and utilization (Hansen et al., 2006). Fiber may decrease OM and energy digestibility (Jorgensen et al., 1996), possibly because of reduced retention time and increased endogenous loss of CP (Hansen et al., 2006). Insoluble fibers may decrease digestibility of OM and energy more than soluble fibers (Hansen et al., 2006). These results are influenced by the ability of microbes to absorb the by-products of fermentation, which is dependent on the solubility of NSPs entering the large intestine, with soluble fibers having greater retention times and greater carbohydrate availability (Latymer et al. 1990; Bach Knudsen et al., 1993).

Fiber also has negative effects on energy digestibility (Urriola et al., 2013). Increasing inclusion rate of wheat bran resulted in decreasing total tract digestibility due to decreases in DM and OM digestibility (Wilfart et al., 2007). The degree of energy reduction has been calculated as a 1% decrease in energy digestibility for each 1% increase in NDF concentration in the diet (Le Gall et al., 2009; Urriola et al., 2013). Several factors modify fiber's effect on energy digestibility. Lignin specifically reduces energy digestibility (Wenk et al., 2001). Additionally, solubility affects energy digestibility with soluble fibers having a lesser effect on the reduction of energy digestibility (Le Gall et al., 2009; Mroz et al., 2013). Decrease in energy digestibility may also be partly attributed to reduced lipid and N digestibility, as a consequence of increased fiber concentration.

The effect of fiber on lipid digestibility is varied. Lipid digestibility, in some instances, increases with its inclusion level in the diet (Dégen et al., 2009; Kil et al., 2010). Nonetheless, multiple experiments that paired increasing lipid concentration with increasing fiber concentration have resulted in few consistent effects on fecal lipid digestibility (Dégen et al., 2009; Bach Knudsen and Hansen, 1991). Results of an experiment using a combination of triticale, wheat, and wheat bran as a fiber source indicated an improvement in the apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD)** of lipids (Högberg and Lindberg, 2004). However, there is limited evidence that a large increase in fiber concentration in a diet, particularly in the form of wheat bran, can enhance pancreatic secretion and lipase activity potentially increasing lipid digestibility (Dégen et al., 2009). Conversely, results of other experiments indicate that wheat bran reduced ATTD of ether extract (Wilfart et al., 2007). It is also possible that the source of fiber and its solubility play a role in effects of fiber on lipid digestibility.

The primary change in N digestibility with the inclusion of fiber is the shift of excretion of N from urine to feces, as bacteria utilize N produced through fermentation (Zervas and Zijlstra, 2002; Urriola et al., 2013). Increases in the concentration of fiber in diets have been associated with decreases in AA digestibility, although apparent N digestibility was not changed in diets with fiber compared with control diets (Dilger et al., 2004). The reasons for these effects are assumed to be that dietary fiber induces losses of endogenous nutrients in the form of digestive enzymes, enterocytes, and mucins (Dilger et al., 2004). Results of other studies have confirmed that the apparent total tract digestibility of CP decreases, and N content in feces increases, as fiber in the diet increases (Hansen et al., 2006; Cervantes-Pahm et al., 2014). Water-holding capacity of fibers can directly affect the ileal endogenous N and AA excretion (Souffrant, 2001).

Although fiber's effects on N and AA digestibility are dependent on the source, nature, chemical composition, and physico-chemical properties of the fiber (Souffrant, 2001), fiber influences both mucin production and endogenous losses of AA (Satchithanandam et al., 1990). The effects of fiber on AA digestibility are important to consider, especially with regard to Thr, because gastrointestinal tissues and mucin secretions have high concentrations of Thr (Wang et al., 2007). As the gastrointestinal mass increases, mucin production will increase. Mucin is excreted by specialized mucosal epithelial cells called goblet cells lining the intestinal tract where it serves as a buffer, a barrier to pathogens, and a proteolytic barrier (Satchithanandam et al., 1990; Bansil et al., 1995; Wang et al., 2010). Mucin is composed of glycoproteins in a ratio of 3 parts carbohydrate to 1 part AA residue of either Ser or Thr (Bansil et al., 1995). The increase in mucin production that is observed if fiber is included in the diet can have 2 effects on the utilization of nutrients in an animal. As mucin production increases, the increased protein

synthesis results in an increase in nutritional requirements. In addition, the increase in production of mucin in response to increased dietary fiber may also impair nutrient absorption, particularly absorption of cholesterol and glucose due to increased resistance of the unstirred water layer composed primarily of mucin (Satchithanandam et al., 1990).

Fiber solubility is a major factor in the effect fiber has on AA secretion and losses and insoluble fibers have a greater effect on increasing mucin production than soluble fiber (Satchithanandam et al., 1990). However, soluble fibers elicit greater increases in secretion of saliva, gastric juices, pancreatic juice, and bile than insoluble fibers (Wenk, 2001). It is theorized that the increased activity of these secretory organs results in their enlargement (Wenk, 2001). This increased metabolic activity can have a negative effect on nutrient requirements, but is balanced to some degree by increases in digestibility (Wenk, 2001). Because up to 50% of dietary Thr is retained by the intestine of healthy pigs (Wang et al., 2010), and because an increase in dietary fiber concentration can increase the size of the intestine (Younoszai et al., 1978), it is likely that a larger intestine may require greater amounts of Thr. Additionally, the increase of substrate for microbial fermentation in the form of fiber has negative effects on the hosts utilization of protein (Libao-Mercado et al., 2008). Besides increased secretion of mucus glycoproteins, there is also increased epithelial cell turnover if fiber is included in the diet. Any immune stimulation due to increased numbers of microbes will also result in increased AA requirements.

FIBER AND THREONINE

Fiber may cause an increase in the requirement of Thr by increasing endogenous loss of Thr (Satchithanandam et al., 1990). Diets low in fiber elicit endogenous losses of Thr between 0.51 and 0.73 g Thr/kg DM with endogenous loss increasing as fiber concentration increases (Blank et al., 2012). The majority of experiments conducted to determine the maintenance requirement for Thr in growing pigs were conducted using diets with low levels of fiber (NRC, 1998), but as fiber increases, dietary requirement of Thr will also increase (Blank et al., 2012). Accounting for increased intestinal loss of Thr, it is estimated that an increase of 120g NDF/kg diet in an 80 kg pig can result in a 2-fold increase in the daily requirement for digestible Thr for maintenance (Blank et al., 2012).

The exact reasons for increases in the Thr requirement as a function of increasing fiber remain unknown; however, several potential factors have been identified. A significant portion of the increased Thr requirement in fibrous diets is the result of increased endogenous losses. Primarily, the abrasive nature of undigested feed, particularly as insoluble fiber, increases endogenous protein loss (de Lange et al., 1989). This is of particular relevance to the Thr requirement as a significant component of abrasion-induced endogenous losses is Thr-rich mucin proteins lining the gastrointestinal tract (de Lange et al., 1989). Between 20 and 30% of AA residues in mucins are Thr (Brosnan and Brosnan, 2013). Unlike other streams of endogenous AA loss, mucin proteins are poorly digested and the AA in mucin, therefore, are not reabsorbed. Instead, these AA are passed into the hindgut where they are subject to microbial fermentation or excretion (de Lange et al., 1989). It has also been theorized that certain fibers have stimulatory effects on microbial fermentation in the hindgut, which may increase the endogenous loss of Thr (Sakata, 1987; Zhu et al., 2005). Microbial fermentation results in the production of SCFA. *In*

vivo data in rats indicate that SCFA stimulated epithelial proliferation of the intestine, which increased the weight of this organ (Sakata, 1987). The combination of increased gastrointestinal tissue mass and the concomitant increased mucin production will result in increased Thr requirements. Dietary fiber, therefore, has a greater negative effect on utilization of Thr for protein deposition than of other AA, and diets with increased fiber may reduce the estimated Thr utilization efficiency by 9% (Zhu et al., 2005).

CONCLUSION

Threonine is an indispensable AA that is needed for both tissue protein accretion and necessary for maintenance. Threonine is also the greatest indispensable AA component of endogenous secretions in pigs (Stein et al., 1999). Because Thr is present in high concentrations in mucin (Satchithanandam et al., 1990), a large component of the endogenous secretions are likely to be mucins. A major cause of the increased mucin is the abrasion induced by diets high in fiber (Satchithanandam et al., 1990). Increased mucin secretion will result in increased mucin production, and thus, in an increased Thr requirement. Because Thr is the greatest indispensable AA component of endogenous secretion and because endogenous protein primarily consists of mucin, it is assumed that the requirement for Thr will increase as the concentration of dietary fiber increases. However, the requirement for Lys is assumed to remain unchanged or only increase slightly as the concentration of dietary fiber increases. Thus, it is our hypothesis that as fiber concentration increases in the diet, the optimal Thr:Lys ratio will also increase.

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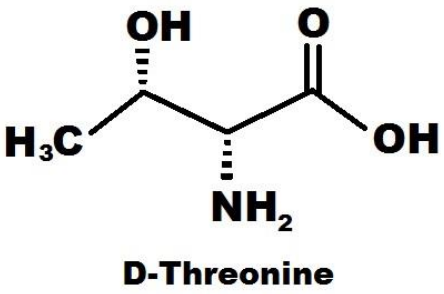
FIGURES

Figure 2.1.

D-Threonine molecule.



L-Threonine molecule.

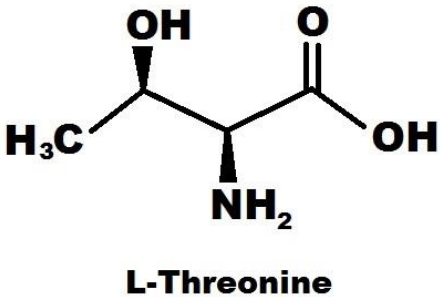
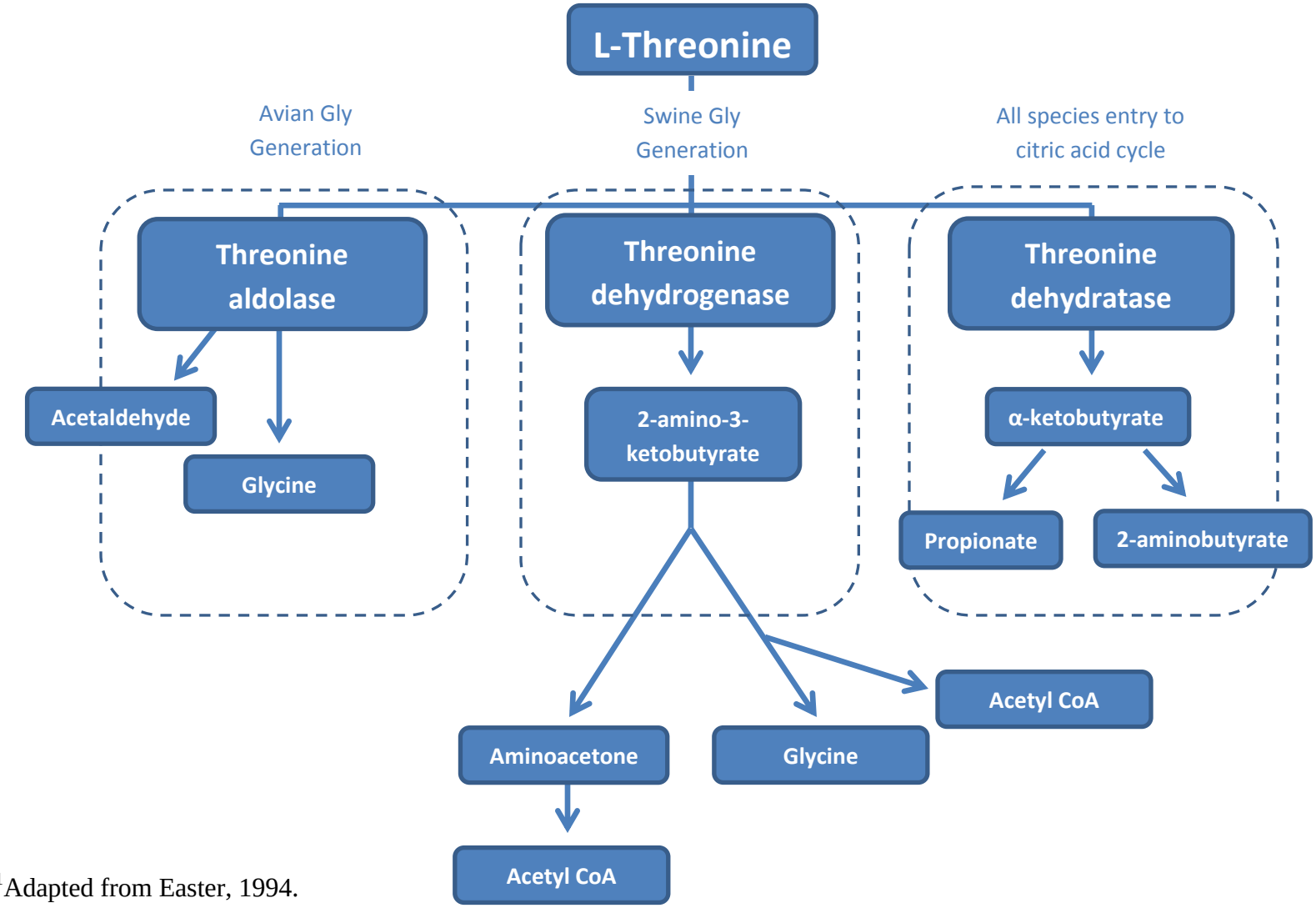


Figure 2.2. L-Threonine Metabolism[1]



[1]Adapted from Easter, 1994.

TABLES

Table 2.1. Crude protein, Lys, and Thr concentration (%) of selected feed ingredients[1]

Ingredient	CP	Lys	Thr
Barley	11.33	0.40	0.36
Canola meal, expelled	35.19	1.58	1.22
Corn, yellow dent	8.24	0.25	0.28
Corn DDGS, 6-9% oil	27.36	0.90	0.99
Cottonseed meal	39.22	1.50	1.36
Field peas	22.17	1.63	0.83
Oats	11.16	0.49	0.42
Palm kernel meal	14.39	0.36	0.47
Rice	7.87	0.35	0.23
Rye	11.66	0.43	0.30
Sorghum	9.36	0.20	0.30
Soybean hulls	10.27	0.66	0.09
Soybean meal, dehulled, solvent extracted	47.73	2.96	1.86
Triticale	13.60	0.46	0.41
Wheat, hard red	14.46	0.39	0.40

[1]Values from NRC (2012).

Table 2.2. Ideal protein for indispensable AA in diets for 3 weight categories[1]

Amino Acid	10 to 20 kg	20 to 50 kg	50 to 110 kg
Lys	100	100	100
Arg	42	30	18
His	32	32	32
Ile	60	60	60
Leu	100	100	100
Met + Cys	60	62	64
Phe + Tyr	95	95	95
Thr	65	67	70
Trp	17	18	19
Val	68	68	68

[1]Table adapted from Baker, 1997.

Table 2.3. Proposed Thr:Lys ratios for growing and finishing pigs

Authors	Response Parameters	Thr:Lys Ratio
Mitchell et al., 1968	N-balance	67:100
Henry and Rerat, 1970	Growth	56:100
Lougnon and Brette, 1971	Growth	57:100
Sowers and Meade, 1972	Growth and plasma free Thr	49:100
Taylor et al., 1975	Growth, carcass, and PUN	59:100
ARC, 1981	Meta-analysis	56:100 – 60:100
Li et al., 1998	Growth, plasma free Thr, and PUN	67.5:100
NRC, 1998	Meta-analysis	60:100
Chang et al., 2000	Growth and PUN	60:100 – 70:100
Pedersen et al., 2003	Growth, PUN, and N-balance	62:100 – 66:100
Wecke and Liebert, 2009	N-balance	61:100
NRC, 2012	Meta-analysis	60:100
Zhang et al., 2013	Growth, plasma free Thr, and PUN	63:100 – 70:100
Xie at al., 2013	Growth and PUN	67:100 – 75:100

CHAPTER 3

ESTIMATION OF THE REQUIREMENT FOR STANDARDIZED ILEAL DIGESTIBLE LYSINE IN 25 TO 50 KG GILTS

ABSTRACT: An experiment was conducted to determine the standardized ileal digestible (SID) Lys requirement for gilts from 25 to 50 kg BW. Seventy gilts (initial BW: 24.54 ± 3.28 kg) were used in a growth assay with 2 pigs per pen and 7 pens per treatment. Diets were formulated using corn and soybean meal as the sole sources of AA. Under the assumption that Lys is the first limiting AA in corn-soybean meal diets, soybean meal concentration was increased at the expense of corn to increase SID Lys in the diets. Five treatments with calculated SID Lys levels of 0.80%, 0.93%, 1.06%, 1.19%, and 1.32% were formulated using values from NRC (2012). Accuracy of diet formulation was confirmed by analyzing diets for total Lys. Daily feed allocations were recorded and individual pig weights were recorded at the beginning and at the conclusion of the experiment. Results indicated that ADG increased ($P < 0.05$) quadratically and G:F increased linearly ($P < 0.05$) as SID Lys increased from 0.80% to 1.32%. Broken-line and curvilinear-plateau regression analyses were used to estimate the requirement for SID Lys. Results indicated that 1.08% SID Lys was needed to maximize ADG and 1.10% SID Lys was needed to maximize G:F. Thus, results of this experiment indicate that the SID Lys requirement for 25-50 kg growing gilts is slightly greater than the recent estimate of 0.98% reported by NRC (2012). Under the conditions of this experiment, the requirement for SID Lys for 25 to 50 kg gilts is approximately 1.09%.

Key words: amino acids, corn, lysine, pigs, requirement, soybean meal

INTRODUCTION

Lysine, along with Leu, are considered the AA with the greatest requirements by growing pigs, but because of the relatively high concentration of Leu in most feed ingredients, Lys is considered the first limiting AA in swine diets (Baker, 1997). Due to the high requirement for Lys, ideal protein theories typically express AA requirements in relation to the requirement for Lys (Baker, 1997). Thus, to determine a particular AA requirement according to the ideal protein ratio, it is necessary to first determine the requirement for Lys.

The requirement for Lys by pigs may be determined using only corn and soybean meal, because the concentration of Lys can be increased by increasing the level of soybean meal in the diets. Because it is assumed that Lys is the first limiting AA in a corn-soybean meal diet, it is possible to titrate Lys and determine the requirement for Lys using this approach (Cline et al., 2000; Shelton et al., 2011). In addition, because the Lys requirement will change as daily protein deposition rate changes among genotypes, it is important to determine the Lys requirement of specific genotypes. It was the objective of this study to determine the requirement for Lys in 25 to 50 kg growing gilts.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

A total of 70 gilts (G-Performer × Fertilis 25, Genetiporc, Alexandria, MN; initial BW: 24.54 ± 3.28 kg) were allotted to 5 diets with 7 pen replicates per diet and 2 pigs per pen. Five diets were based on corn and soybean meal – they contained 0.80, 0.93, 1.06, 1.19, and 1.32 %

standardized ileal digestible (**SID**) Lys (Tables 3.1 and 3.2). The different diets were created by changing the proportion of corn and soybean meal in the diets using principles described by Cline et al. (2000). Values for the SID of AA in corn and soybean meal were from NRC (2012).

Pigs were housed in pens with 50% solid concrete floor and 50% concrete slats. There were a feeder and a nipple drinker in each pen and the room was temperature controlled. Pigs had free access to feed and water throughout the experiment. Daily feed allocations were recorded and individual pig weights were recorded at the beginning of the experiment and at the end of the experiment 33 d later. The amount of feed left in the feeders was also recorded as pigs were weighed off of the experiment.

The ADG, the ADFI, and the G:F were calculated for each pen of pigs and for each treatment group at the conclusion of the experiment (Table 3). The concentration of AA in the diets were analyzed and the daily intake of Lys was calculated for each treatment group.

Chemical Analyses

All diets were analyzed for CP (method 990.03; AOAC Int., 2007) and AA. Amino acids were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, U.S.) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007).

Statistical Analyses

Data were analyzed by ANOVA using the UNIVARIATE and MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Treatment means were calculated using the LS Means test and separated using the PDIFF option. Broken line analyses were performed using the NLIN procedure of SAS (Robbins et al., 2006) for all variables that had significant linear effects, and quadratic line analyses were performed using the NLIN procedure of SAS for all variables with significant quadratic effects. Broken line analyses and quadratic analyses were used to determine the Lys requirement for pigs using ADG and G:F as the response criteria. The average of Lys required to maximize ADG and G:F was considered the Lys requirement of the pigs.

RESULTS AND DISCUSSION

Confirming the Lys requirement is an important first step in determining the requirements of any other AA in terms of a ratio to Lys. This determination makes it possible to formulate diets in which Lys will be a limiting AA, because only if Lys is limiting will it be possible to determine the correct ratio of other AA. Determining the Lys requirement in the same population as the AA ratio is being determined enables for an accurate determination of the AA to Lys ratio. Using corn and soybean meal diets made it possible to determine the Lys requirement in gilts using ingredients that are commonly used in commercial diets, and also offers some control for potential ingredient effects in subsequent confirmation and titration diets using these ingredients. Variation between calculated and analyzed values of concentration of Lys decreased as the concentration of Lys increased within diets. However, all variation was at or below the 20% analytical variation limit for Lys defined by AAFCO (2005).

Average daily gain increased quadratically ($P < 0.05$) as SID Lys increased from 0.80% to 1.32% (Table 3). Average daily feed intake was not influenced by dietary treatments, but G:F increased (linear; $P < 0.05$) as the concentration of Lys in the diets increased. Quadratic regression estimated the optimal SID Lys requirement at 1.08% for ADG (Figure 1). Broken-line regression estimated the optimal SID Lys requirement for G:F at 1.10% (Figure 2).

There are several statistical analyses utilized for determination of AA requirements. One of the most common methods is the broken-line regression method. Broken-line regression has been criticized for its generally lower requirement estimates when compared to other methods of analyses (Baker et al., 2002; Parr et al., 2003). Because the broken line regression determines the break-point at an average for the test population, it has been suggested that its determinations for requirement levels are below the true requirement for a significant portion of the population (Parr et al., 2003). However, because of this relatively conservative estimate, it has been cited by some as the preferred method for determination of AA ratios (Baker at al., 2002).

Another common method for AA requirement determination is the quadratic or curvilinear model. The quadratic model fits a quadratic line to the data and determines the requirement as the apex of the line. Typically, the quadratic model results in greater requirement estimates than broken-line models (Baker et al., 2002; Parr et al., 2003). Naturally, requirements determined by the quadratic models will meet the needs of a larger portion of the population, however, it will do so at the cost of overestimating the needs for a major portion of the population. Additionally, quadratic models have been criticized for their potentially subjective breakpoints (Baker et al., 2002). To control for the overestimation in quadratic models, a subjective method of using 90% of the estimated requirement has been suggested (Parr et al.,

2003). Another, more objective, form of the quadratic model, the quadratic broken-line, involves forcing the quadratic model to reach a plateau at the requirement (Robbins et al., 2006).

To overcome the weaknesses of both the broken-line and quadratic models, the combined broken-line and quadratic model was proposed (Baker et al., 2002; Parr et al., 2003). This method employs both broken-line and quadratic lines that are fitted to the data, and the requirement is determined as the first intersection of the quadratic line with the plateau region of the broken-line. It has been suggested that this combined analysis provides a more robust requirement estimate, which is adequate for a larger portion of the population than the broken-line method, but at the same time more conservative than the quadratic line method (Baker et al., 2002; Parr et al., 2003; Nemechek et al. 2012). Utilizing this combined method yields requirement estimates very similar to requirements estimated as 90% of quadratic model estimates, with the benefit of being completely objective (Parr et al., 2003).

Despite the combined broken-line and quadratic methodology's robustness, one of the primary limitations of this method is that it requires both significant linear and quadratic effects for the response parameters that are considered. In the case of the current data, none of the response parameters had both significant linear and quadratic effects. Thus, the combined broken-line and quadratic analyses could not be used. Therefore, the effect that was significant determined the method of statistical analyses utilized. A significant linear effect resulted in broken-line analysis for the requirement estimate, whereas a significant quadratic effect resulted in quadratic broken-line analysis.

Our data resulted in a quadratic increase in ADG as a function of increasing Lys concentration. The drop in ADG in pigs fed the diet with the highest concentration of SID Lys

may be due to a reduction in ADFI caused by the high concentration of Lys. Fitting a quadratic line to the model, the asymptote represented the optimal SID Lys percentage for maximizing ADG and was determined to be 1.08%. For G:F, our data demonstrated a linear increase as a function of increasing Lys concentration. Fitting a broken-line model to the data, the plateau represented the optimal SID Lys percentage for maximizing G:F and was determined to be 1.10%. As a consequence, the estimate for the SID Lys requirement was determined as an average of the values determined by ADG and G:F as 1.09%.

Our requirement of 1.09% SID Lys is substantially greater than what is suggested by the requirement tables in NRC (2012). There are several potential reasons for this observation. The NRC recommendation of 0.98% SID Lys is for both barrows and gilts, whereas our estimate is for gilts only. Gilts may require more Lys than barrows as they approach the finishing period because of reduced feed intake and increased lean deposition as compared with barrows (Cline et al., 2000). The NCR-42 Committee on Swine Nutrition determined that gilts between 35 and 105 kg required greater Lys concentration in the diet to maximize gain than did barrows (Cromwell et al., 1993). Additionally, in an experiment with mixed-sex pens, post hoc analysis indicated that gilts gained faster than barrows (Cromwell et al., 1996). However, our requirement estimate of 1.09% is also significantly greater than the 1.00% that is suggested by the model (NRC, 2012). Nevertheless, NRC values are based on several studies over a broad spectrum of genetics, whereas our requirement is based on a population of a particular Genetiporc line that is optimized for maximum growth rates. The requirement estimated in this experiment, however, is in agreement with those determined from a series of experiments conducted in commercial facilities using gilts at 3 different weight categories (Shelton et al., 2011). Like our experiment, those experiments increased the Lys concentration in the diets by altering the levels of corn and

soybean meal. For gilts from 38 to 65 kg, quadratic analyses determined the SID Lys level that maximized ADG and G:F at 1.1%, and broken-line analyses indicated levels of 1.03% and 1.05%, respectively. It is also possible that synthetic experimental diets do not adequately reflect the CP and energy levels in corn and because of this cannot be directly applied to conventional corn-soybean meal diets (Baker et al., 2002). In the present experiment, test diets were formulated using corn and soybean meal, which better represent commercial diets, and thus may be indicative of animal requirements and performance in commercial settings.

CONCLUSION

The SID Lys requirement to optimize ADG in 25 to 50 kg growing gilts is 1.08%, and the SID Lys requirement to optimize G:F in 25 to 50 kg growing gilts is 1.10%. Thus, by c the two estimates, an SID Lys of 1.09% should optimize both ADG and G:F. Additionally, because both linear and quadratic models were averaged it is expected that this estimate is not an overestimation for required Lys but a requirement that is representative of a majority of the population.

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TABLES

Table 3.1. Ingredient composition of experimental diets based on corn and soybean meal

	Standardized ileal digestible Lys, %				
Ingredient, %	0.80	0.93	1.06	1.19	1.32
Ground corn	68.55	63.3	58.17	52.97	47.78
Soybean meal, 48% CP	25.55	30.8	36.1	41.4	46.68
Soybean oil	3.00	3.00	3.00	3.00	3.00
Ground limestone	0.90	0.90	0.925	0.925	0.94
Dicalcium phosphate	1.30	1.20	1.10	1.00	0.90
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[1]	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00

[1] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 3.2. Analyzed chemical composition of experimental diets based on corn and soybean meal

	Standardized ileal digestible Lys, %				
Item, %	0.80	0.93	1.06	1.19	1.32
ME, kcal/kg[1]	3,421	3,419	3,416	3,414	3,412
CP, %	17.41	19.52	21.65	23.79	25.91
Ca[1], %	0.72	0.72	0.72	0.72	0.72
P[1], %	0.60	0.61	0.61	0.62	0.62
Digestible P[1], %	0.34	0.34	0.34	0.34	0.34
Indispensable AA, %					
Arg	1.18	1.33	1.39	1.52	1.65
His	0.47	0.52	0.55	0.58	0.63
Ile	0.78	0.88	0.93	1.00	1.08
Leu	1.70	1.85	1.92	2.01	2.12
Lys	1.00	1.14	1.21	1.33	1.46
Met	0.29	0.31	0.32	0.34	0.36
Met + Cys	0.59	0.63	0.65	0.69	0.72
Phe	0.96	1.07	1.12	1.20	1.29
Thr	0.70	0.78	0.82	0.89	0.96
Trp	0.19	0.21	0.24	0.27	0.31
Val	0.87	0.97	1.01	1.09	1.17
Dispensable AA, %					
Ala	0.99	1.07	1.11	1.17	1.24

Table 3.2. (cont.)

Asp	1.82	2.06	2.18	2.39	2.61
Cys	0.30	0.32	0.33	0.35	0.36
Glu	3.37	3.75	3.91	4.19	4.50
Gly	0.78	0.86	0.90	0.97	1.05
Pro	1.17	1.24	1.27	1.33	1.40
Ser	0.83	0.91	0.96	1.02	1.11
Tyr	0.62	0.68	0.69	0.75	0.80

[1]Values not analyzed, but based on calculations by composition (NRC, 2012).

Table 3.3. Performance of pigs fed increasing levels of standardized ileal digestible Lys

	Standardized ileal digestible Lys, %						Contrasts (*P*-value)	
	0.80	0.93	1.06	1.19	1.32	SEM	Linear	Quadratic
Initial BW, kg	24.55	24.59	24.53	24.58	24.46	0.72	0.94	0.94
ADG, g	782	809	825	846	794	17.36	0.27	0.03
ADFI, g	1,758	1,826	1,738	1,775	1,658	92.74	0.12	0.20
G:F	432	444	462	465	467	13.13	0.003	0.28
Final BW, kg	53.60	54.52	54.99	55.74	53.92	0.80	0.33	0.03

FIGURES

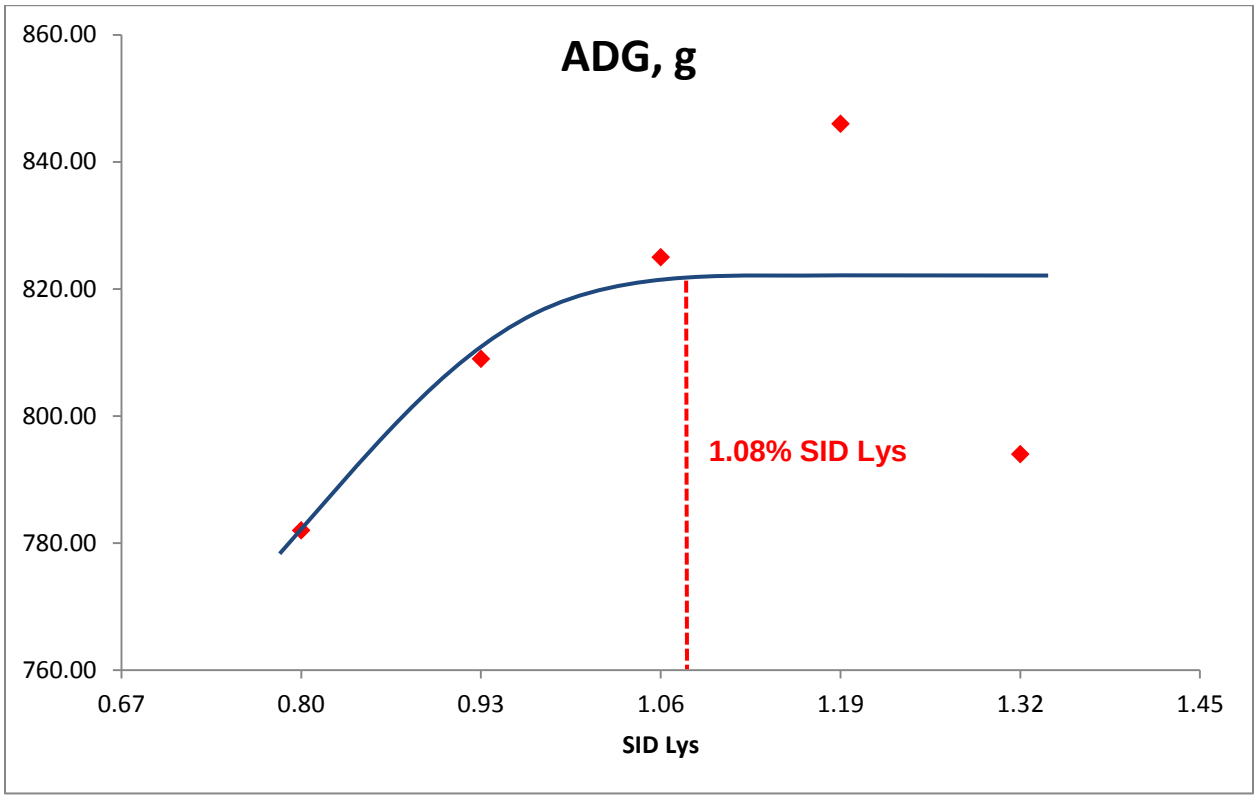

Figure 3.1. Fitted curvilinear plateau plot of ADG as a function of standardized ileal digestible (**SID**) Lys

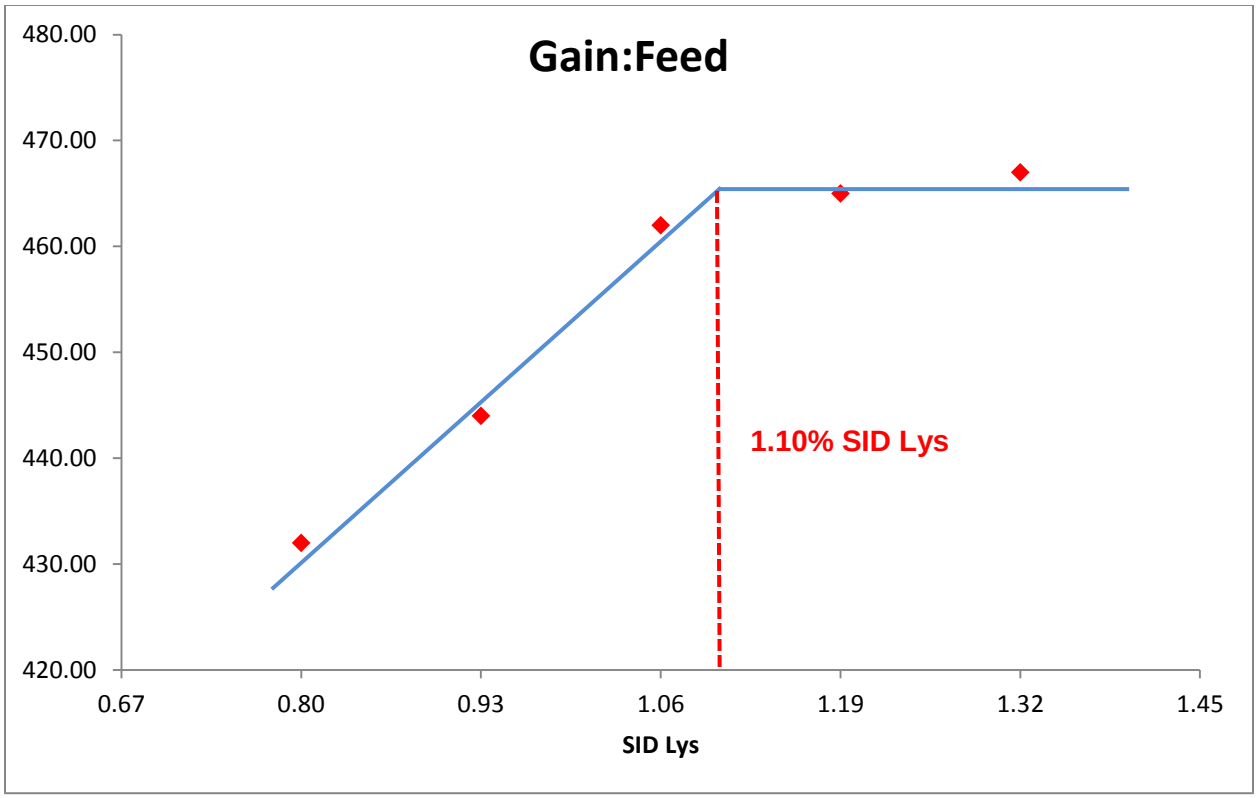

Figure 3.2. Fitted broken-line plot of G:F as a function of standardized ileal digestible (**SID**) Lys

CHAPTER 4

STANDARDIZED ILEAL DIGESTIBILITY OF AMINO ACIDS IN CORN, SOYBEAN MEAL, FIELD PEAS, FISH MEAL, AND SOYBEAN HULLS FED TO 25-50 KG GROWING GILTS

ABSTRACT: An experiment was conducted to determine the apparent ileal digestibility (**AID)** and standardized ileal digestibility (**SID**) of AA in corn, soybean meal, field peas, fish meal, and soybean hulls. Six ileal-cannulated gilts (initial BW: 26.5 ± 0.74 kg) were allotted to a 6 × 6 Latin square design with 6 diets and 6 periods. A N-free diet was formulated to determine basal endogenous losses of AA and CP and to enable the calculation of SID of AA. The remaining diets were formulated with each test ingredient as the sole source of AA, with the exception that the soybean hulls were included in a diet that also contained soybean meal to compensate for the low CP in soybean hulls. The AID and SID values were calculated in the soybean hulls diet using the difference procedure whereas AID and SID in the other ingredients were calculated using the direct procedure. The SID of Lys was greater ($P < 0.05$) in field peas, fish meal, and soybean meal than in corn and soybean hulls. The SID of Trp was greater ($P < 0.05$) in corn than in soybean meal, and greater ($P < 0.05$) in soybean meal than in field peas. The SID of His, Lys, and Trp were less ($P < 0.05$) in soybean hulls than in all other ingredients. These data indicate that the SID of most indispensable AA is not different between field peas, fish meal, and soybean meal, whereas the SID of some indispensable AA is less in soybean hulls than in other ingredients.

Key words: amino acids, field peas, pigs, soybean hulls, soybean meal, standardized ileal digestibility

INTRODUCTION

Determining AA requirements, and consequently the optimal AA:Lys ratio in pigs, requires precise formulation of diets and accurate measures of AA in the ingredients. Determining the optimal Thr:Lys ratio requires diets low both in Thr and Lys, and therefore, ingredients low in Thr must be used.

Corn and soybean meal (**SBM**) are commonly used sources of energy and protein in conventional swine diets (Baker, 1997). Soybean meal has a relatively high concentration of Lys and Trp, whereas corn protein has high concentrations of the sulfur containing AA (NRC, 2012). Field peas is an excellent source of dietary protein in growing pig diets (Stein et al., 2004), although the concentration of the sulfur containing AA is relatively low. Diets containing field peas, therefore, often need to be supplemented with synthetic Met. Compared with conventional sources of dietary protein, field peas also has a lower Thr:Lys ratio. Fish meal is also a commonly used source of high quality protein, which has a relatively low concentration of Thr. Both field peas and fish meal, therefore, are protein sources that may be used in Thr requirement studies. Soybean hulls (**SBH**) is a source of soluble and insoluble fiber that may be used in diets fed to pigs. There is, however, very limited information about the digestibility of AA in SBH.

It was the objective of this experiment to determine the apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of AA in corn, SBM, field peas, fish meal, and SBH fed to growing female pigs.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Animals, Housing, and Experimental Design

Six growing gilts (G-Performer × Fertilis 25, Genetiporc, Alexandria, MN; initial BW: 26.5 ± 0.74 kg) were equipped with a T-cannula in the distal ileum and allotted to a 6 × 6 Latin square design with 6 diets and six 7-day periods in each square. There were 6 replicate pigs per treatment. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had solid-sided walls and fully slatted tri-bar floors. A feeder and a nipple drinker were installed in each pen.

Ingredients, Diets, and Feeding

A locally-grown commercial hybrid of yellow dent corn was obtained from the University of Illinois Feed Mill, Champaign, IL (Table 1). Field peas and fish meal (Select Menhaden) were obtained from commercial sources (Central Ingredients, West Bend, WI; Omega Protein Corp., Houston, TX). Soybean meal was procured from Solae, Gibson City, IL, and SBH was obtained from Archer Daniels Midland, Decatur, IL.

Six diets were prepared (Tables 2 and 3). A N-free diet was formulated to determine basal endogenous losses of AA and to enable the calculation of SID of AA. Four diets were formulated containing corn, SBM, field peas, or fish meal as the sole source of AA, and one diet contained SBM and SBH as the sources of AA. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.4% chromic oxide as an indigestible marker. All pigs were fed once daily at 0700 h at 3 times the

estimated energy requirement for maintenance (i.e., 197 kcal ME/$kg^{0.60}$; NRC, 2012). Water was available at all times.

Data Recording and Sample Collection

Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was recorded, as well. The initial 5 d of each period were considered an adaptation period to the diet. Ileal digesta were collected for 8 h on d 6 and 7 using procedures described by Stein et al. (1998). In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta - or at least once every 30 minutes - and immediately frozen at –20^{o}C to prevent bacterial degradation of AA in the digesta. Upon completion of each experimental period, animals were deprived of feed overnight and the following morning, a new experimental diet was offered.

Chemical Analyses

At the conclusion of each period, ileal samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. A sample of each diet was collected as well. Digesta samples were lyophilized and finely ground prior to chemical analysis. All samples of diets and digesta were analyzed for DM (method 930.15; AOAC Int., 2007), chromium (method 990.08; AOAC Int., 2007), and AA [method 982.30 E (a, b, c); AOAC Int., 2007]. A sample of each ingredient was analyzed for DM, CP, and AA.

Calculations and Statistical Analyses

Apparent ileal digestibility values for AA in the protein sources were calculated using equation [1] (Stein et al., 2007):

$$AID\ (\%) = [1 - [(AAd/AAf) \times (Crf/Crd)] \times 100 \quad \textbf{[1]}$$

where AID is the apparent ileal digestibility of an AA (%), AAd is the concentration of that AA in the ileal digesta DM, AAf is the AA concentration of that AA in the feed DM, Crf is the chromium concentration in the feed DM, and Crd is the chromium concentration in the ileal digesta DM.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 2007):

$$IAA_{end} = [AAd \times (Crf/Crd)] \quad \textbf{[2]}$$

where IAA_{end} is the basal endogenous loss of an AA (mg per kg DMI). The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the IAA_{end} of each AA, standardized ileal AA digestibility values were calculated using equation [3] (Stein et al., 2007):

$$SID = [(AID + IAA_{end})/AAf] \quad \textbf{[3]}$$

where SID is the standardized ileal digestibility value (%).

Values for AID and SID of AA in the diets containing corn, SBM, fishmeal, or field peas also represent the AID and SID of AA in these ingredients because all dietary AA were provided by the test ingredients. However, the diet containing SBH also contained SBM and values for the AID and SID of AA in SBH were calculated using the difference procedure (Fan and Sauer, 1995).

Data were analyzed using the Proc MIXED procedure of SAS (SAS Institute Inc., Cary, NC). An analysis of variance was conducted with pigs, periods, and diets as the main effects. The pig was the experimental unit for all analyses and an α-value of 0.05 was used to assess statistical significance. Mean values for each diet were calculated using the LSMeans statement.

When significant differences were detected, treatment means were separated using the Least Significant Difference test in Proc MIXED.

RESULTS

All pigs stayed healthy throughout the experiment and consumed their diets readily. Gross chemical composition of the ingredients was generally in agreement with published values (NRC, 2012).

The AID of all indispensable AA were least ($P < 0.05$) in SBH (Table 4). The AID of Lys was greater ($P < 0.05$) in field peas and fish meal than in corn and SBM. The AID of Trp was greater ($P < 0.05$) in fish meal, corn, and SBM, than in field peas. The AID of Met was greater ($P < 0.05$) in corn than in field peas and SBM. Field peas and SBM had the greatest ($P < 0.05$) AID of Thr, but the AID of His was greater ($P < 0.05$) in corn, field peas, and SBM than in fish meal.

The AID of all dispensable AA were least ($P < 0.05$) in SBH. The AID of Ala and Cys were greatest ($P < 0.05$) in corn, but the AID of Asp was greatest ($P < 0.05$) in field peas, followed by SBM, fish meal, and corn. The AID of Glu was greater ($P < 0.05$) in field peas and corn than in fish meal. The AID of Gly was greater ($P < 0.05$) in field peas, fish meal, and SBM than in corn.

The SID of Lys was greater ($P < 0.05$) in field peas, fish meal, and SBM than in corn and SBH (Table 5). The SID of Met was greatest ($P < 0.05$) in SBH, and greater ($P < 0.05$) in corn than in fish meal or SBM. The SID of Thr was greater ($P < 0.05$) in corn than in fish meal. There were no differences ($P < 0.05$) in the SID of Thr among field peas, SBM, and SBH but the SID of Trp was greater ($P < 0.05$) in corn than in SBM, and greater ($P < 0.05$) in SBM than in field

peas. The SID of His was greater ($P < 0.05$) in corn than in SBM, and greater ($P < 0.05$) in soybean meal than in fish meal, and greater ($P < 0.05$) in fish meal than in SBH. The mean of indispensable AA was greater ($P < 0.05$) in corn than in fish meal and SBM, and was least ($P < 0.05$) in SBH.

The SID of Ala was greatest ($P < 0.05$) in corn, and the SID of Asp was greater ($P < 0.05$) in field peas and corn than in SBM, and greater ($P < 0.05$) in SBM than in fish meal, and greater ($P < 0.05$) in fish meal than in SBH. The SID of Cys was greater ($P < 0.05$) in corn than in field peas and SBM, and greater ($P < 0.05$) in field peas and SBM than in fish meal, and greater ($P < 0.05$) in fish meal than in SBH. The SID of Glu was greater ($P < 0.05$) in corn than in fish meal or SBM, and greater ($P < 0.05$) in fish meal and SBM than in SBH. The SID of Gly was greater ($P < 0.05$) in corn than in fish meal, and greater ($P < 0.05$) in fish meal than in SBM. The mean of dispensable AA was not different ($P < 0.05$) among cron, SBM, fish meal, and field peas, but SBH had the least ($P < 0.05$) SID for the mean of dispensable AA.

DISCUSSION

The ingredients used in this experiment were chosen because of their relatively low Thr: Lys ratios. Soybean hulls were included in the diets as a source of insoluble and soluble fiber, to determine effects of fiber on the pig's Thr requirement. All ingredients were procured in single batches that were used in a series of Thr requirement studies. Thus, all ingredients were used to formulate diets on an SID basis that were limiting in Lys and Thr. However, to ensure the precision of diet formulation, the AA concentration and digestibility of the specific batches of these ingredients needed to be determined.

Corn

The corn used in this experiment had concentrations of CP and AA in agreement with published values (Stein et al., 2001; Almeida et al., 2011; NRC, 2012; Petersen et al., 2014) with relatively high levels of sulfur containing AA and a low concentration of Lys when compared with SBM.

The AID of all indispensable AA, with the exception of Lys, was slightly greater in corn used in this experiment than in corn used in some previous experiments (Almeida et al., 2011; NRC, 2012). The SID of all indispensable AA were greater in corn than expected (Stein et al., 2001; Almeida et al., 2011; NRC, 2012), but in agreement with values reported more recently (Petersen et al., 2014).

Soybean Meal

The SBM used in the present experiment had concentrations of CP and AA that were in agreement with values observed in previous experiments (Cervantes-Pahm and Stein, 2010; NRC, 2012; Sulabo et al., 2013a;b; Petersen et al., 2014), indicating that the SBM used in this experiment was of similar quality to that used in previous experiments. The AID and SID of all AA were in agreement with the majority of previous data (Cervantes-Pahm and Stein, 2010; Sulabo et al., 2013a;b; Petersen et al., 2014). However, values for both the AID and SID of AA were lower than values suggested by the NRC (2012). The likely cause for this discrepancy is the fact that the values listed in the NRC are determined by averaging numerous published values over various geographic regions, whereas the papers in agreement procured soybean meal from similar regions. Thus, the differences are likely due to source variation as a result of growing location and condition of the soybean crop.

Field Peas

Field peas used in this experiment also had concentrations of CP and AA that concur with published values (Stein et al., 2004; NRC, 2012), with a high concentration of Lys, relatively low concentrations of Met, Cys, and Trp, and a low Thr:Lys ratio. The values for AID of AA obtained in this experiment was slightly greater than expected (Stein and Bohlke, 2007; NRC, 2012). The SID of most indispensable AA was also somewhat greater for all indispensable AA than expected (Stein and Bohlke, 2007; NRC, 2012; Petersen et al., 2014). There are several potential reasons for the greater digestibility of AA in field peas used in this experiment. Values by NRC (2012) are averages of several studies and thus may not represent individual variation among ingredient sources. Additionally, the presentation of diets, in terms of feed preparation and processing, may affect the digestibility of nutrients (Stein and Bohlke, 2007). Particle size, pelleting, extrusion, heat treatment, etc., may also affect AA digestibility, even when AA concentrations are not changed. Overall, however, the AID and SID of AA in field peas was within the range of previously published data (Stein et al., 2004; Stein and Bohlke, 2007; NRC, 2012; Petersen et al., 2014), and, as other experiments have indicated, not different from that of SBM (Stein et al., 2004).

Fish Meal

Fish meal used in this experiment had CP and AA concentrations that were in agreement with published values (Cervantes-Pahm and Stein, 2010; NRC, 2012; Sulabo et al., 2013b). As in the other experiments, the fish meal used in this experiment had a relatively high concentration of Lys, Met, and Thr, and a relatively low Thr:Lys ratio.

The AID of all indispensable AA, except Lys, Met and Phe, were less than some previous data (Sulabo et al., 2013b); however, data from this experiment were in good agreement with

other values (Cervantes-Pahm and Stein, 2010). The AID of all indispensable AA, except Thr, were in agreement with values reported by NRC (2012). However, all nutrient compositions for fish meal reported by NRC (2012) were determined by combining all data available for fish meal, irrespective of species, whereas the fish meal used in this study was composed of fish from the *Brevoortia* or *Ethmidium* genera (Menhaden).

The SID of indispensable AA were also less than some previous data (Sulabo et al., 2013b), but in good agreement with other data (Cervantes-Pahm and Stein, 2010). The SID of all indispensable AA, with the exception of Arg and Trp, which were greater in this study, were similar to values reported by NRC (2012). Overall, the AID and SID of AA in fish meal was within the range of previously published data (Cervantes-Pahm and Stein, 2010; NRC, 2012; Sulabo et al., 2013b)

Soybean Hulls

Soybean hulls used in this experiment contained less CP and AA than expected (NRC, 2012; Stewart et al., 2013). It is possible that this is a function of differences in the techniques used among soybean crushing plants to dehull soybeans.

The difference procedure was used to determine the digestibility of AA in SBH. Accuracy and precision of digestibility values determined using the difference procedure are dependent on the accuracy of the results of the ingredient in the basal diet. The SBM diet was considered the basal diet in this experiment and the values obtained for AID and SID of AA in SBM were in agreement with published values (Cervantes-Pahm and Stein, 2010; NRC, 2012; Sulabo et al., 2013a;b). To the best of our knowledge, there is limited data on AA digestibility of soybean hulls. This is likely due to the fact that soybean hulls is a relatively uncommon feed stuff in growing pigs diets, due to its high fiber content and low CP. Typically, SBH are used in

diets for beef cattle where they can be included as a replacement for grain and hay. The fiber in soybean hulls can be relatively rapidly fermented in cattle and thus can be a valuable source of energy.

CONCLUSION

Results of this experiment indicate that SBM, field peas, and fish meal are excellent sources of AA in swine diets, with high concentrations and digestibility of AA. Concentration and digestibility of indispensable AA indicate that field peas can be used as a replacement for some or all of the SBM in a swine ration. The high quality of fish meal protein, as indicated by the high concentration and digestibility of indispensable AA, indicates that even low levels of fish meal can increase the protein quality and concentration of a swine diet. Results indicate that the protein in corn also has relatively high SID of AA, and because of the high concentration of sulfur AA in corn protein, the combination of corn with SBM, an ingredient that is relatively low in sulfur AA concentration, can be beneficial in swine diets. Although this experiment confirms a relatively high digestibility of AA in SBH, its value in swine diets is limited by its low concentration of CP and AA and its high fiber content.

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TABLES

Table 4.1. Analyzed nutrient composition of field peas, fish meal, corn, soybean meal (SBM), and soybean hulls (SBH)

Item	Corn	SBM	Field peas	Fish meal	SBH
DM, %	91.90	92.58	92.19	93.76	91.21
CP, %	8.66	47.44	22.11	62.84	8.59
Indispensable AA, %					
Arg	0.44	3.36	1.67	3.67	0.34
His	0.25	1.19	0.50	1.44	0.22
Ile	0.32	2.15	0.89	2.51	0.30
Leu	1.07	3.60	1.52	4.35	0.54
Lys	0.31	2.85	1.54	4.80	0.56
Met	0.16	0.63	0.19	1.66	0.08
Phe	0.45	2.41	1.03	2.42	0.33
Thr	0.30	1.82	0.79	2.50	0.27
Trp	0.09	0.63	0.17	0.56	0.05
Val	0.42	2.29	0.99	2.95	0.38
Total	3.81	20.93	9.29	26.86	3.07
Dispensable AA, %					
Ala	0.65	2.01	0.92	3.76	0.35
Asp	0.59	5.17	2.33	5.40	0.70

Table 4.1. (cont.)

Cys	0.18	0.63	0.29	0.50	0.13
Glu	1.64	8.32	3.47	7.75	0.82
Gly	0.40	1.95	0.94	4.25	0.73
Pro	0.75	2.44	0.83	2.90	0.42
Ser	0.41	2.05	0.89	2.18	0.43
Tyr	0.34	1.48	0.63	1.87	0.31
Total	4.96	24.05	10.30	28.61	3.89
Total AA	8.77	44.98	19.59	55.47	6.96

Table 4.2. Ingredient composition of experimental diets (as-is basis)

Ingredient, %	Diet					
	Corn	Soybean meal	Field peas	Fish meal	Soybean hulls	N-free
Corn	96.58	-	-	-	-	-
Soybean meal, 48%	-	30.00	-	-	22.50	-
Field peas	-	-	65.00	-	-	-
Fish meal	-	-	-	23.00	-	-
Soy Hulls	-	-	-	-	35.00	-
Soybean oil	-	3.00	3.00	-	3.00	4.00
Solka floc	-	-	-	-	-	4.00
Dicalcium phosphate	1.50	1.40	1.30	-	2.10	2.40
Limestone	0.82	0.75	0.90	-	0.10	0.50
Sucrose	-	20.00	20.00	20.00	20.00	20.00
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40
Cornstarch	-	43.75	8.70	55.90	16.20	67.50
Magnesium oxide	-	-	-	-	-	0.10

Table 4.2. (cont.)

Potassium carbonate	-	-	-	-	-	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin micromineral premix [1]	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.0	100.00

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 4.3. Analyzed nutrient composition of experimental diets (as-fed basis)

Item	Corn	Soybean meal	Field peas	Fish meal	Soybean hulls	N-free
DM, %	92.71	94.37	94.41	94.25	93.87	93.98
CP, %	8.16	13.9	13.78	15.08	13.11	0.29
Indispensable AA, %						
Arg	0.41	0.95	1.14	0.84	0.91	0.01
His	0.23	0.34	0.34	0.34	0.36	0.00
Ile	0.31	0.62	0.60	0.60	0.63	0.01
Leu	1.04	1.05	1.04	1.06	1.06	0.06
Lys	0.30	0.85	1.05	1.15	0.90	0.02
Met	0.15	0.17	0.13	0.39	0.16	0.00
Phe	0.44	0.69	0.71	0.61	0.71	0.01
Thr	0.29	0.55	0.54	0.56	0.51	0.01
Trp	0.09	0.18	0.12	0.16	0.17	0.04
Val	0.40	0.65	0.67	0.70	0.68	0.01
Total	3.66	6.05	6.34	6.41	6.09	0.17
Dispensable AA, %						
Ala	0.63	0.61	0.64	0.92	0.63	0.02
Asp	0.56	1.53	1.58	1.28	1.48	0.02
Cys	0.15	0.18	0.18	0.11	0.09	0.00

Table 4.3. (cont.)

Glu	1.57	2.47	2.36	1.93	2.33	0.05
Gly	0.38	0.59	0.67	1.08	0.78	0.02
Pro	0.71	0.67	0.57	0.67	0.68	0.00
Ser	0.37	0.64	0.63	0.50	0.65	0.01
Tyr	0.33	0.42	0.47	0.42	0.47	0.01
Total	8.66	7.11	7.10	6.91	7.11	0.13
Total AA	49.94	13.16	13.44	13.32	13.20	0.30

Table 4.4. Apparent ileal digestibility (AID) of AA in field peas, fish meal, corn, soybean meal (SBM), and soybean hulls (SBH) fed to pigs[1]

	Ingredients					Pooled SEM	*P*-value
Item	Corn	SBM	Field peas	Fish meal	SBH		
Indispensable AA, %							
Arg	76.23^{c}	85.48^{a}	88.40^{a}	81.02^{b}	34.5^{d}	1.82	<0.05
His	84.61^{a}	84.28^{a}	86.69^{a}	81.38^{b}	44.5^{c}	1.20	<0.05
Ile	80.79^{a}	82.68^{a}	82.38^{a}	81.51^{a}	55.8^{b}	1.25	<0.05
Leu	89.49^{a}	82.99^{b}	83.82^{b}	83.15^{b}	59.0^{c}	1.14	<0.05
Lys	55.27^{c}	79.89^{b}	85.45^{a}	82.98^{ab}	44.1^{d}	2.14	<0.05
Met	87.31^{a}	83.08^{bc}	82.26^{bc}	85.31^{ab}	69.3^{d}	1.51	<0.05
Phe	86.73^{a}	84.39^{ab}	85.29^{a}	81.49^{b}	67.1^{c}	1.21	<0.05
Thr	70.56^{bc}	75.40^{a}	76.73^{a}	74.44^{ab}	28.6^{d}	1.92	<0.05
Trp	79.93^{a}	82.92^{a}	74.30^{b}	82.57^{a}	2.7^{c}	1.88	<0.05
Val	76.55	77.39	78.21	76.98	60.9	1.64	0.2043
Mean	80.90^{a}	81.86^{a}	83.87^{a}	81.12^{a}	50.3^{b}	1.24	<0.05
Dispensable AA, %							
Ala	82.98^{a}	74.75^{b}	77.32^{b}	76.91^{b}	32.9^{c}	1.80	<0.05
Asp	75.95^{c}	80.13^{b}	83.55^{a}	74.97^{c}	30.6^{d}	1.40	<0.05
Cys	77.89^{a}	71.45^{b}	72.00^{b}	52.89^{c}	-166.8^{d}	2.53	<0.05
Glu	88.00^{a}	84.71^{ab}	86.89^{a}	82.40^{b}	35.0^{c}	1.46	<0.05
Gly	39.83^{b}	54.67^{a}	57.85^{a}	63.41^{a}	-8.7^{c}	5.13	<0.05
Ser	78.11^{a}	81.12^{a}	80.04^{a}	74.30^{b}	31.9^{c}	1.69	<0.05

Table 4.4. (cont.)

Tyr	85.39[a]	81.32[b]	84.87[a]	81.07[b]	49.7[c]	1.34	<0.05
Mean	82.55[a]	80.96[ab]	81.15[ab]	77.89[b]	70.59[c]	1.88	<0.05
Total AA	81.78[a]	81.37[a]	81.19[a]	79.45[a]	73.08[b]	1.85	<0.05

[a-d]Means within a row lacking a common superscript letter differ.

[1]Data are least square means of 6 observations per treatment.

Table 4.5. Standardized ileal digestibility (SID) of AA in field peas, fish meal, corn, soybean meal (SBM), and soybean hulls (SBH) fed to pigs[1,2]

	Ingredients					Pooled SEM	*P*-value
Item	Corn	SBM	Field peas	Fish meal	SBH		
Indispensable AA, %							
Arg	100.31[a]	96.00[ab]	96.52[ab]	92.90[bc]	114.8[d]	1.82	<0.05
His	93.26[a]	90.24[b]	92.65[ab]	87.34[c]	69.5[d]	1.20	<0.05
Ile	90.66[a]	87.71[ab]	87.57[ab]	86.70[b]	83.4[c]	1.25	<0.05
Leu	94.06[a]	87.61[b]	88.48[b]	87.72[b]	83.0[c]	1.14	<0.05
Lys	73.14[b]	86.31[a]	90.64[a]	87.72[a]	69.8[c]	2.14	<0.05
Met	92.14[b]	87.42[c]	87.94[bc]	87.20[c]	97.1[a]	1.51	<0.05
Phe	92.94[a]	88.41[b]	89.21[b]	86.04[bc]	89.2[b]	1.21	<0.05
Thr	89.24[a]	85.42[ab]	86.94[ab]	84.26[b]	85.9[ab]	1.92	<0.05
Trp	95.03[a]	90.60[b]	85.83[c]	91.21[ab]	76.4[d]	1.88	<0.05
Val	90.18[b]	85.93[c]	86.49[bc]	84.90[c]	100.5[a]	1.64	<0.05
Mean	92.55[a]	89.03[b]	90.72[ab]	87.88[b]	88.4[c]	1.24	<0.05
Dispensable AA, %							
Ala	94.36[a]	86.70[b]	88.72[b]	84.82[b]	86.9[b]	1.80	<0.05
Asp	90.47[a]	85.55[b]	88.79[a]	81.43[c]	62.6[d]	1.40	<0.05
Cys	90.11[a]	81.82[b]	82.36[b]	69.82[c]	-82.0[d]	2.53	<0.05
Glu	94.25[a]	88.75[b]	91.12[ab]	87.57[b]	67.1[c]	1.46	<0.05
Gly	99.15[a]	93.56[ab]	92.11[ab]	84.62[b]	70.8[c]	5.13	<0.05
Ser	92.31[a]	89.47[a]	88.53[ab]	84.98[b]	65.1[c]	1.69	<0.05

Table 4.5. (cont.)

Tyr	93.00[a]	87.41[b]	90.31[ab]	87.15[b]	71.7[c]	1.34	<0.05
Mean	90.28[a]	89.24[a]	89.45[a]	86.41[a]	78.83[b]	2.55	<0.05
Total AA	89.10[a]	89.14[a]	88.80[a]	87.11[ab]	80.79 [b]	2.59	<0.05

[a-d]Means within a row lacking a common superscript letter differ.

[1]Data are least square means of 6 observations per treatment.

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of DMI) of AA were as follows: Arg, 1.06; His, 0.21; Ile, 0.33; Leu, 0.51; Lys, 0.58; Met, 0.08; Phe, 0.29; Thr, 0.58; Trp, 0.15; Val, 0.59; Ala, 0.77; Asp, 0.88; Cys, 0.20; Glu, 1.06; Gly, 2.43; Ser, 0.57; Tyr, 0.27.

CHAPTER 5

CONFIRMATION OF LIMITATIONS OF LYSINE AND THREONINE IN DIETS FOR 25 TO 50 KG GROWING GILTS

ABSTRACT: An experiment was conducted to confirm that certain diet formulations used for 25 to 50 kg growing gilts were deficient in only the AA Thr and Lys. One hundred twenty gilts (initial BW: 24.84 ± 3.39 kg) were allotted to 10 diets using a randomized complete block design with 6 pen replicates per diet and 2 pigs per pen. Five of the diets were considered low-fiber with inclusion of 15% corn starch and 5 of the diets were considered high-fiber with 15% soybean hulls replacing the corn starch in these diets. Within each level of fiber, the 5 different diets were formulated by changing the proportion of synthetic Thr and Lys in the diets using principles described by Hahn and Baker (1995). Two of the diets formulated using corn, field peas, and soybean meal served as the low-fiber and high-fiber control diets and these diets met all AA requirements. Two basal diets, 1 with low fiber and 1 with high fiber, were formulated to provide 0.98% standardized ileal digestible Lys and 0.45% standardized ileal digestible Thr, whereas all other AA were supplied at 105% the NRC (2012) requirements for 25 to 50 kg growing gilts. Six additional diets, 3 with low fiber and 3 with high fiber, were formulated exactly as the basal diets with the exception that synthetic Lys, synthetic Thr, or both synthetic Lys and Thr were added to the diets. Average daily gain was greater in the high-fiber Thr supplemented diet than in the low-fiber control diet, low-fiber basal diet, and low-fiber Thr supplemented diet. Average daily feed intake was greatest for pigs fed the high-fiber basal diet and least in pigs fed the low-fiber Thr supplemented diet. There were no differences in ADFI among the other dietary treatments. Gain:feed was greatest in the low-fiber Lys and Thr supplemented diet and least in the low-fiber

basal diet; additionally, G:F was greater in the high-fiber Lys and Thr supplemented diet than in the high-fiber basal diet. There were no differences in G:F among dietary treatments. These data indicate that the diets were at least marginally deficient in terms of Lys and Thr concentration.

Key words: amino acids, lysine, pigs, threonine

INTRODUCTION

An approach to determine AA requirements in pigs has been proposed utilizing titration. To facilitate further titration experiments to determine the optimal Thr:Lys ratio in growing gilts, diets deficient in only Lys and Thr must be developed. It is, therefore, necessary to develop a diet that is below the requirement for the AA that will be titrated. Subsequently, identical diets with the exception of increasing levels of the deficient AA can be formulated. To isolate the growth response changes in response to increasing the concentration of a specific AA, it has to be the only deficient AA in the diet. It was the objective of this experiment to confirm that the diets used are deficient in only the AA Thr and Lys.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Animals, Housing, and Experimental Design

A total of 120 gilts (G-Performer × Fertilis 25, Genetiporc, Alexandria, MN; initial BW: 24.84 ± 3.39 kg) were allotted to 10 diets using a randomized complete block design with 6 pen replicates per diet and 2 pigs per pen. Pigs were housed in pens with 50% solid concrete floor and 50% concrete slats. There was a feeder and a nipple drinker in each pen and the room was temperature controlled. Pigs had free access to feed and water throughout the experiment.

Ingredients, Diets, and Feeding

All ingredients used in the diets in this experiment came from the same batches as those used in the experiment of chapter 4. Thus, the standardized ileal digestibility (**SID**) of AA in all ingredients that were determined in these experiments were used for the formulation of the diets in this experiment.

All 10 diets were formulated using corn, field peas, and soybean meal. Five of the diets were considered low-fiber diets with inclusion of 15% corn starch and 5 of the diets were considered high-fiber with 15% soybean hulls replacing the corn starch in these diets (Tables 5.1 and 5.2). Within each level of fiber, the 5 different diets were formulated by changing the proportion of synthetic Thr and Lys in the diets using principles described by Hahn and Baker (1995). Two of the diets served as the low-fiber and high-fiber control diets and these diets met all AA requirements. A previous experiment determined the requirement for SID Lys in 25 to 50 kg growing gilts of the same genetics at 1.09%. Both control diets were formulated at an SID Thr level of 0.66%, which is 105% of the requirement (NRC, 2012) for 25 to 50kg growing pigs. All other indispensable AA were also included at least 105% of the requirement. Two basal diets, 1 with low fiber and 1 with high fiber, were formulated to provide 0.98% SID Lys and 0.45% SID

Thr, whereas all other AA were supplied at 105% the NRC (2012) requirements for 25 to 50 kg growing gilts. Six additional diets, 3 with low fiber and 3 with high fiber, were formulated exactly as the basal diets with the exception that synthetic Lys (Lys supplemented), synthetic Thr (Thr supplemented), or both synthetic Lys and Thr (Lys and Thr supplemented) were added to the diets. The diets with only increased synthetic Lys provided 1.14% SID Lys and 0.45% SID Thr. The diets with only increased synthetic Thr provided 0.98% SID Lys and 0.66% SID Thr. The diets with increased synthetic Lys and Thr provided 1.14% SID Lys and 0.66% SID Thr.

Data Recording

Daily feed allocations were recorded and individual pig weights were recorded at the beginning of the experiment and at the end of the experiment 28 d later. The amount of feed left in the feeders was also recorded as pigs were weighted off the experiment. The ADG, ADFI, and G:F were calculated for each pen of pigs and for each treatment group at the conclusion of the experiment. The concentration of AA in the diets were analyzed and the daily intake of Lys and Thr were calculated for each treatment group.

Chemical Analyses

All diets were analyzed for CP (method 990.03; AOAC Int., 2007) and AA. Amino acids were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, U.S.) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after

NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007).

Statistical Analyses

Normality of data was verified and outliers were tested using the UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC). Data were analyzed by ANOVA using the UNIVARIATE and MIXED procedures of SAS (SAS Inst. Inc.) with pen as the experimental unit. The statistical model included diet as the fixed effect and block as the random effect. Treatment means were calculated using the Least Square Means test and separated using the PDIFF function of the MIXED procedure of SAS (SAS Inst. Inc.). Comparisons between low- and high-fiber treatments were performed using orthogonal polynomial contrasts. Statistical significance and tendency were considered at $P < 0.05$ and at $0.05 < P \leq 0.10$, respectively.

RESULTS AND DISCUSSION

Initial BW of pigs did not differ among dietary treatments (Table 5.3). Final BW was greatest ($P < 0.05$) for pigs fed the high-fiber Thr supplemented diet, and least ($P < 0.05$) in the low-fiber basal diet. There were no differences in final BW among other dietary treatments. Average daily gain was greater ($P < 0.05$) in the high-fiber Thr supplemented diet than in the low-fiber control diet, low-fiber basal diet, and low-fiber Thr supplemented diet. Average daily feed intake was greatest ($P < 0.05$) for pigs fed the high-fiber basal diet and least in pigs fed the low-fiber Thr supplemented diet. There were no differences in ADFI among the other dietary treatments. Gain:feed was greatest in the low-fiber Lys and Thr supplemented diet and least in the low-fiber basal diet, and G:F was greater ($P < 0.05$) in the high-fiber Lys and Thr supplemented diet than in the high-fiber basal diet. There were no differences in G:F among the

other dietary treatments. Final BW was least ($P < 0.05$) in the low-fiber basal diet, and greatest in the high-fiber Thr supplemented diet. There were no differences in final BW among all other dietary treatments.

Contrasts among dietary treatments between fiber levels indicated no differences (Table 4), with the exception of greater ADG ($P < 0.01$) and greater ADFI ($P < 0.01$) in the high-fiber Thr supplemented diets when compared with the low-fiber Thr supplemented diets. However, the combination of the increase in ADG and ADFI, resulted in no differences in G:F between both low- and high-fiber Thr treatments. The final BW trended towards an increase in final BW ($P = 0.08$) in the high-fiber Thr treatment when compared with the low-fiber Thr treatment. This increase is likely due to the increased ADG in pigs fed the high-fiber Thr treatment.

Although an increase in ADFI is contrary to traditional thought in terms of the feed intake of high-fiber diets and the effects of gut fill, it is possible that the increased intake is the result of compensatory eating by the animals in an effort to compensate for reduced ME in the high-fiber diets. Additionally, this increased intake can potentially explain the trend for increased ADG in the high-fiber diets ($P = 0.08$).

The purpose of this experiment was to determine if the diets formulated for this experiment to be deficient in Lys and/or Thr were actually deficient in those AA and to evaluate the response of the animals based on various growth performance parameters. It was expected that there would be no differences in ADG between the basal diets and the Lys or Thr supplemented diets, and that the Lys and Thr supplemented diets would result in similar values for ADG as the control diets. Although the results of the experiment do not conclusively indicate major deficiencies in the diets, the data do indicate that the diets were marginally deficient in Lys

and Thr. The differences in ME between low- and high-fiber diets may have resulted in the variations in feed intake that was observed, and thus compromised clear growth patterns. Correcting for feed intake, by limit-feeding as opposed to *ad libitum* may have helped to mitigate the effects of the energetic variations between the low- and high-fiber formulations.

CONCLUSION

Although the results of this experiment were not as definitive as expected, results do reveal some information regarding the future formulation of diets, particularly for use in Thr titration studies. The results emphasize the need for isocaloric treatments to minimize the effects of possible energy deficiencies and any resulting compensatory feeding. Additionally, results of this experiment represent the adaptability of swine and their ability to maximize their ADG among diets of varying protein quality and fiber concentration. This ability also indicates that a titration study may need to encompass a rather large range of AA concentrations to capture meaningful and significant responses with growth performance parameters.

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TABLES

Table 5.1. Ingredient composition of experimental diets (as-fed basis)

	Low-fiber					High-fiber				
	Control	Basal	Lys	Thr	Lys - Thr	Control	Basal	Lys	Thr	Lys - Thr
Ground corn	30.45	40.07	39.86	39.90	39.68	32.64	42.2	42.00	42.04	41.84
Field peas	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
Soybean meal, 48% CP	18.0	8.0	8.0	8.0	8.0	16.0	6.0	6.0	6.0	6.0
Fish meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-Lysine HCl	0.14	0.22	0.43	0.22	0.43	0.125	0.21	0.41	0.21	0.41
DL-Methionine	0.13	0.21	0.21	0.21	0.21	0.20	0.23	0.23	0.23	0.23
L-Threonine	0.04	-	-	0.175	0.175	0.03	-	-	0.165	0.165
L-Tryptophan	-	0.045	0.045	0.045	0.045	-	0.05	0.05	0.05	0.05

Table 5.1. (cont.)

L-Valine	-	0.05	0.05	0.05	0.05	-	0.025	0.025	0.025	0.025
L-Histidine	-	0.015	0.015	0.015	0.015	-	0.01	0.01	0.01	0.01
Corn starch	15.0	15.0	15.0	15.0	15.0	-	-	-	-	-
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Soybean hulls	-	-	-	-	-	15.0	15.0	15.0	15.0	15.0
Limestone	0.86	0.84	0.84	0.84	0.84	0.67	0.62	0.62	0.62	0.62
Dicalcium phosphate	0.68	0.85	0.85	0.85	0.85	0.68	0.95	0.95	0.95	0.95
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66

Table 5.1. (cont.)

IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 5.2. Analyzed energy and nutrient composition of diets

	Low-fiber					High-fiber				
	Control	Basal	Lys	Thr	Lys - Thr	Control	Basal	Lys	Thr	Lys - Thr
ME[1], kcal/kg	3521	3518	3511	3513	3505	3221	3216	3209	3211	3204
NE[1], kcal/kg	2539	2587	2582	2583	2577	2329	2375	2370	2371	2366
Ca[1], %	0.70	0.69	0.69	0.69	0.69	0.70	0.71	0.71	0.71	0.71
STTD P[1], %	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34
CP, %	19.25	15.35	15.32	15.34	15.31	19.78	15.87	15.85	15.86	15.83
Indispensable AA, %										
Arg	1.42	1.11	1.11	1.12	1.10	1.37	1.06	1.10	1.09	1.08
His	0.55	0.47	0.41	0.47	0.48	0.56	0.47	0.47	0.47	0.41
Ile	0.83	0.65	0.64	0.64	0.65	0.82	0.64	0.66	0.66	0.63
Leu	1.59	1.34	1.32	1.30	1.34	1.57	1.33	1.35	1.35	1.33
Lys	1.30	1.10	1.31	1.12	1.28	1.28	1.13	1.24	1.10	1.32
Met	0.34	0.39	0.44	0.42	0.37	0.38	0.43	0.47	0.42	0.44
Phe	0.93	0.74	0.73	0.72	0.74	0.92	0.73	0.74	0.74	0.72
Thr	0.77	0.59	0.60	0.71	0.76	0.76	0.59	0.60	0.71	0.74

Table 5.2. (cont.)

Trp	0.20	0.19	0.20	0.19	0.19	0.20	0.18	0.19	0.21	0.21
Val	0.92	0.77	0.76	0.77	0.79	0.93	0.77	0.77	0.79	0.75
Dispensable AA, %										
Ala	0.95	0.82	0.81	0.81	0.83	0.96	0.83	0.84	0.83	0.84
Asp	2.03	1.58	1.60	1.57	1.55	2.02	1.57	1.59	1.61	1.58
Cys	0.26	0.23	0.22	0.22	0.22	0.28	0.23	0.23	0.22	0.25
Glu	3.27	2.64	2.59	2.57	2.63	3.18	2.56	2.58	2.61	2.54
Gly	0.87	0.71	0.70	0.72	0.71	0.96	0.77	0.80	0.78	0.81
Pro	0.97	0.82	0.82	0.81	0.83	0.98	0.83	0.84	0.84	0.86
Ser	0.85	0.69	0.70	0.67	0.70	0.86	0.70	0.70	0.71	0.72
Tyr	0.52	0.41	0.45	0.42	0.43	0.58	0.48	0.50	0.50	0.52

[1]Values not analyzed, but based on calculations by composition (NRC, 2012).

Table 5.3. Performance of pigs fed AA supplemented diets of low- and high-fiber[1]

	Low-fiber					High-fiber					
	Control	Basal	Lys	Thr	Lys - Thr	Control	Basal	Lys	Thr	Lys - Thr	SEM
Initial BW, kg	24.84[a]	24.88[a]	24.70[a]	25.00[a]	24.90[a]	24.87[a]	24.85[a]	24.72[a]	25.05[a]	24.63[a]	0.82
ADG, g	805[b]	765[b]	849[ab]	778[b]	857[ab]	836[ab]	841[ab]	841[ab]	912[a]	824[ab]	40.63
ADFI, g	1,993[bcd]	2,102[abcd]	1,979[cd]	1,900[d]	1,982[bcd]	2,073[abcd]	2,266[a]	2,125[abc]	2,201[ab]	1,969[cd]	105.92
G:F	420[ab]	384[b]	434[a]	448[a]	457[a]	422[ab]	386[b]	414[ab]	434[a]	441[a]	21.72
Final BW, kg	49.39[ab]	48.14[b]	49.81[ab]	48.64[ab]	51.06[ab]	50.31[ab]	50.56[ab]	50.43[ab]	52.27[a]	49.73[ab]	1.68

[1]Data are means of 6 observations per treatment.

[a-c]Within a row, means without a common superscript letter are different (*P*-value of 0.05).

CHAPTER 6

EFFECTS OF FIBER ON THE OPTIMAL THREONINE:LYSINE RATIO FOR 25 TO 50 KG GROWING GILTS

ABSTRACT: An experiment was conducted to determine effects of fiber on the optimal standardized ileal digestible (SID) Thr:Lys ratio for gilts from 25 to 50 kg BW. One hundred ninety-two gilts were used in a growth assay with 2 pigs per pen and 8 pens per treatment. A low-fiber basal diet was formulated with approximately 0.40% SID Thr and 0.90% SID Lys. Five additional diets were formulated by adding crystalline L-Thr to the basal diet in increments of 0.08% to create diets containing approximately 0.49, 0.57, 0.65, 0.73, and 0.81% SID Thr, respectively. A high-fiber basal diet was also formulated by including 15% soybean hulls to the low-fiber basal diet at the expense of corn starch and 5 additional diets were formulated by adding crystalline Thr to this diet. Thus, diets with SID Thr:Lys ratios at 45:100, 54:100, 63:100, 72:100, 81:100, and 90:100 were used. Daily feed allocations were recorded and individual pig weights were recorded at the beginning and at the conclusion of the experiment. In both the low-fiber and high-fiber diets, ADG and G:F increased both linearly and quadratically ($P < 0.01$ and $P < 0.05$, respectively), as the concentration of Thr increased in the diets. For pigs fed the low-fiber diets, combined broken-line and quadratic analyses estimated the optimum SID Thr:Lys ratio as 0.66 and 0.63 for ADG and G:F, respectively. For the pigs fed high fiber diets, combined broken-line quadratic analyses estimated the optimum SID Thr:Lys requirement as 0.71 and 0.63 for ADG and G:F, respectively. Thus, the ideal Thr:Lys ratio for 25 to 50 kg growing gilts in order to optimize G:F is 0.63, regardless of fiber content, but to optimize ADG, the ideal Thr:Lys ratio is 0.66 in low-fiber diets and 0.71 in high fiber diets.

Key words: amino acids, fiber, ideal protein, pigs, requirement, threonine

INTRODUCTION

Threonine is the second or third limiting AA in most common swine diets (Easter, 1994). Because of the relatively high requirement for Thr by pigs, there is a need to determine the specific requirement to reduce diet costs, to maximize animal performance, and to prevent unnecessary excretion of excess N from the animal. Because of high concentrations of Thr in endogenous losses of AA (Stein et al., 1999), it is possible that inclusion of fibrous ingredients in diets will increase the Thr requirement for growing pigs (Zhu et al., 2005), but the effects of high fiber diets on the Thr:Lys ratio still have to be determined.

Threonine is a unique AA in several respects. It is the indispensable AA in greatest concentration in endogenous secretions (Stein et al., 1999), and it is one of only 5 AA that are both glucogenic and ketogenic (Easter, 1994). Threonine can also be used for *in vivo* synthesis of Gly in the pig (Easter, 1994). Additionally, Thr is present in high concentrations in digestive enzymes, mucins, and immunoproteins (Easter, 1994).

Requirements for AA by pigs may be determined using titration (Cline et al., 2000; Shelton et al., 2011). By utilizing diets deficient only in Thr and titrating increasing levels of Thr in subsequent diets it is expected that the growth response of the animals will allow for determination of the optimum requirement for Thr. Thus, by titrating Thr in low-fiber as well as high-fiber diets, it was assumed that the effect of fiber on the Thr requirement of the animals can be quantified. Therefore, it was the objective of this experiment to determine the effects of dietary fiber on the optimum Thr:Lys ratio for 25 to 50 kg growing gilts.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

A total of 192 female pigs with initial BW: 26.29 ± 4.64 kg (G-Performer × Fertilis 25, Genetiporc, Alexandria, MN) were used. There were 2 pigs per pen and 8 pen replicates per diet. Performance of pigs was measured during a 28 d growth assay. Pigs were housed in pens with 50% concrete slats. There was a feeder and a nipple drinker in each pen and the room was temperature controlled. Pigs had free access to feed and water throughout the experiment.

Ingredients, Diets, and Feeding

All ingredients used in the diets used in this experiment came from the same batches as those used in the experiments described in chapters 4 and 5. The standardized ileal digestibility (**SID**) of AA in all ingredients were determined in the experiment described in chapter 4, and those values were used for the formulation of the diets in this experiment.

A low-fiber basal diet was formulated with approximately 0.40% SID Thr and 0.90% SID Lys. This level of Lys was chosen because the results of the Lys titration experiment described in chapter 3 indicated that the requirement for SID Lys for 25 to 50 kg gilts is 1.09% and the results of the experiment described in chapter 5. Five additional diets were formulated by adding crystalline L-Thr to the basal diet in increments of 0.08% to create diets containing approximately 0.49, 0.57, 0.65, 0.73, and 0.81% SID Thr, respectively (Tables 1 and 2). Thus, diets with SID Thr:Lys ratios at 45:100, 54:100, 63:100, 72:100, 81:100, and 90:100 were used. It was believed that both the linear and the plateau regions of the growth curve would be represented by these inclusion levels of Thr (NRC, 2012). A high-fiber basal diet was also

formulated by adding 15% soybean hulls to the low-fiber basal diet at the expense of corn starch and 5 additional diets were formulated by adding crystalline Thr to this diet, as explained for the low-fiber diets.

Data Recording and Sample Collection

Feed allocations were recorded and individual pig weights were recorded at the beginning of the experiment and at the end of the experiment. The amount of feed left in the feeders was recorded as pigs were weighed off the experiment. The ADG, ADFI, and G:F were calculated for each pen of pigs and for each treatment group at the conclusion of the experiment. The concentration of AA in the diets was analyzed and the daily intake of Thr was calculated for each treatment group.

Chemical Analyses

All diets were analyzed for CP (method 990.03; AOAC Int., 2007) and AA. Amino acids were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc.; Pleasanton, CA, U.S.) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007).

Statistical Analyses

Normality of data was verified and outliers were tested using the UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC). Data were analyzed by ANOVA using the

UNIVARIATE and MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Treatment means were calculated using the LS Means statement and means were separated using the PDIFF option in SAS. Pen was the experimental unit for all analyses.

Data were subjected to broken line analysis and non-linear quadratic regression equations were developed to establish inflection points for ADG and G:F as previously described (Robbins et al., 1979; Baker et al., 2002; Robbins et al., 2006). The concentration of Thr that resulted in the first intercept value of the quadratic regression line and the plateau value from the broken line analysis was used to calculate the ideal SID Thr:Lys ratio for ADG and G:F. The average of these values within fiber levels was determined to be the ideal SID Thr:Lys ratio. Linear and quadratic effects of inclusion of Thr in diets of low or high fiber were determined using orthogonal polynomial contrasts.

RESULTS

In the low-fiber diets ADG and G:F increased both linearly and quadratically ($P < 0.01$ and $P < 0.05$, respectively), as the concentration of Thr increased in the diets (Table 3). Similarly, in the high-fiber diets ADG and G:F increased both linearly and quadratically ($P < 0.001$ and $P < 0.05$, respectively), as the concentration of Thr increased in the diets. Final BW increased linearly in both low-fiber and high-fiber diets ($P < 0.01$ and $P < 0.05$, respectively) as Thr concentration in diets increased. There were no effects of Thr level on ADFI in all low-fiber diets, but ADFI increased linearly ($P < 0.05$) as Thr concentration increased in high-fiber diets. Overall, ADG was greater in pigs fed high fiber diets than in pigs fed low fiber diets.

For pigs fed the low-fiber diets, broken-line analyses estimated the optimum SID Thr:Lys ratio as 0.60 and 0.59 for ADG and G:F, respectively (Figures 1 and 2). For pigs fed high-fiber diets, broken-line analyses estimated the optimum SID Thr:Lys requirement as 0.66 and 0.55 for ADG and G:F, respectively (Figures 3 and 4). For pigs fed the low-fiber diets, quadratic analyses estimated the optimum SID Thr:Lys ratio as 0.76 and 0.73 for ADG and G:F, respectively (Figures 1 and 2). For the pigs fed high-fiber diets, quadratic analyses estimated the optimum SID Thr:Lys requirement as 0.80 and 0.75 for ADG and G:F, respectively (Figures 3 and 4). For pigs fed the low-fiber diets, combined broken-line and quadratic analyses estimated the optimum SID Thr:Lys ratio as 0.66 and 0.63 for ADG and G:F, respectively (Figures 1 and 2). For the pigs fed high-fiber diets, combined broken-line quadratic analyses estimated the optimum SID Thr:Lys requirement as 0.71 and 0.63 for ADG and G:F, respectively (Figures 3 and 4).

DISCUSSION

Several methods are commonly used to determine AA requirements in animals, but the most common method is to use some form of broken-line analyses. In broken-line analyses the determinations for requirements are defined as the intersection point between the slope and plateau portion of the models for specific response parameters (Robbins et al., 1979; Baker et al., 2002; Robbins et al., 2006). Although relatively objective, these models are very conservative and typically do not estimate requirements at levels that represent the majority of the population in question but rather for an average of the test population, and thus, often provide an estimate lower than that which would be sufficient for the majority of the population (Robbins et al., 1979; Baker et al., 2002; Parr et al., 2003).

Another common model used for determination of AA requirements is the quadratic model. This model fits a quadratic line to the model and estimates the requirement as the level determined by the apex of the curve. This model generally results in greater requirement estimates than those determined using broken-line analyses, and will represent a requirement that is sufficient for a larger proportion of the population (Robbins et al., 1979; Baker et al., 2002). However, as a consequence of the use of this relatively subjective model for determination, the quadratic model may overestimate the needs for a large portion of the population (Baker et al., 2002). However, by using a combination of the broken-line and the quadratic model the strengths of both broken-line and curvilinear models are combined (Baker et al., 2002). In this model, the data will be fit to both a broken-line model and a quadratic model. The requirement is not determined at the breakpoint or the apex of the models, but rather the first intersection of the plateau region of the broken-line model and the curvilinear model (Parr et al., 2003). This model combines the strengths of both models by offering a means for not dramatically over or under-estimating the requirement of a given population, but also determines said requirement through the use of a reproducible method and objectively calculated value (Parr et al., 2003). The obvious limitation of the use of this combined model is that it requires the data to both have significant linear and quadratic effects; otherwise any benefits conferred by objectivity are lost. For these reasons, the requirements determined through the use of the combined models will be considered the best estimations of the requirement for this particular population of pigs.

The Thr:Lys ratios estimated by the combined models for G:F and ADG in both low- and high-fiber diets were in agreement with previously published values (Mitchell et al., 1968; Li et al., 1998; Chang et al., 2000; Pedersen et al., 2003; NRC, 2012; Zhang et al., 2013). However,

the objectives of this study were not only to determine the ideal Thr:Lys ratio for 25 to 50 kg growing gilts, but to also determine the effects of fiber on that requirement.

The estimated requirement for the ideal Thr:Lys ratio for optimizing G:F was the same for both low- and high-fiber diets at 0.63. This indicates that the requirement for Thr was not affected by the inclusion of soybean hulls in the diet. However, the estimated requirement for the ideal Thr:Lys ratio for optimizing ADG was greater for pigs fed the high-fiber diets (0.71) than for the pigs fed the low fiber diets (0.66). This increase in the estimated requirement indicates that the presence of soybean hulls, a source of both soluble and insoluble fiber, in the diet increases the requirement for Thr in the growing pig. Although there is limited data available regarding the particular effects of fiber on the Thr requirement of animals, there are several potential reasons for this increased requirement.

Fiber may have negative effects on energy digestibility (Wenk, 2001; Urriola et al., 2013; Cervantes-Pahm et al., 2014), and possibly also on lipid digestibility (Zervas and Zijlstra, 2002; Urriola et al., 2013) and N digestibility (Bach Knudsen and Hansen, 1991; Degén et al., 2009; Kil et al., 2010; Cervantes-Pahm et al., 2014). In addition, fiber has varying physicochemical properties dependent on its composition, particularly its solubility (Dikeman and Fahey, 2006). Inclusion of fibrous ingredients can have significant effects on transit rate and passage time of digesta, particularly in the case of insoluble fiber (Dikeman and Fahey, 2006). Generally, an increase in fiber concentration in the diets will cause decreases in transit time of digesta due to an increased passage rate (Rose and Hamaker, 2011). The abrasive nature of fiber also increases endogenous protein losses, a large portion of which is composed of Thr-rich mucins lining the digestive tract (de Lange et al., 1989).

It has been theorized that certain fibers can have stimulatory effects on microbial activity in the hindgut, which in turn, results in increased production of short chain fatty acids (Sakata, 1987; Zhu et al., 2005). Short chain fatty acids may stimulate proliferation of intestinal cells and increase the weight of the organ (Sakata, 1987) which will result in a simultaneous increase in mucin production, and therefore, in increased loss of Thr.

The increased production and excretion of Thr-rich mucins and the potentially increased growth of the lower GI in response to fiber may be some of the reasons for the greater requirement of Thr in animals fed fibrous diets (de Lange et al., 1989; Easter, 1994; Sakata, 1987). Additionally, the negative effects of fiber on N and AA utilization are well documented and may explain the increased requirement (Bach Knudsen and Hansen, 1991; Dégen et al., 1999; Dikeman and Fahey, 2006; Rose and Hamaker, 2011).

CONCLUSION

The ideal Thr:Lys ratio for 25 to 50 kg growing gilts to optimize G:F is 0.63, regardless of fiber content. However, to optimize ADG the fiber content of diets should be considered when determining the amount of Thr to be included in the diet. For 25 to 50 kg growing gilts fed fibrous diets, the ideal Thr:Lys ratio is 0.71 to optimize ADG, but if low-fiber diets are fed, the ideal Thr:Lys ratio is 0.66 to optimize ADG. Although, the specific reasons for the increase in the requirement relative to fiber level are not entirely clear, it is supported by biological plausibility, and is of special importance to the modern swine industry as the practice of feeding higher fiber diets becomes increasingly common.

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TABLES

Table 6.1. Ingredient composition of experimental diets

	Low fiber						High fiber					
	Thr:Lys ratio											
	0.45	0.54	0.63	0.72	0.81	0.90	0.45	0.54	0.63	0.72	0.81	0.90
Ground corn	57.17	57.07	56.99	56.90	56.825	56.745	55.15	55.06	54.98	54.9	54.82	54.73
Field peas	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal, 48% CP	5.25	5.25	5.25	5.25	5.25	5.25	3.25	3.25	3.25	3.25	3.25	3.25
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Corn starch	15.0	15.0	15.0	15.0	15.0	15.0	-	-	-	-	-	-
Soy hulls	-	-	-	-	-	-	15.00	15.00	15.00	15.00	15.00	15.00
Soy oil	1.0	1.0	1.0	1.0	1.0	1.0	5.25	5.25	5.25	5.25	5.25	5.25

Table 6.1. (cont.)

Limestone	0.80	0.80	0.80	0.80	0.80	0.80	0.62	0.62	0.62	0.62	0.62	0.62
Dicalcium phosphate	1.02	1.02	1.02	1.02	1.02	1.02	0.95	0.95	0.95	0.95	0.95	0.95
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-Lysine HCl	0.39	0.39	0.39	0.39	0.39	0.39	0.38	0.38	0.38	0.38	0.38	0.38
DL-Methionine	0.24	0.24	0.24	0.24	0.24	0.24	0.27	0.27	0.27	0.27	0.27	0.27
L-Threonine	-	0.09	0.17	0.25	0.33	0.41	-	0.09	0.17	0.25	0.33	0.42
L-Tryptophan	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
L-Isoleucine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
L-Valine	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.14	0.14
L-Phenylalanine	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07

Table 6.1. (cont.)

Histidine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Vitamin - mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 6.2. Analyzed nutrient composition of diets

	Low fiber						High fiber					
	Thr:Lys ratio											
	0.45	0.54	0.63	0.72	0.81	0.90	0.45	0.54	0.63	0.72	0.81	0.90
ME[1], kcal/kg	3,404	3,401	3,398	3,395	3,393	3,390	3,318	3,315	3,312	3,310	3,307	3,304
NE[1], kcal/kg	2,522	2,519	2,517	2,515	2,513	2,510	2,519	2,517	2,515	2,513	2,510	2,508
Ca[1], %	0.70	0.70	0.70	0.70	0.70	0.70	0.69	0.69	0.69	0.69	0.69	0.69
STTD P[1], %	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.32	0.32	0.32	0.32	0.32
CP, %	12.64	12.63	12.63	12.62	12.61	12.61	12.81	12.80	12.79	12.79	12.78	12.77
Indispensable AA, %												
Arg	0.81	0.83	0.79	0.80	0.77	0.78	0.73	0.79	0.78	0.73	0.72	0.72
His	0.39	0.39	0.38	0.37	0.36	0.39	0.37	0.39	0.38	0.37	0.38	0.36
Ile	0.63	0.65	0.60	0.61	0.57	0.62	0.60	0.60	0.60	0.60	0.56	0.56
Leu	1.17	1.18	1.14	1.16	1.13	1.13	1.10	1.15	1.15	1.12	1.06	1.04
Lys	0.92	0.92	0.94	1.03	0.90	0.99	0.92	0.96	1.00	0.95	0.92	0.90
Met	0.47	0.22	0.47	0.39	0.43	0.42	0.44	0.43	0.50	0.41	0.43	0.42
Met + Cys	0.67	0.42	0.66	0.58	0.61	0.61	0.64	0.63	0.70	0.60	0.61	0.60

Table 6.2. (cont.)

Phe	0.69	0.66	0.66	0.65	0.64	0.63	0.65	0.64	0.65	0.63	0.61	0.64
Thr	0.47	0.57	0.58	0.75	0.83	0.88	0.45	0.65	0.61	0.70	0.81	0.72
Trp	0.18	0.18	0.18	0.18	0.18	0.19	0.17	0.16	0.16	0.16	0.15	0.13
Val	0.72	0.76	0.74	0.74	0.73	0.75	0.69	0.74	0.72	0.69	0.70	0.67
Dispensable AA, %												
Ala	0.74	0.74	0.72	0.72	0.71	0.71	0.69	0.73	0.72	0.70	0.67	0.66
Asp	1.18	1.21	1.14	1.16	1.13	1.14	1.10	1.17	1.16	1.11	1.08	1.08
Cys	0.20	0.20	0.19	0.19	0.18	0.19	0.20	0.20	0.20	0.19	0.18	0.18
Glu	2.15	2.18	2.09	2.12	2.07	2.08	1.95	2.06	2.02	1.98	1.90	1.87
Gly	0.60	0.59	0.58	0.57	0.57	0.57	0.63	0.66	0.64	0.62	0.60	0.62
Pro	0.76	0.76	0.75	0.73	0.73	0.73	0.71	0.76	0.75	0.72	0.70	0.69
Ser	0.55	0.56	0.54	0.54	0.53	0.53	0.54	0.57	0.56	0.54	0.52	0.51
Tyr	0.39	0.36	0.35	0.36	0.34	0.34	0.40	0.41	0.41	0.40	0.41	0.40

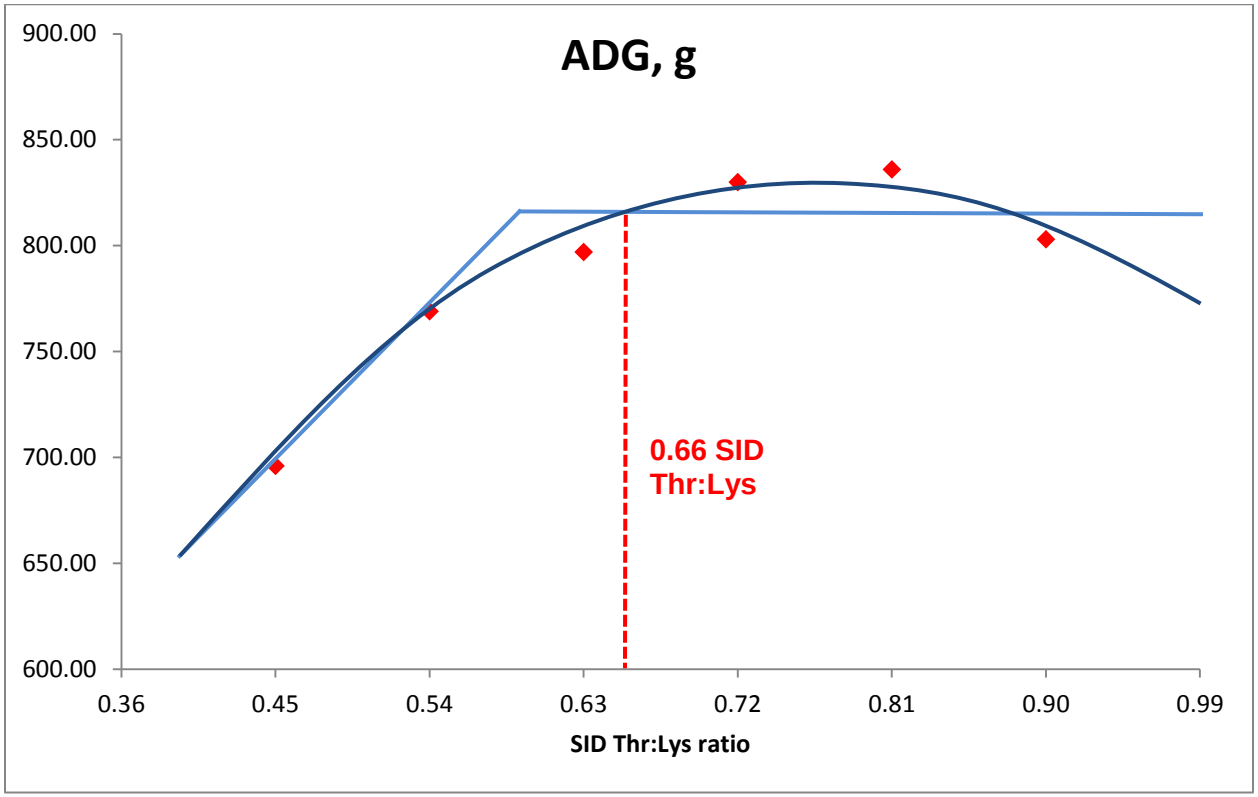
[1]Values not analyzed, but based on calculations by composition.

Table 6.3. Growth performance of pigs fed experimental diets [1]

	Thr:Lys							Contrasts (*P*-value)	
	0.45	0.54	0.63	0.72	0.81	0.90	SEM	Linear	Quadratic
Low Fiber									
Initial BW, kg	26.16	26.68	26.68	25.73	26.73	26.39	0.80	0.963	0.969
ADG, g	696	769	797	830	836	803	28.9	<0.01	<0.05
ADFI, g	1,785	1,799	1,777	1,812	1,862	1,830	102	0.376	0.917
G:F	382	423	450	455	445	435	20.9	0.001	<0.001
Final BW, kg	45.66	49.43	49.56	49.5	50.13	50.36	1.15	<0.01	0.132
High Fiber									
Initial BW, kg	26.47	26.85	26.72	26.57	26.28	26.72	1.31	0.924	0.964
ADG, g	763	882	878	900	933	915	35.48	<0.001	<0.05
ADFI, g	1,828	1,872	1,835	1,864	1,945	1,989	64.9	<0.05	0.409
G:F	421	461	465	472	470	464	12.91	<0.01	<0.01
Final BW, kg	48.14	51.86	51.67	52.08	52.70	52.64	1.61	<0.05	0.145

[1]Data are means of 8 observations per treatment.

FIGURES



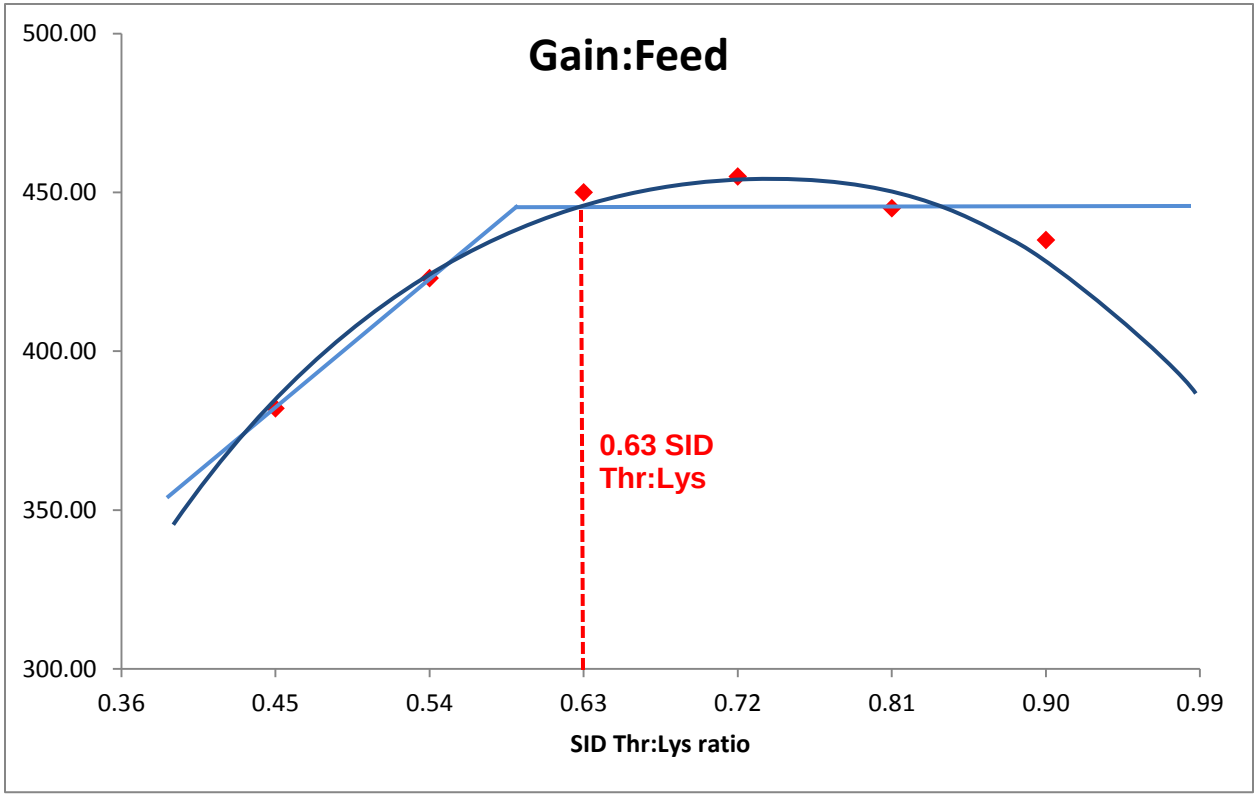
Broken-line estimate:

0.60 SID Thr:Lys

Quadratic curve estimate:

0.76 SID Thr:Lys

Figure 6.1. Fitted linear breakpoint and quadratic plots of ADG as a function of standardized ileal digestible Thr to Lys ratio with observed treatment means in pigs fed low fiber diets.



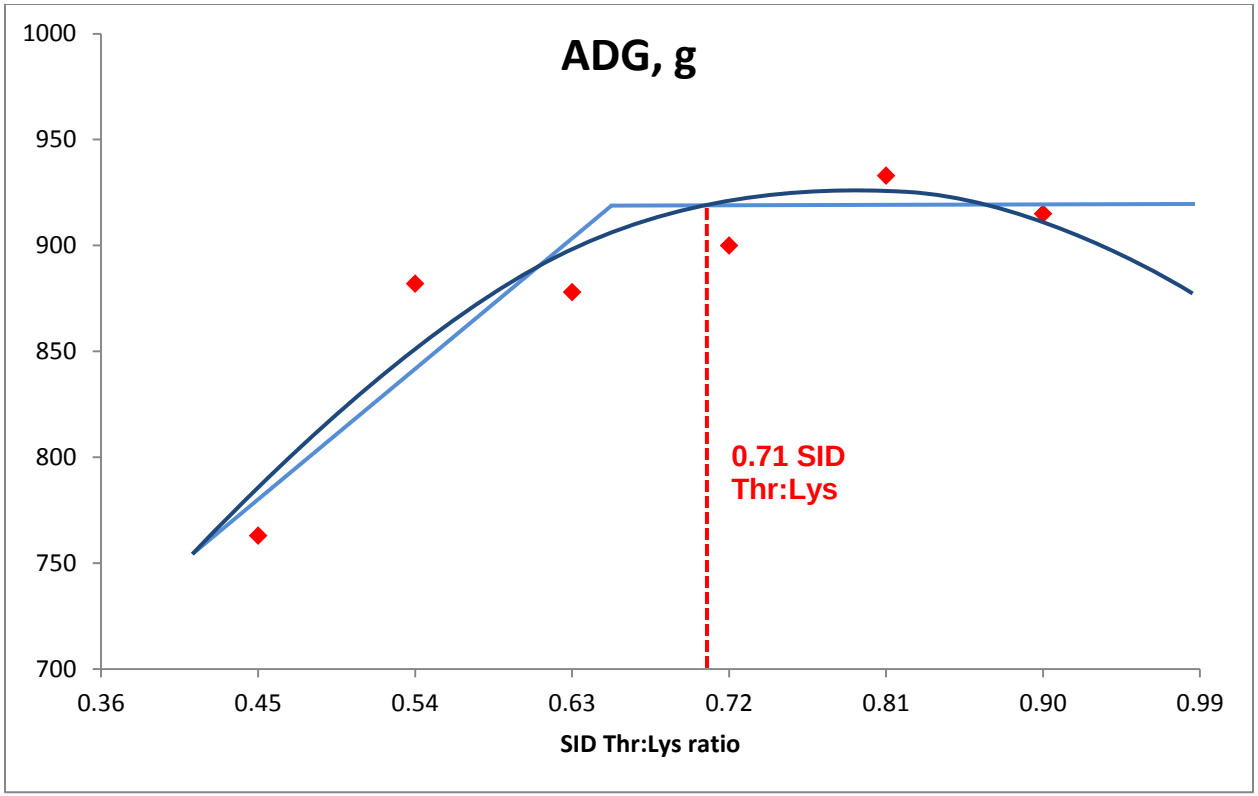
Broken-line estimate:

0.59 SID Thr:Lys

Quadratic curve estimate:

0.73 SID Thr:Lys

Figure 6.2. Fitted linear breakpoint and quadratic plots of G:F as a function of standardized ileal digestible Thr to Lys ratio with observed treatment means in pigs fed low fiber diets.



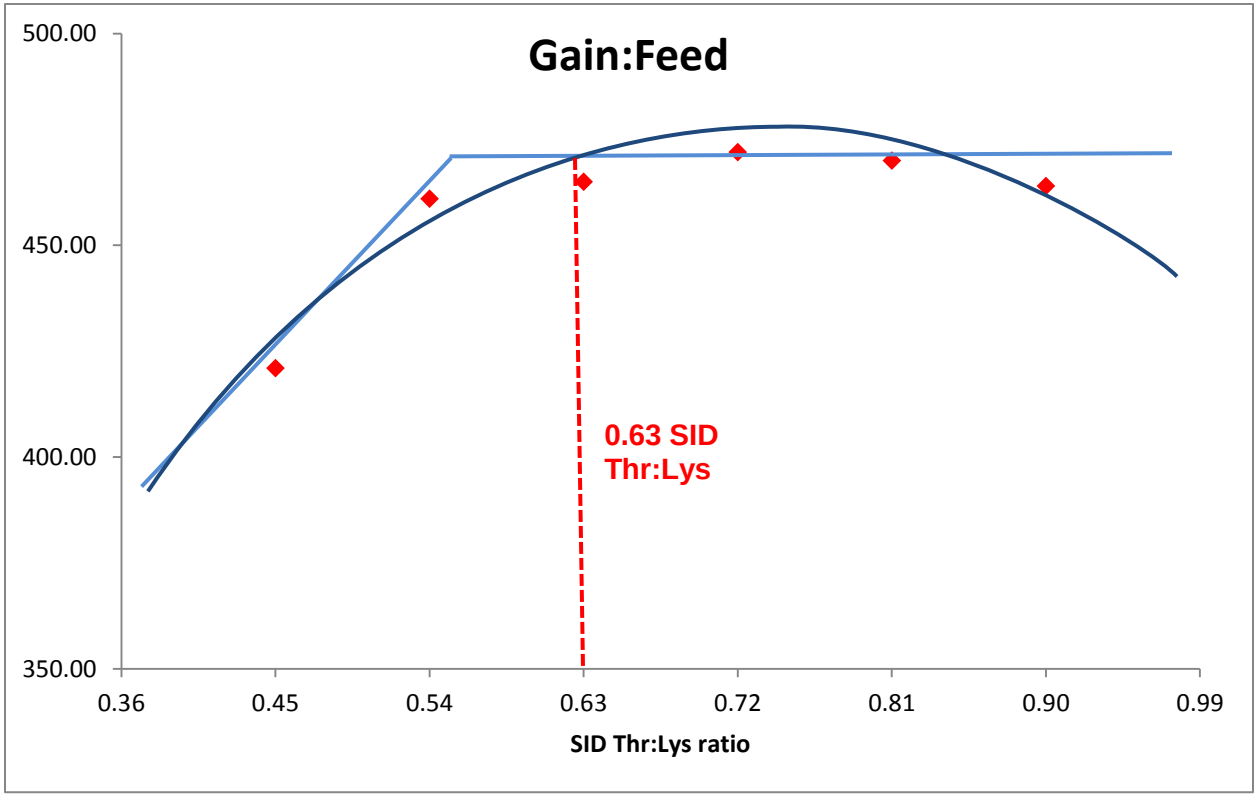
Broken-line estimate:

0.55 SID Thr:Lys

Quadratic curve estimate:

0.75 SID Thr:Lys

Figure 6.3. Fitted linear breakpoint and quadratic plots of ADG as a function of standardized ileal digestible Thr to Lys ratio with observed treatment means in pigs fed high fiber diets.



Broken-line estimate:

0.55 SID Thr:Lys

Quadratic curve estimate:

0.75 SID Thr:Lys

Figure 6.4. Fitted linear breakpoint and quadratic plots of G:F as a function of standardized ileal digestible Thr to Lys ratio with observed treatment means in pigs fed high fiber diets.

CHAPTER 7

EFFECTS OF FIBER LEVEL ON NITROGEN BALANCE OF GROWING PIGS FED DIETS THAT ARE VERY DEFICIENT OR MARGINALLY DEFICIENT IN THREONINE

ABSTRACT: An experiment was conducted to quantify the effects of fiber on the Thr requirement of 25 to 50 kg growing gilts by measuring the flow and retention of N in animals fed diets with different concentrations of Thr and fiber. Thirty-six growing gilts (initial BW: 29.0 ± 0.74 kg) were housed in metabolism cages that allowed for the total, but separate collection, of urine and feces from each pig. Pigs were allotted to 4 diets with 9 replicate pigs per diet utilizing a randomized complete block design with 3 blocks of 12 pigs. Four diets were prepared using a 2 × 2 factorial arrangement. Two diets were low-fiber diets and 2 diets were high-fiber diets. Soybean hulls were included at 15% in the high-fiber diets, whereas corn starch was included at 15% in the low-fiber diets. Within each level of fiber, 1 diet was formulated to contain standardized ileal digestible (**SID**) Thr at a Thr:Lys ratio of 0.45, whereas the other diet was formulated to contain Thr at a SID Thr:Lys ratio of 0.60. Experimental diets were fed for 14 d. The initial 7 d were an adaptation period to the diets. Urine and fecal samples were collected during the following days according to the marker to marker method. Intake of N was less ($P < 0.05$) in pigs fed high-fiber diets than in pigs fed low-fiber diets. Output of N in feces was greater ($P < 0.05$) from pigs fed high-fiber diets, but output of N in urine was greater ($P < 0.05$) from pigs fed low-fiber diets. The ATTD of N was greater ($P < 0.05$) from pigs fed low-fiber diets than in pigs fed high-fiber diets, and retention of N was greater ($P < 0.05$) in pigs fed low-fiber diets than in pigs fed high-fiber diets. There was greater ($P < 0.05$) output of N in urine from

pigs fed low-Thr diets than from pigs fed high-Thr diets, and there was greater ($P < 0.05$) N retention in pigs fed high-Thr diets compared with pigs fed low-Thr diets. There was also an interaction ($P < 0.05$) between fiber level and Thr for output of N in feces with N output increasing ($P < 0.05$) as Thr in the high-fiber diet increased, whereas this was not the case for the low-fiber diet. Results indicate that higher fiber diets may require greater concentrations of Thr than low-fiber diets.

Key words: amino acids, fiber, gilts, lysine, nitrogen, pigs, threonine

INTRODUCTION

The ideal Thr:Lys ratio can be determined by responses other than growth performance. By measuring the flow, and particularly the retention, of N in animals fed identical diets, with the exception of varying levels of Thr, one can determine an animal's requirement for Thr. Additionally, by feeding diets with varying Thr concentrations in addition to varying levels of fiber, effects of fiber on the flow and retention of N, and thus Thr, can be quantified.

Effects of fiber on N digestibility are well documented (Zervas and Zijlstra, 2002; Dilger et al., 2004; Urriola et al., 2013). The primary change when fiber is included in the diet is a shift of N excretion from the urine to the feces, as bacteria utilize N produced through fermentation (Zervas and Zijlstra, 2002; Urriola et al., 2013). An increase in fiber concentration in diets has also been associated with decreases in AA digestibility, despite no changes in apparent N digestibility in diets with fiber compared with control diets (Dilger et al., 2004). However, results of other studies have confirmed that the apparent total tract digestibility (**ATTD**) of CP decreases, and N content in feces increases, as the concentration of fiber in the diets increases

(Hansen et al., 2006; Cervantes-Pahm et al., 2014). Effects of fiber on N and AA digestibility depend on the source and physico-chemical properties of the fiber (Souffrant, 2001). Fiber may also influence both the mucin production and endogenous losses of AA in an animal (Satchithanandam et al., 1990). This is of special importance when the Thr requirement is in question, because of the high concentration of Thr in gastrointestinal tissues and mucin secretions (Wang et al., 2007). Both the potential increase in mucin production and the increased endogenous losses due to increased fiber in the diet raises the possibility that the Thr requirement is increased in animals fed high fiber diets, but this hypothesis has not been verified. Therefore, the objective of this experiment to quantify the effects of fiber on the Thr requirement by measuring the flow and retention of N in animals fed diets with different concentrations of Thr and fiber.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Animals, Housing, and Experimental Design

Thirty-six growing gilts (G-Performer × Fertilis 25, Genetiporc, Alexandria, MN; initial BW: 29.0 ± 0.74 kg) were housed in metabolism cages that were equipped with a feeder and a nipple-drinker, fully slatted floors, solid-sided walls, a screen floor, and urine trays. The trays allowed for the total, but separate collection, of urine and feces from each pig. Pigs were housed in an environmentally controlled room. Pigs were allotted to 4 diets with 9 replicate pigs per diet utilizing a randomized complete block design. There were 3 blocks of 12 pigs each.

Ingredients, Diets, and Feeding

All ingredients in the diets used in this experiment were from the same batch as those used in the experiments described in chapters 4, 5, and 6. The standardized ileal digestibility (**SID**) of AA in all ingredients were determined in the experiment described in chapter 4, and those values were used for the formulation of the diets in this experiment.

Four diets were prepared using a 2 × 2 factorial arrangement (Tables 2 and 3). Two diets were low-fiber diets and 2 diets were high-fiber diets. Within each level of fiber, 1 diet was formulated to contain Thr at a SID Thr:Lys ratio of 0.45, whereas the other diet was formulated to contain Thr at a SID Thr:Lys ratio of 0.60. These ratios were used based on the results of a prior Thr titration study using gilts of the same BW and genetics and were believed to be approximately 70% and 95% of the optimum Thr:Lys ratio. Diets were formulated so that the diet with the greatest SID Thr:Lys ratio within each level of fiber was expected to result in the greatest Lys, and thus, N retention. The 4 diets were formulated using corn, SBM, field peas, and fish meal. Soybean hulls were included at 15% in the high-fiber diets, whereas corn starch was included at 15% in the low-fiber diets. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All pigs were fed twice daily at 0800 h and 1600 h. All pigs were fed 810g per feeding for a total of 1,620 g of feed daily. This feeding level was believed to be the above 90% of *ad libitum* feed intake for gilts based on the ADFI observed in a previous experiment in which similar diets were fed. Water was available at all times.

Data Recording and Sample Collection

Pig weights were recorded at the beginning of each period and at the conclusion of the period. The amount of feed supplied each day was recorded and feed refusals were recorded, as

well. Experimental diets were fed for 14 d. The initial 7 d were considered an adaptation period to the diets. Urine and fecal samples were collected during the following 5 days according to standard procedures for the marker to marker method (Adeola, 2001). Daily collections of feces were immediately frozen at -20ºC. Daily collections of urine were weighed and mixed, and a 20% subsample was frozen at -20ºC.

Chemical Analyses

At the conclusion of the experiment, fecal samples were dried in a forced-air oven and finely ground before analysis. Diets and ingredients were analyzed for CP (method 990.03; AOAC Int., 2007) and AA [method 982.30 E (a, b, c); AOAC Int., 2007]. Fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007) and CP (method 990.03; AOAC Int., 2007). Urine samples were filtered and analyzed for CP (method 990.03; AOAC Int., 2007). All samples were analyzed in duplicate, with the exception of analysis for AA.

Calculations and Statistical Analyses

Retention of N (Nr) for each pig was calculated using Eq. [1] (Pedersen et al., 2007):

$$Nr = \{[Ni – (Nf + Nu)] / Ni] \times 100\%, \quad \textbf{[1]}$$

where Nr is the retention (%) of N, Ni is the intake (g) of N from d 8 to 14, Nf is the fecal output (g) of N from d 8 to 14, and Nu is the urinary output (g) of N from d 8 to 14.

The apparent total tract digestibility for N was calculated using Eq. [2] (Pedersen et al., 2007):

$$ATTD = [(Fi – Ff)/Fi] \times 100\%, \quad \textbf{[2]}$$

Where ATTD is the apparent total tract digestibility for N (%), Fi is the total N intake (g), and Ff is the total fecal output of N (g).

Data were analyzed as a 2 way ANOVA, with 2 levels of fiber (high and low) and 2 SID Thr:Lys ratios (0.45 and 0.60) using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Interactions between fiber level and the SID Thr:Lys ratio were included in the model. Mean values for each diet were calculated using the LSMeans statement and means were separated when interaction was significant. The pig was the experimental unit for all analyses and an α-value of 0.05 was used to assess statistical significance.

RESULTS

All pigs stayed healthy throughout the experiment and readily consumed their diets. The total intake of N was greater ($P < 0.05$) for pigs fed the low-fiber diets than pigs fed high-fiber diets (Table 3). Output of N in the feces was greatest ($P < 0.05$) in pigs fed the high-fiber, high-Thr diet and least ($P < 0.05$) in pigs fed the 2 low-fiber diets. Output of N in urine was greatest ($P < 0.05$) in pigs fed the low-fiber, low-Thr diet and least ($P < 0.05$) in pigs fed the high-fiber, high-Thr diet. The ATTD of N was greatest ($P < 0.05$) in pigs fed the low-fiber diet and least ($P < 0.05$) in pigs fed the high-fiber diet. Retention of N was greatest ($P < 0.05$) in pigs fed the low-fiber, high-Thr diets and least ($P < 0.05$) in pigs fed the high-fiber, low-Thr diets. Retention of N was greater ($P < 0.05$) in pigs fed the low-fiber, high-Thr diet than in pigs fed the low-fiber, low-Thr diet. Additionally, retention of N was greater ($P < 0.05$) in pigs fed the high-fiber, high-Thr than in pigs fed the high-fiber, low-Thr diets.

The ATTD of N was greater ($P < 0.05$) from pigs fed the low-fiber diets than in pigs fed the high-fiber diets, and the retention of N was greater ($P < 0.05$) in pigs fed the low-fiber diets than in pigs fed the high-fiber diets. There was greater ($P < 0.05$) output of N in the urine from pigs fed the low-Thr diets than from pigs fed the high-fiber diets, and there was greater ($P < 0.05$) N retention in pigs fed the high-Thr diets compared with pigs fed the low-fiber diets. There was also an interaction ($P < 0.05$) between fiber level and Thr for the output of N in feces with N output increasing ($P < 0.05$) as Thr in the high fiber diet increased, whereas this was not the case for the low-fiber diet.

DISCUSSION

The increased fecal N excretion from the high-fiber diets compared with low-fiber diets is consistent with observation from other studies and represents the shift in excretion of N from the urine to the feces as microbial utilization of N increases (Dilger et al., 2004). The increased N output in the feces of pigs fed high-fiber diets compared with pigs fed low-fiber diets may represent microbial proteins generated in the hindgut and the potentially negative effect that microbial substrates in the form of fiber in the hindgut can have on an animal's utilization of protein (Libao-Mercado et al., 2008). Increased viscosity due to soluble fibers increases the thickness of the muscularis layer, increases mucosal mass, and increases overall protein deposition in the gastrointestinal tract (Larsen et al., 1994; Stark et al., 1996; Zhu et al., 2005). Changes in viscosity of the digesta and passage rate as a result of fiber in the diet may also explain the increased fecal N excretion. It is possible that the increased N in the feces of pigs fed the high-fiber diets may have been the result of increased endogenous losses of proteins due to

the abrasive nature of fiber in the diets (de Lange et al., 1989), or the stimulatory effects of fiber on microbial fermentation (Libao-Mercado et al., 2008; Sakata, 1987).

The decrease in N output in the urine of pigs fed the high-fiber, high-Thr diet compared with pigs fed the high-fiber, low-Thr diets is consistent with the increase in N output in the feces. However, regardless of the fiber-level, pigs fed the high-Thr diets had reduced N output in the urine when compared with the low-Thr diets, which is indicative of the increased utilization of N from the diet as Thr level approached the requirement of the animal. The substantial decrease in urinary N for pigs fed the low-fiber, high-Thr diet compared with pigs fed the high-fiber, high-Thr diet indicates that the requirement for Thr may be greater in high-fiber diets than in low-fiber diets.

Nitrogen retention in pigs fed the high-Thr diets compared with pigs fed the low-Thr diets indicates that a SID Thr:Lys ratio of 0.45 is inadequate to meet the requirements of 25 to 50 kg growing gilts, as the concomitant increase in AA supplementation and N retention indicates that the N in the diet is being used more efficiently. Because of the decrease in the N retained in pigs fed the high-fiber diets, compared with pigs fed the low-fiber diets, there is an indication that the requirement for Thr increases as fiber level increases.

CONCLUSION

Results of this experiment indicate that a SID Thr:Lys ratio of 0.45 is below the requirement for optimal protein accretion in 25 to 50 kg growing gilts. Results also indicate that dietary fiber affects the flow and retention of N in the pig. Results indicate that fiber in the diet increases total N output and simultaneously decreases urinary N excretion by shifting N-

excretion towards fecal excretion. This experiment also confirmed that the inclusion of fiber in the diet will reduce the ATTD of N.

The increase in N retention in pigs fed the high-Thr diets indicates that those diets provided for the pigs that were closer to the requirement of the animals than the low-Thr diets. The ratio of 0.60 SID Thr:Lys is in agreement with the NRC's recommendation for the Thr requirement of 25 to 50 kg growing pigs (2012). However, the difference in N retention between the high-Thr diets indicates that the animals on the high-fiber, high-Thr diets were not receiving enough Thr to meet the requirement of the animals. Therefore, the present data indicate that a higher fiber diet may require a greater inclusion level of Thr relative to Lys.

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TABLES

Table 7.1. Ingredient composition of experimental diets (as-fed basis)

	Low-fiber		High-fiber	
	0.45 SID Thr:Lys	0.60 SID Thr:Lys	0.45 SID Thr:Lys	0.60 SID Thr:Lys
Ground corn	57.17	57.01	55.15	55.01
Field peas	15.0	15.0	15.0	15.0
Soybean meal, 48% CP	5.25	5.25	3.25	3.25
Fish meal	3.0	3.0	3.0	3.0
L-Isoleucine	0.09	0.09	0.09	0.09
L-Lysine HCl	0.39	0.39	0.38	0.38
DL-Methionine	0.23	0.23	0.27	0.27
L-Threonine	-	0.14	-	0.1425

Table 7.1. (cont.)

L-Tryptophan	0.06	0.06	0.07	0.07
L-Valine	0.16	0.16	0.14	0.14
L-Histidine	0.06	0.06	0.06	0.06
Corn starch	15.0	15.0	-	-
Soybean oil	1.0	1.0	5.25	5.25
Soybean hulls	-	-	15.0	15.0
Limestone	0.8	0.8	0.62	0.62
Dicalcium phosphate	1.02	1.02	0.95	0.95
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.30	0.30	0.30	0.30

Table 7.1. (cont.)

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 7.2. Analyzed nutrient composition of diets

	Low-fiber		High-fiber	
	0.45 SID Thr:Lys	0.60 SID Thr:Lys	0.45 SID Thr:Lys	0.60 SID Thr:Lys
ME[1], kcal/kg	3404	3399	3318	3314
NE[1], kcal/kg	2522	2518	2519	2516
Ca[1], %	0.69	0.69	0.70	0.71
STTD P[2], %	0.33	0.33	0.33	0.34
CP, %	14.37	14.89	13.92	13.86
Indispensable AA, %				
Arg	0.82	0.96	0.82	0.80
His	0.39	0.47	0.42	0.43
Ile	0.59	0.63	0.61	0.61

Table 7.2. (cont.)

Leu	1.16	1.23	1.17	1.19
Lys	0.98	1.10	1.05	1.04
Met	0.39	0.46	0.52	0.50
Phe	0.68	0.73	0.70	0.69
Thr	0.47	0.64	0.48	0.59
Trp	0.19	0.18	0.16	0.15
Val	0.73	0.79	0.74	0.74
Dispensable AA, %				
Ala	0.71	0.76	0.73	0.74
Asp	1.16	1.35	1.19	1.17
Cys	0.19	0.21	0.20	0.20

Table 7.2. (cont.)

Glu	2.15	2.37	2.11	2.13
Gly	0.57	0.63	0.64	0.65
Pro	0.77	0.79	0.77	0.79
Ser	0.55	0.61	0.58	0.58
Tyr	0.42	0.45	0.44	0.44

[1]Values not analyzed, but based on calculations by composition (NRC, 2012).

[2]STTD = standardized total tract digestible.

Table 7.3. Nitrogen balance in pigs fed Thr supplemented diets of low- and high-fiber[1]

	Low-fiber		High-fiber			*P*-value		
	0.45 SID Thr:Lys	0.60 SID Thr:Lys	0.45 SID Thr:Lys	0.60 SID Thr:Lys	Pooled SEM	Fiber level	Thr level	Fiber level × Thr level
N intake, g/5 d	1,138	1,156	1,015	1,069	30	<0.05	0.17	0.48
N output in feces, g/5 d	236^{bc}	220^{c}	257^{b}	295^{a}	16	<0.05	0.30	<0.05
N output in urine, g/5 d	183	143	164	94	13	<0.05	<0.05	0.22
ATTD[2] of N, %	80.1	81.6	75.6	73.2	1.46	<0.05	0.69	0.06
N retention, %	64.5	69.77	59.5	64.9	2.07	<0.05	<0.05	0.99

[1]Data are means of 9 observations per treatment, except for the treatment with high-fiber and 0.60 SID Thr:Lys, which had only 7 observations.

[2]ATTD = apparent total tract digestibility.

CHAPTER 8

CONCLUSIONS

The rising cost of swine production due to the increases in prices of feed ingredients encourages producers to explore alternative sources of protein and energy for swine diets. Many of these alternative ingredients have considerably different protein quality and greater fiber content when compared with traditional feed ingredients, like soybean meal. Because of this it is important to understand the relationship between fiber and AA, and particularly to determine how increased fiber in the diet can affect the protein requirement of an animal.

The objectives of this research were to define an optimal standardized ileal digestible (**SID**) Thr:Lys ratio for 25 to 50 kg growing gilts fed commercially representative diets, while exploring and attempting to quantify the effects of fiber on this ratio. Although the effects of fiber on nutrient digestibility and endogenous losses have been explored, the effects on the actual ideal SID Thr:Lys ratio had yet to be determined.

Combined, the results of the experiments indicate that the optimal SID Thr:Lys ratio is greater in pigs fed high-fiber diets than in pigs fed low-fiber diets. Thus, to maximize performance, the concentration of digestible Thr should be increased if fiber levels of diets are increased. Results of these experiments are important because not only is the cost of AA supplementation great, but modern practices in the swine industry indicate that feeding of higher-fiber ingredients will become more common. Understanding the relationships between fiber and AA utilization and requirements will enable producers to improve efficiency by feeding to the requirements of the animals and will help to reduce waste by minimizing the N excreted by animals.