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Evaluation of the threonine requirement and the bioavailability of threonine in feedstuffs in pregnant sows

by

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ABSTRACT

Current recommendations for amino acid intake during pregnancy are for a constant amino acid intake throughout. However, the demand for amino acids changes from maternal tissue growth in early gestation to fetal, conceptus and mammary tissue development in late gestation. The availability of amino acids from feed ingredients are based on growing pig data, although recent evidence suggests that mature animals have a greater capacity to digest and absorb amino acids. Therefore, this thesis investigated the threonine requirement of sows in gestation and the availability of threonine (Thr) in common feed ingredients fed directly to sows using the indicator amino acid oxidation technique.

The Thr requirement in early gestation was determined to be 5.0 to 6.0 g/d, at least 40% below current recommended Thr requirements, whereas the requirement for Thr in late gestation was determined to be 12.3 to 13.6 g/d, close to 30% above current recommendations. These results suggest that current sow feeding recommendations (i.e. constant level of AA throughout gestation) result in over- and under-feeding AA in early and late gestation, respectively. The metabolic availability of Thr in corn and barley fed to growing pigs was 82.2 and 115.3%, respectively, whereas when fed to pregnant sows, the metabolic availability of Thr in corn and barley was 88.0 and 89.3%, respectively. The > 100% availability of Thr from barley was likely due to the effect of barley on the demand for Thr for production of mucin and mucous proteins. The results indicate that the availability of amino acids from feed ingredients is greater when fed to sows than when fed to growing pigs.

In conclusion, current sow amino acid requirement recommendations do not appropriately reflect actual amino acid demand during pregnancy. The deficiency in dietary amino acids during late gestation may result in maternal lean tissue catabolism to support fetal growth. The greater

availability of amino acids from feed ingredients in sows may reduce the degree of amino acid deficiency in late gestation under current feeding programs. Application of phase feeding sows during pregnancy will more closely meet the demand for amino acids and may improve sow reproductive longevity.

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List of Abbreviations

AA, amino acid(s)

APE, atom percent excess

BCAA, branched-chain amino acids

BW, body weight

IAAO, indicator amino acid oxidation

Lys, lysine

MA, metabolic availability

NPPU, net post-prandial utilization

PDCAAS, protein digestibility-corrected amino acid score

Phe, phenylalanine

PPU, post-prandial utilization

PUN, plasma urea nitrogen

Thr, threonine

Trp, tryptophan

1.0 Introduction

Nutritional information contained in the NRC (1998) provides the basis for feed and nutritional programs for all stages of swine production; however, the research on which sow nutrient requirement recommendations were based was completed in the 1970's and 1980's. Each year improvements in swine genetics and management of the breeding herd results in higher production/sow/year. The increase in production is a result of many factors including greater lean tissue gain, more piglets per litter, a reduction in non-productive days and reduced days to market (CCSI, 2008). Since 1980, backfat at 100 kg has dropped 44% and days to 100 kg has dropped 17%. As well, litter size has increased 0.25 piglets/litter each year over the last 6 years alone (CCSI, 2008). Thus, as lean gain and reproductive performance of the sow increases so too will the requirement for nutrients in the diet. Studies examining whole body amino acid (AA) requirements less than 10 years old are limited and recent data by Samuel et al. (2008) suggest that the maintenance lysine requirement of sows is 30% higher than NRC (1998) estimates. The lysine requirement was estimated to be 30% higher in gestating sows (Dourmad and Étienne, 2002; Srichana, 2006) and 35% higher in lactating sows (Srichana, 2006) than NRC (1998) estimates.

The cost of using outdated nutritional data when formulating feeding programs for the reproductive herd may come in the form of reduced reproductive longevity, lower lifetime pig output, premature culling, or reduced growth potential in the offspring. Genetic selection for lean tissue growth may have influenced the breeding potential of the sow and made her more vulnerable to nutritional deficiencies (Close and Cole, 2000). Evidence of this increased vulnerability can be seen in the high sow replacement rate

(close to 50%) with half of the first and second parity culled animals for failing to come into oestrus or conceiving (Close and Cole, 2000). Deficient dietary protein in gestation can increase the loss of body protein that occurs during lactation and may reduce ovarian function in the subsequent rebreeding (Clowes et al., 2003). Greater losses of body protein in lactation are associated with extended wean-to-estrus interval (King, 1987) and lower subsequent litter size (Touchette et al., 1998). Although it is well understood that nutritional status in one parity can have consequences on subsequent rebreeding and litter size, very few studies examining nutrition of the breeding herd are carried through more than one gestation/lactation cycle.

The Canadian hog industry has suffered severe financial losses over the last 3 yrs due to high feed costs from increased demand for feed grains from the ethanol industry, the strengthening Canadian dollar and low hog prices (Lidster et al., 2009). Feed costs represent 60% of the total cost of hog production (Lazaruk, 2009) with sows consuming 20% of the total feed consumed (Moehn et al., 2009). Thus improving current nutrient recommendations for sows has the potential to provide substantial savings in feed costs (Moehn et al., 2009).

The requirement for a particular AA in gestation depends on the body maintenance requirement, requirement for maternal weight gain and conceptus gain (NRC, 1998) and efficiency of retention. The current NRC (1998) estimate of a single AA level throughout gestation assumes that the relative influence of each factor remains constant. However, as gestation progresses the influence of maternal lean tissue gain on the total gestational gain decreases and the influence of fetal and mammary tissue development increase. Maternal carcass protein gain made up > 75% of total protein gain from 0 to 60 d

gestation but fell to < 50% of total protein gain from 60 to 115 d gestation (Kim et al., 2009), whereas, fetal protein gain increased 18-fold from 70 to 114 d (4.63 g/d) compared to 0 to 70 d gestation (0.25 g/d; McPherson et al., 2004). The change in demand for AA from maternal gain to fetal and conceptus gain along with the substantial increase in sow productivity indicates that nutrient requirements for today's high producing sow need to not only be re-evaluated but examined in early and late gestation separately.

The animal's AA requirement and the potential of feedstuffs to supply those AA in metabolically available form (feedstuff amino acid bioavailability) are intertwined. To fully utilize the knowledge of one, we must also have a complete knowledge of the other thus a complete nutritional program also includes an accurate estimate of the ability of the diet (i.e. feed ingredients) to supply the required nutrients. Amino acid digestibility is the most common method for assessing the quality of feed ingredients to supply dietary AA in pigs (Stein et al., 2007). Published digestibility estimates are based on growing pig data (NRC, 1998); however, adult sows have a greater capacity to digest and absorb dietary protein than growing pigs (Noblet and Shi, 1993; Stein et al., 2001). Therefore, in order to adequately re-evaluate amino acid recommendations for gestating sows, feed quality evaluation must be included.

1.1 Assessing amino acid requirements

There are two methods of determining AA requirements: empirical and factorial. The empirical method is a dose-response relationship using a basal diet adequate in all

nutrients except the AA being measured where a physiological response to graded addition of the limiting AA is measured.

There are 3 problems with the empirical method (Moughan and Fuller, 2003): 1. the method assumes the requirement of all other nutrients are known. Since this is not the case, the basal diet must include an excess of all other nutrients as a safety margin; however, these excesses may create an imbalance which may affect the metabolic use of the limiting AA. 2. more than one physiological response can be used to determine the requirement and the determined requirement can be different depending on the response measured. 3. the determined requirement can differ depending on the statistical criteria used to determine the breakpoint (i.e. requirement).

When an imbalanced AA diet is ingested, the efficiency of utilization of the most limiting AA for protein synthesis is increased while the efficiency of utilization of the non-limiting essential AA is reduced (Soliman and King, 1969). However, the efficiency of utilization of the most limiting AA is not affected by moderate dietary deficiency (70 to 90% of requirement) of the most limiting AA (Moehn et al., 2004). By nature of the experiment, AA requirement studies must temporarily create an imbalance (i.e. 1 AA limiting protein synthesis while all others are in excess); however, extreme dietary excesses of non-limiting AA should be avoided. To minimize the potential negative effects of AA imbalance, dietary levels of all other nutrients are usually set slightly higher than the most recent published requirement (i.e. 120% of NRC, 1998). Crystalline AA are commonly used to balance the supply of dietary AA. The use of crystalline AA in a conventional diet to estimate AA requirement may lead to an overestimation, particularly when animals are fed once daily because the efficiency of utilization of

crystalline AA is affected by feeding frequency (Batterham, 1979). Crystalline AA pass through the stomach and are absorbed in the small intestine more rapidly than protein-bound AA (Rolls et al., 1972); however, protein synthesis requires that all essential AA are available to the tissues at the same time (Wang and Fuller, 1989). However, when the experimental diets are based on highly digestible protein sources, the protein-bound AA supply is held constant, at least a portion of each essential AA is provided in the diet as crystalline AA and the subjects are fed more than once a day the error associated with the requirement estimates can be assumed to be minimal (Wang and Fuller, 1989).

In order to determine AA requirements, changes to a certain metabolic criterion in response to graded intakes of the test AA must be measured (Pencharz and Ball, 2003). However, the appropriate response variable is dependent on age, physiological state and the desired animal response. In the young animal, skeletal, musculature and digestive development are of primary importance; whereas in mature animals, the focus is on maintenance of body weight and body condition. In growing animals, the desired response is maximal lean tissue growth thus methods which estimate changes in lean tissue growth or body weight are most advantageous (Baker, 1986). However, in the breeding animal, optimal fetal and conceptus growth and milk production along with maternal lean gain and health status are of primary concern (NRC, 1998). Therefore, methods which estimate only a change in lean tissue growth may not adequately reflect the breeding animal's requirement for a particular AA. The response variable selected must appropriately reflect the desired animal response to the test nutrient and allow meaningful extrapolation to the practical setting (Baker, 1986). The level of AA to maximize growth or development of 1 response variable does not necessarily maximize

gain of a different response variable. For example, in growing pigs, the dietary threonine requirement to maximize lean tissue gain was lower than the requirement to maximize immune function (Defa et al., 1999). Therefore, the method used to determine the requirement should be selected carefully and be sensitive enough to reasonably measure changes in all the metabolic criteria of interest.

Statistical criteria used to determine the breakpoint include: the level of addition of test AA that produces the maximum response or the minimum level of addition of test AA that is not statistically different from the level that produces the maximum response (Moughan and Fuller, 2003). These criteria can be evaluated statistically using a paired-comparison test or a broken-line regression model (Baker, 1986). The use of paired-comparison tests of adjacent points to determine the requirement is weak because the upper curvilinear area of a growth curve is where the greatest animal to animal variation occurs (Baker, 1986). Broken-line regression models to determine nutrient requirements include the one or two slope broken-line model or the curvilinear response curve (Robbins et al., 2006). Broken-line regression provides a breakpoint estimate and standard error. The breakpoint is defined as the level of nutrient intake above which there is no significant change in the dependent response variable (Robbins et al., 2006). Broken-line models may not adequately describe the response when there is no definite plateau; however, breakpoints, by definition, are not a component of the curvilinear model (Baker, 1986). Baker et al. (2002) suggest that the first point at which the curvilinear line intersects the plateau of the one slope fitted broken line is a good objective estimate of AA requirement.

The factorial method for determining AA requirements is based on mathematical models which sum the requirement of AA for maintenance and growth and correct for the inefficiency of AA utilization (Moughan and Fuller, 2003). Therefore, the factorial approach provides a more flexible estimation of requirement at the whole animal level (Moughan and Fuller, 2003); however, its development relies on solid values determined from the empirical approach (Close and Cole, 2000). The lysine requirement for maintenance was recently determined to be 30% higher than NRC (1998) recommendations (Srichana, 2006; Samuel et al., 2008), thus the factorial method would underestimate the requirement for maintenance alone by 30%. Given the outdated empirical data on which current sow requirements are based, new empirical data are necessary to develop improved factorial models.

1.2 Physiological responses to assess amino acid requirements

Dietary AA are used for protein synthesis thus lean tissue gain is typically the metabolic criterion of focus in AA requirement studies and response variables selected reflect this focus (i.e. body weight gain, feed efficiency, nitrogen balance). However, as discussed previously, in the gestating sow, fetal and conceptus gains and maternal immune status are additional response variables of primary interest. As well as adequately representing the metabolic criterion of interest, the response variable selected must respond rapidly to changes in dietary AA level in order to evaluate AA requirement in early and late gestation, separately.

Numerous response variables have been used to determine amino acid requirements in gestating or lactating sows including body weight gain with back fat measurements

and litter gain (Cooper et al., 2001 a,b), plasma urea nitrogen (Soltwedel et al., 2006) nitrogen balance (Dourmad and Étienne, 2002) and only recently, indicator amino acid oxidation (IAAO; Samuel et al., 2010). Body weight gain and feed efficiency may be selected due to their ease of measurement; however, weight gain correlates well with nitrogen balance in young growing animals but the correlation between body weight (BW) and lean tissue decreases as BW increases (Baker, 1986). This is likely because maintenance makes up a greater proportion of the requirement for nutrients as BW increases. Therefore, in the gestating sow, BW and feed efficiency are less sensitive parameters and are less desirable as the dependent response variable.

Metabolic criterion such as plasma urea nitrogen (PUN), nitrogen balance or IAAO are more direct measures of protein retention (Brown and Cline, 1974; Ball and Bayley, 1986; Fuller and Garlick, 1994). Plasma urea nitrogen is a measure of AA balance and changes in PUN reflect total nitrogen utilization where urea excretion is minimized when the requirement for the first limiting AA is met, resulting in reduced catabolism of excess AA (Brown and Cline, 1974). Time post feeding and feeding regime (*ad libitum* vs meal fed) can affect the PUN concentration (Eggum, 1970; Cai et al., 1994). A minimum of 4 h post feeding was necessary to reach a plateau in PUN levels in meal-fed pigs (Eggum, 1970; Cai et al., 1994). However, when fed *ad libitum*, PUN concentrations were almost constant with only 8% fluctuation in daily PUN concentrations compared to 32% in meal-fed pigs (Cai et al., 1994). Although PUN responds rapidly to changes in dietary AA level, requiring < 3 d adaptation time (Brown and Cline, 1974; Dourmad and Étienne, 2002), its use in AA requirement studies has been limited, possibly due to challenges with obtaining blood samples in animals.

1.2.1 Nitrogen balance

Estimation of nitrogen balance is calculated as nitrogen intake minus nitrogen output. Nitrogen intake is the nitrogen supplied by the diet and nitrogen output is measured as the nitrogen lost in the urine and feces. Nitrogen balance can refer to maximal nitrogen retention, where further intakes of nitrogen result in no change in nitrogen retention, absolute nitrogen retention (i.e. 0.5 g N/d) or nitrogen equilibrium (i.e. 0 g N/d retained). The requirement determined by nitrogen balance is sensitive to the level of nitrogen retention selected (Fuller and Garlick, 1994). For example, AA requirements needed to maintain positive nitrogen retention (0.5 g nitrogen/d) were estimated to be 1.9 to 4.9 times higher than that required for apparent nitrogen equilibrium (Hegsted, 1963).

The nitrogen balance method has been commonly used to estimate AA requirements; however, inaccuracies in measuring nitrogen intakes and losses result in an underestimation of the AA requirement (Fuller and Garlick, 1994). Particularly in animals, nitrogen intake is often overestimated due to feed spillage and nitrogen loss is underestimated due to incomplete measurement of the sources of nitrogen loss. Nitrogen is lost in feces, urine, secretions, gaseous ammonia, skin and hair. Fecal and urinary nitrogen can be measured relatively simply and thus are typically the only measurements of nitrogen loss (Fuller and Garlick, 1994); however, for more accurate calculation of nitrogen balance an estimate of obligatory nitrogen losses is required. A number of studies have estimated the obligatory nitrogen losses in humans at different stages of development (Pencharz et al., 1977; Tontisirini et al., 1981; Kaneko and Koike, 1983) but limited similar data for swine was found making accurate estimate of nitrogen losses in pigs difficult.

Nitrogen equilibrium is taken to imply AA equilibrium; however, the AA composition of the body may not be constant when amino acid-deficient diets are fed (Fuller and Garlick, 1994). Lysine per 16 g nitrogen in whole-body protein was lower when pigs were fed diets deficient in lysine than when given lysine-adequate diets; whereas, the glycine and arginine content in whole-body protein increased when fed lysine-deficient diets (Batterham et al., 1990). Therefore, the rate of retention of AA in whole-body protein per increment of test AA intake may not equal the rate of nitrogen retention.

In relation to sow AA requirements, a further challenge with nitrogen balance is the 7 to 10 d adaptation time required for the body urea pool to stabilize after a change in dietary nitrogen content (Rand et al., 1976). As previously discussed, the requirement for AA is likely to be higher in late gestation compared to early gestation (McPherson et al., 2004). The dramatic increase in protein requirement in gestation in sows that occurs in the last trimester (80 to 115 d of gestation; McPherson et al., 2004) suggests that the estimation of AA requirements in late gestation should be conducted over a maximum period of 35 d. In order to utilize at least 5 levels of test AA, > 4 levels of test AA are necessary for accurate estimate of the requirement (i.e breakpoint; Baker, 1986), a nitrogen balance study would require a collection period of 35 to 50 d in each of early and late gestation which is beyond the limit of the late gestation period as defined above.

Although, implementation of nitrogen balance studies can be completed with relative ease in animal studies, the methodological and physiological challenges with nitrogen balance studies have resulted in discontinuance of its use in humans (WHO, 2007).

1.2.2 Indicator amino acid oxidation

The IAAO method is based on the principle that excess AA cannot be stored such that AA are either utilized for protein synthesis or must be oxidized. When one indispensable AA is deficient for protein synthesis, all other indispensible AA are in excess and must be oxidized (Elango et al., 2008a). The adequacy of the supply of the deficient AA is measured indirectly by the oxidation of another carboxy-labeled ($^{13 \text{ or } 14}C$) indispensible AA (Kim et al., 1983) called the indicator AA. As the intake of the deficient AA increases, the oxidation of the indicator AA decreases linearly until the requirement for the limiting AA is reached (Ball and Bayley, 1984). Increases of the test limiting AA beyond the requirement do not increase protein synthesis further, and there is no further change in oxidation of the indicator AA (Figure 1.2.1); thus IAAO reaches a plateau. The inflection point between the linear decrease and plateau in oxidation represents the estimated average requirement and is determined with the use of bi-phase linear regression analysis (Elango et al., 2008a,b). Ball and Bayley, 1986 demonstrated that the change in oxidation of the indicator AA (L-[1-^{14}C] phenylalanine) was inversely proportional to the incorporation of L-[1-^{14}C] phenylalanine into liver proteins (i.e. protein synthesis) (Figure 1.2.1)

The IAAO technique has been extensively developed to determine AA requirements and was chosen as the gold standard by the World Health Organization for determination of AA requirements of humans (WHO, 2007; Pencharz and Ball, 2003). Initial development of the IAAO technique was in young pigs using ^{14}C phenylalanine to determine the requirement for histidine (Kim et al., 1983) and tryptophan (Ball and Bayley, 1984a) and was validated against the traditional method of nitrogen balance

(Kim et al., 1983). Extensive work followed to adapt the IAAO technique in humans including the use of a stable rather than radioactive isotope (Zello et al., 1993), the route of isotope administration: intravenous vs oral delivery (Bross et al., 1998; Kriengsinyos et al., 2002), the time of adaptation required for the level of test AA (Zello et al., 1990) and the time of adaptation necessary for the level of dietary protein (Thorpe et al., 1999). Thus the current protocol involves a 2-d adaptation to a fixed daily protein intake followed by at least 8-h adaptation to the test AA intake on the d of study (Elango et al., 2008a; 2009). Since then the IAAO technique has been used in the pig model to assess the affect of enteral vs parenteral nutrition on AA requirement (Bertolo et al., 1998), the development of advanced total parenteral nutrition solutions (Brunton et al., 2007), the effect of gut AA utilization on whole body AA requirements (Law et al., 2007) and the interanimal variability of AA requirements (Bertolo et al., 2005; Moehn et al., 2008).

Indicator amino acid oxidation relies on selection of an appropriate indicator AA. There are 3 key criteria when selecting an AA as indicator 1) an essential AA, 2) the labeled carbon is irreversibly lost as CO_2 during oxidation, preferably during the first steps of AA catabolism and 3) the AA should undergo no other significant reactions apart from oxidation to CO_2 and incorporation into protein (Zello et al., 1995). Since accurate measurement of label in the free AA pool and the end-products of degradation are essential to the oxidation technique, ubiquitously labeled carbon atoms would not be appropriate (Zello et al., 1995). Threonine (Soliman and King, 1969), [^{14}C-methyl]-methionine **(**Brookes et al., 1972**)** lysine (Ball and Bayley, 1984a), leucine (Hsu et al., 2006; Kurpad et al., 1998) and phenylalanine (Kim et al., 1983; Ball and Bayley, 1984a) have all been used as indicator AA.

Although threonine and methionine are essential AA, they were determined to be inadequate indicator AA. The methyl group of [^{14}C-methyl]-methionine could be incorporated into many reactions besides production of $^{14}CO_2$ (Brookes et al., 1972) and threonine has 2 main pathways for catabolism (Ballevre et al., 1990). End products of [1-^{13}C] threonine oxidation could be $^{13}CO_2$ or ^{13}C-glycine thus the rate of $^{13}CO_2$ appearance would not provide a quantitative measure of threonine catabolism (Zhao et al., 1986).

Leucine has a larger, more variable pool size than Phe or lysine and plays a significant role in the regulation of protein synthesis (Garlick and Grant, 1988) as well as hormonal secretion (Rocha et al., 1972). Therefore, leucine has a direct influence on the partitioning between protein synthesis and oxidation, the measurement parameter. As well, leucine oxidation was not sensitive to increasing Phe intake when intake of Phe was limiting protein synthesis and leucine was in excess of requirement (Hsu et al., 2006).

Although lysine meets all 3 requirements for an indicator AA, the body pool size is larger and more variable and has more steps involved in its catabolism prior to oxidation of the carboxyl carbon compared to Phe (Neale and Waterlow, 1974). The L-[1-^{13} or 1-^{14}C]-Phe (Kim et al., 1983; Ball and Bayley, 1984a,b), in the presence of excess tyrosine, has been used most often because it satisfies all 3 key criteria and has a number of advantages: the free pool size is comparatively small with a quick turn-over rate (Neale and Waterlow, 1974), and the size of the intracellular free Phe pool is tightly regulated (Flaim et al., 1982). Phenylalanine oxidation occurs in the liver and thus responds to dietary AA entering the liver (Ball and Bayley, 1984a), as well, the primary first step of Phe oxidation is decarboxylation (Chamruspollert et al., 2002). Phenylalanine oxidation was more responsive to dietary changes than lysine or leucine (Neale and

Waterlow, 1974). The advantage of the short half-life of the free Phe pool is that it responds rapidly to changes in test AA intake. Ball and Bayley (1984b) showed in baby pigs that the AA requirement could be determined with as little as 4 h of adaptation to the diet. In sows and growing pigs, Phe oxidation responded within 1 – 2 days of a change in test AA intake, and remained constant for up to 10 d after a change in diet (Moehn et al., 2004). Therefore, 2 d of adaptation to a new dietary test AA level are deemed sufficient. Recently, Elango et al., (2009) showed in humans that the adaptation period to the test AA can be even shorter (~ 8 h) provided the subjects are prior adapted to a standard protein intake. The short adaptation time makes IAAO, using Phe as the indicator AA, particularly advantageous for determining AA requirements during rapidly changing physiological states (i.e. gestation and lactation) and in vulnerable populations (i.e. very young and elderly).

1.3 Amino acid availability from feedstuffs

Amino acid bioavailability has been defined as the percentage of AA digested and absorbed in a form available for utilization for the purpose of maintenance or growth of tissue (ARC, 1981; Batterham, 1992; Fan, 1994; Danfaer and Fernández, 1999). There is no direct measure of AA bioavailability (Stein et al., 2007). However, numerous methods have been used to estimate protein quality of animal and human food ingredients: fecal and ileal AA digestibility, AA availability using slope-ratio assays, protein digestibility-corrected amino acid score, net postprandial protein utilization and postprandial protein utilization (Stein et al., 2007, Elango et al., 2009b). Each method has its own unique set of advantages and disadvantages.

1.3.1 Amino acid digestibility

In pig nutrition, ileal AA digestibility is currently most widely used as an estimate of dietary protein quality. Ileal AA digestibility is comparatively easy to determine, and yields results for all AA in a reasonably short time frame; however, ileal AA digestibility coefficients are highly dependent on an accurate estimate of ileal endogenous AA losses (Stein et al., 2005). Ileal AA digestibility coefficients can be referred to as apparent, standard or true ileal AA digestibility depending on the method used to estimate ileal endogenous AA losses (Stein et al., 2007).

The reader is directed to Stein et al. (2007) for a detailed description of the terminology and problems with estimation of ileal endogenous AA losses. Briefly, ileal endogenous AA losses can be divided into 2 components: basal and specific (Nyachoti et al., 1997). Basal losses represent inevitable (diet independent) AA losses; whereas specific AA losses are losses induced by specific feed ingredient characteristics, above the basal losses; therefore, are considered diet dependent AA losses (Stein et al., 2007). Numerous factors such as dietary fiber and crude protein content, antinutritional factors, body weight and composition of protein-free diet can all influence the estimate of endogenous AA losses (Stein et al., 2007). Amino acid digestibility, in its most accurate form (true ileal digestibility), can only measure AA disappearance from the gut (Stein et al., 2007) and thus does not account for first-pass metabolism or AA absorbed in a chemical complex unsuitable for metabolism. If the degree of first-pass metabolism is different between AA then the measurement of the pattern of AA in the diet, or the residual AA in the digesta, will not reflect the availability of AA to the extra-intestinal

tissue (Stoll et al., 1998). Heat processing of feedstuffs can result in AA forming chemical complexes (i.e. lysine and Maillard reaction products) that can be digested and absorbed but cannot be utilized by the animal for protein synthesis (Carpenter and Booth, 1973).

Surgical implantation of a simple t-cannula is a popular technique for collection of ileal digesta due to the ease of surgical procedure and minimal trial-to-trial variation (Knabe et al., 1989) and the surgical procedure has been well described for growing pigs (Decuypere et al., 1977; Gargallo and Zimmerman, 1980). Ileal cannulation of pregnant sows was successfully demonstrated by Stein et al., (1998). The digesta flow through the cannula was maintained for up to 3 parities; however, ~ 25% of the cannulated sows developed peritonitis shortly before parturition presumably due to increased pressure on the intestine from the expanding uterus causing an intestinal rupture (Stein et al., 1989).

Development of a new method of estimating AA bioavailability that can account for the inadequacies of the ileal AA digestibility method, and eliminate the need for surgical modification, has the potential to significantly advance feed quality evaluation.

1.3.2 Protein quality assessment in human nutrition

The protein digestibility-corrected amino acid score (PDCAAS) was proposed as the method of choice for assessing protein quality in humans (FAO, 1991). However, there are a number of limitations with the PDCAAS method: does not account for conditionally indispensable AA, utilizes fecal rather than ileal digestibility, does not account for variations in digestibility between entire protein and individual AA nor the effect of heat processing or antinutitional factors on digestibility (Elango et al., 2009).

For example, the apparent ileal digestibility of individual AA and crude protein was reduced with the inclusion of fiber but the degree of reduction was dependent on the fiber source with wheat bran and barley having the greatest reduction in digestibility of protein and AA (Myrie et al., 2008). The availability of lysine was 38% lower in heat-treated peas compared to unheated peas (Moehn et al., 2005). Due to the disadvantages of the PDCAAS method, new technologies need to be developed. Stable isotope based methods have the potential to more accurately estimate the nutritional value of food proteins.

Within the last decade, 3 new technologies using stable isotope techniques have been developed: net postprandial protein utilization (NPPU; Gausserès et al., 1997; Bos et al., 1999), postprandial protein utilization (PPU; Millward et al., 2000, 2002), and metabolic availability (MA; Moehn et al., 2005, 2007). Net postprandial protein utilization is calculated as the ileal digestibility of ^{15}N-labeled dietary protein corrected for ^{15}N -labeled AA deaminated in the body nitrogen pool, i.e. plasma urea and urinary nitrogen (Gausserès etal., 1997). Although NPPU is a major advancement in protein quality evaluation, it is limited to foods which can be intrinsically labeled with ^{15}N, requires collection of ileal digesta via naso-intestinal intubation and does not estimate digestibility of individual AA. These limitations indicate that routine application of this technique is unlikely.

The PPU method expresses protein quality in terms of the efficiency of nitrogen utilization, i.e. nitrogen utilization/nitrogen intake (Millward et al., 2002). Nitrogen utilization is determined from ^{13}C-labeled leucine utilization (leucine intake – cumulative leucine oxidation) assuming a leucine:nitrogen ratio of 625 mg/g nitrogen in body tissue protein. Leucine and nitrogen balances are estimated based on ^{13}C-leucine oxidation

using the cumulative difference between postabsorptive and fed-state leucine oxidation rates. The method described above involves several assumptions which have not been validated. Since it is not possible to estimate the proportion of leucine oxidation in the 6 h postprandial period arising from exogenous (dietary) or endogenous sources, the PPU for nitrogen present in the test meal cannot be accurately determined (Kurpad and Young, 2003). Furthermore, the administration route of isotope labeled AA or proteins to estimate digestion and absorption of food protein should be the same as that of the test protein (i.e. orally).

Metabolic availability is calculated as the ratio of the slope of the response (tracer oxidation) of a test protein to the slope of the response of a reference protein (Elango et al., 2009b). It is based on minimal assumptions that have been validated in animals (Ball and Bayley, 1986; Moehn et al., 2005), can be used to assess different protein sources and measures bioavailability of individual AA (Moehn et al., 2007). Details and advantages of the MA method are discussed in the following section.

1.3.3 Slope ratio assays and metabolic availability

Assays of AA bioavailability that measure parameters of growth (i.e. slope ratio assays) are considered the standard against which other methods of AA availability are judged (Lewis and Bayley, 1995). The advantages of slope ratio assays that measure parameters of protein synthesis are that they measure a response that has practical and economic consequences and account for the effect of digestion, absorption and metabolic utilization of the AA provided by the protein source (Lewis and Bayley, 1995). Therefore, such assays measure the effect of protein quality on the entire metabolism and thus are likely to be equally applicable in animal or human nutrition. The importance of

the effect of protein quality on metabolic utilization of AA has been shown by Batterham (1992) who demonstrated that, in growing pigs, heat-damaged protein supported lower protein retention than a control diet despite similar AA digestibility. However, practical use of AA bioavailability growth assays is limited by time, cost and the limitation of examining only one AA at a time (Batterham, 1992).

Metabolic availability utilizes the IAAO technique as the dependent response criterion in a slope ratio assay as a method to improve the practical application of bioavailability growth assays in animal (Moehn et al., 2005, 2007) and human (Humayun et al., 2007) protein quality evaluation of protein sources.

In the IAAO technique, the change in appearance of $^{13}CO_2$ or $^{14}CO_2$ in breath reflects the change of whole body protein synthesis (Ball and Bayley, 1986). At plateau in oxidation, protein synthesis is maximized and would not be expected to respond to changing supply of test AA of differing availability. However, when the intake of the test AA is limiting, the change in indicator oxidation reflects the response of whole body protein synthesis to graded levels (or intakes) of the limiting AA (Moehn et al., 2005). This slope indicates the change in IAAO per unit change in limiting AA. A shallower slope indicates less AA per unit intake is available to support protein synthesis (Figure 1.3.2). Therefore, the relative difference in the rate (slope) of the IAAO between test and reference proteins will be proportional to the whole body MA of the test AA for protein synthesis.

Gabert et al. (2001) suggested that this comparison of the slope of the response from a test protein source to the slope of the response to a defined reference protein source represents relative values only. The MA method utilizes increasing levels of the

crystalline form of the test AA to establish the reference response curve (Moehn et al., 2005). The true ileal digestibility of crystalline AA was determined to be essentially 100% (Baker, 1992). Thus, the slope of the IAAO obtained with the crystalline form of the test AA represents the maximal unit increase in protein synthesis and is equivalent to 100% MA of the test AA. The oxidation of the indicator AA is regressed against the AA intake above that provided by the base diet (supplied by crystalline test AA) and the protein source (Moehn et al., 2005). Therefore, the ratio of the slope of the indicator oxidation due to the protein source compared to the indicator oxidation slope due to the crystalline AA gives the true metabolic utilization of the test AA available from the protein source. In this way, MA takes into account all losses associated with incomplete digestion and absorption, gut endogenous AA losses and absorbed AA which are unavailable due to anti-nutritional factors, damage caused during heat processing (Maillard reaction compounds), D-amino acids, and cross-linked proteins such as lysinoalanine (Elango et al., 2009).

The test of the MA concept using the IAAO technique was conducted by Moehn et al. (2005). Addition of lysine from peas, and even more so from heat-treated peas, to a base diet led to a shallower decrease in oxidation than addition of crystalline lysine (Figure 1.3.1). This indicated a lower availability of lysine in peas and heated peas for protein synthesis. The MA of lysine in peas was calculated to be 88.8% and was similar to previously published estimates of the standard ileal digestibility of lysine in peas (NRC, 1998). Heating peas using a protocol similar to that employed by van Barneveld et al. (1994), who used a traditional slope ratio assay, reduced the lysine availability further, to 54.8% (Moehn et al., 2005). Similarly, Ball et al. (1995) reported impaired lysine

availability in cotton seed meal and soybean meal compared to crystalline lysine. Therefore, using the IAAO in a slope-ratio assay allows determination of the availability of AA in vivo, and is capable, contrary to digestibility studies, to detect reduced protein quality of heat-treated feedstuffs.

Determination of MA requires a number of key criteria be met: (1) the test AA must be first limiting, (2) the response to changes in test AA intake must be linear (3) the observed response must not be influenced by other dietary nutrients in the test feed ingredient and (4) an isotopic steady state must be achieved (Batterham, 1992; Littell et al., 1995; Moehn et al., 2005).

Appropriate formulation of the test diets can ensure each of the above criteria are met. The basal diet should contain all nutrients in slight excess, except the AA of interest (Batterham, 1992); therefore, the test AA will be first limiting. The upper limit of the test AA intake, from both the reference and test protein sources, should be below the lower confidence interval of the requirement in every subject (Elango et al., 2009b) or at least 2 SD below requirement (Moehn et al., 2005). Numerous studies measuring different response parameters have shown a linear response to AA intakes below requirement: PUN and nitrogen retention (Coma et al., 1995; Dourmad and Étienne, 2002), IAAO (Ball and Bayley, 1986; Moehn et al., 2005; Humayun et al., 2007) and BW and litter gain (Cooper et al., 2001a,b). In this way, criteria (1) and (2) will be met.

Addition of the test ingredient will alter the dietary AA profile compared to the reference diets. However, with the use of crystalline AA it is possible to obtain similar dietary intakes of all essential AA and crude protein in both reference and test protein diets, thus the only factor driving IAAO becomes the level of test AA intake (Humayan et

al. 2007). As well, the test ingredient should be incorporated at the expense of a non-protein energy source to ensure the response is due to the test AA and not influenced by other nutrients from the test protein (Batterham, 1992) thus satisfying criteria (3).

To satisfy criteria (4), an isotopic state, either primed i.v. or primed, frequent oral dosing of the indicator AA can be used. Moehn et al. (2005) showed that either route of isotope delivery achieved a plateau lasting for 2.5 h, beginning approximately 1.5 h after the start of isotope delivery. Oral dosing of isotope circumvents the need for venous catheterization, and assures that the indicator AA is subjected to the same route of application as the test AA. Therefore, oral isotope delivery with frequent, ½ hourly feeding is the preferred application of IAAO in the determination of MA to obtain an isotopic steady state.

There are 2 important assumptions for statistical evaluation of bioavailability assays: linearity of the response to graded AA intakes and intersection of the regression lines from the reference and protein sources (Littell et al., 1995). As discussed previously, the assumption of linearity of the response to graded AA intakes holds true when dietary AA intake levels are maintained below 2 SD of the mean AA requirement. To ensure intersection of the regression lines of the reference and test proteins, both the crystalline test AA and the test protein source should be added to a semi-synthetic or synthetic basal diet containing the lowest level of test AA. The dependent response variable is then regressed against the AA intake above that provided by the base diet (Moehn et al., 2005).

The MA method, utilizing IAAO, can address many of the limitations of previous bioavailability studies using slope ratio growth assays, particularly inter-animal variation and length of experimental period.

1.3.4 Advantages of metabolic availability for evaluating protein quality

The measurement of AA bioavailability by slope-ratio assay is regarded as the 'gold standard' (Lewis and Bayley, 1995); however, there are problems with its practical implementation. Conventional response criteria used in slope-ratio assays (comparative slaughter, body weight or the N-balance technique) are unethical in humans (comparative slaughter), require large numbers of animals and prolonged periods of time. As discussed previously, in adult humans, errors associated with estimation of zero or minimal nitrogen balance can be substantial. As well, estimates of bioavailability demonstrate a high standard error due to the effect of individual variation among test subjects on the slope of the response and the accuracy of the total AA analysis of the test protein (Batterham, 1992). These problems can be addressed by using the IAAO technique instead of conventional methods. The short adaptation time required for the IAAO technique (<2 d, Moehn et al., 2004) allows repeated measurement of indicator oxidation at all levels of test AA intake within subjects in rapid succession. Therefore, the number of subjects, inter-subject variation and the time-frame of a study can be reduced drastically. Typically the MA of AA in the test feed can be tested within 14 d. The IAAO technique also fulfills the critical requirement that the response criterion used in a slope ratio assay should be a measure of protein retention (Batterham 1992).

An error associated with estimation of protein quality of ingredients is total AA analysis. Total AA analyses can vary from 5 to 10% between laboratories (Sarwar et al., 1983; Batterham, 1992). Ileal digestibility studies require total AA analyses to be conducted on the test diets, the protein-free diet, as well as the collected digesta, potentially multiplying the error associated with AA analysis in the final digestibility estimate. Although some AA analysis is unavoidable when evaluating protein quality, the MA method, necessitates analysis of the test diets only minimizing this error. Additionally, stable and radioactive isotope analyses are measured with great precision and are highly sensitive (Wolfe and Chinkes, 2005) and thus able to detect smaller differences between treatments. The high sensitivity of isotopic analysis allows a minimal numbers of observations necessary to account for differences in the sensitivity of the response variable.

Although the MA provides information for only one AA at a time, it represents the end result of digestion, absorption and metabolic utilization of AA, while digestibility values are only a measure of disappearance in the small intestine. This is particularly important where metabolic availability may be expected to be reduced (i.e. heat-damaged proteins). Furthermore, in pigs, ileal digestibility is determined using surgically modified animals, while MA can be measured in intact pigs. Surgical modification (e.g. T-canula, re-entrant canula, ileal-rectal anastomosis) may disrupt the regular function of the gastro-intestinal tract (Fuller, 1991), and thus yield digestibility values that may deviate from those applicable to intact animals. In human subjects, ileal digesta effluents can be estimated with the use of animal models (i.e. digestibility correction based on rat ileal digestibility estimate, PDCAAS) or can be collected via ileostamates from ileostomy

patients or by nasointestinal tubes in healthy subjects or (Fuller and Tomé, 2005). The use of ilestomy patients or nasointestinal tubes is too demanding for routine application (Fuller and Tomé, 2005) and although animal models have been shown to reasonably estimate ileal digestibility of foods in humans (FAO, 1991; Rowan et al., 1994) the measurement of AA availability in vivo is preferred (FAO, 1991).

Ileal digestibility is the most common measure of protein quality in pig nutrition and the basis for most recommendations for dietary AA supply. Given its comparatively easy determination, it can be expected that ileal digestibility will be the measure of choice for the foreseeable future. In contrast, Batterham (1992) suggests that AA bioavailability values are the most useful for creating AA availability reference tables, evaluating the effect of processing conditions, and as reference values in the development of new techniques. The MA method becomes the method of choice to determine AA availability (e.g. lysine) if losses during and/or after absorption are suspected and where surgical modification is either unethical, e.g. in humans, or impractical, e.g. pregnancy.

Metabolic availability offers a number of advantages over PDCAAS in humans. Metabolic availability is determined in vivo in humans rather than using the rat as a surrogate for estimation of fecal digestibility. As in pigs, MA does not require estimation of either fecal or ileal endogenous losses. The PDCAAS is expressed relative to a reference pattern of essential AA based on the essential AA requirement of pre-school aged children (Schaafsma, 2005). Protein and AA requirements change with age (Schaafsma, 2005); therefore, PDCAAS does not account for differences in digestibility among age groups. Metabolic availability is non-invasive thus can be used to assess

protein quality in different age groups accounting for differences in AA availability with age.

1.3.5 Threonine

Metabolism of dietary AA includes transport into the systemic circulation, utilization for metabolic processes and catabolism. Threonine (Thr) is a polar, short-chain AA with a hydroxyl group attached to the single carbon of the R-group. Within the systemic circulation Thr is used for the synthesis of lean tissue (Wang et al., 2007) and other systemic proteins, such as plasma γ-globulin (Crumpton, et al., 1963; Tenehouse and Deutsch, 1966). Within the gastrointestinal tract, Thr plays an important role in intestinal barrier function (Law et al., 2007; Wang et al., 2007) due to the high concentration of threonine in mucin and mucous proteins (Lien et al., 2001).Threonine is transported into intestinal epithelial cells from the gut lumen by the Na-dependent transporter, System B (Maenz and Patience, 1992) and is catabolized by 2 enzyme pathways, threonine dehydratase (TDH) and L-threonine 3-dehyrogenase (TDG) into 2-ketobutyric acid or glycine and acetyl-CoA, respectively (Ballevre et al., 1990). In pigs, TDG accounts for 80% of Thr degradation (Ballevre et al., 1990). Threonine is one of only 2 essential AA that are not transaminated thus deamination represents irreversible loss of Thr (Elliott and Neuburger, 1950).

Utilization of nutrients by the gut makes up a considerable portion of the final total body requirement of AA (Schaart et al., 2005). In pigs, about 45% of dietary essential AA are utilized by enterocytes (Stoll et al., 1998); however, the first pass metabolism of Thr can range from 60 to 80% of dietary intake (Schaart et al., 2005) and the major

metabolic fate of Thr utilized by intestinal epithelial cells is for the synthesis of mucosal proteins (van der Schoor et al., 2002). Threonine makes up > 40% of the AA within the protein core of mucin (Carlstedt et al., 1993) and the synthesis of mucin in pigs has been correlated to dietary Thr intake (Bertolo et al., 1998). Although systemic and dietary Thr are incorporated into mucosal proteins, dietary Thr is preferentially used by the portal drained viscera (Schaart et al., 2005).

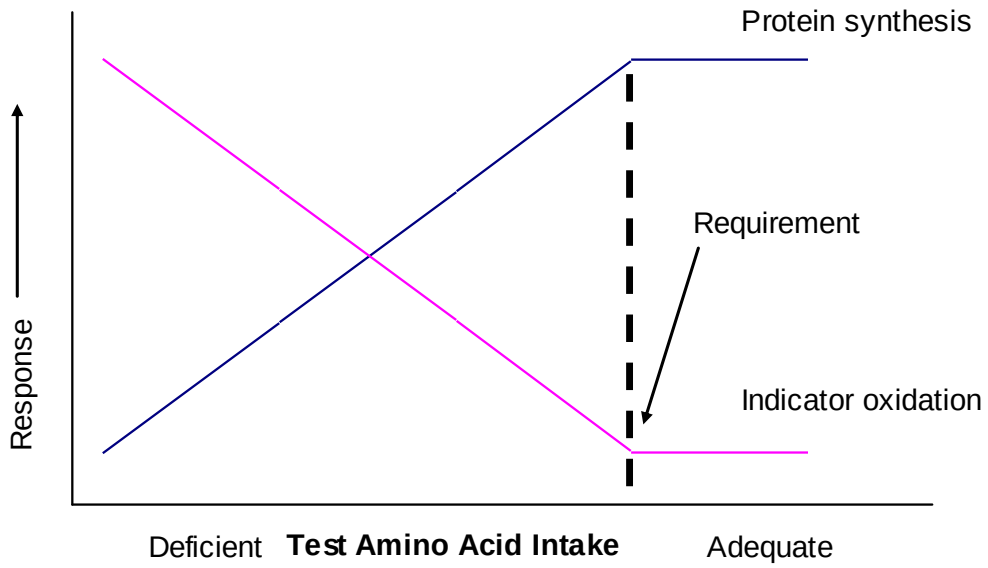
A deficiency in dietary Thr affects body weight gain (Defa et al., 1999; Wang et al., 2006); nitrogen retention (Law et al., 2007) and litter performance (Cooper et al., 2001b) as would be expected of a deficiency in any dietary essential AA in gestation or lactation. However, deficient Thr intake has also been shown to reduce plasma IgG concentration (Cuaron et al., 1984; Defa et al., 1999; Wang et al., 2006), decrease fractional protein synthesis rate of the jejunal mucosa and mucin (Wang et al., 2007), increased paracellular permeability and inflammatory response gene expression (Hamard et al., 2010) and increased liver lipid deposition (Aoyama and Ashida, 1972; Leonard and Speer, 1983). For the gestating sow, the additional effects of a deficient Thr intake may affect her long term health status.

The rate of jejunal mucosal protein synthesis (g/d) and mucin production (%/d) fell 40 to 45% in young pigs fed a Thr deficient diet (Wang et al., 2007). As well, gilts were unable to maintain plasma IgG levels when fed a Thr deficient diet (Cuaron et al., 1984). IgG is the primary immunoglobulin in sow colostrum (Bourne and Curtis, 1973) of which Thr is a major component (Smith and Greene, 1947). In sows, colostral IgG is derived exclusively from the maternal plasma IgG pool rather than de novo synthesis by the mammary gland (Bourne and Curtis, 1973). For the gestating sow, these immunological

consequences of a deficient Thr intake suggest an impaired maternal immune status and may result in increased susceptibility to infection, chronic immune challenge and reduced performance.

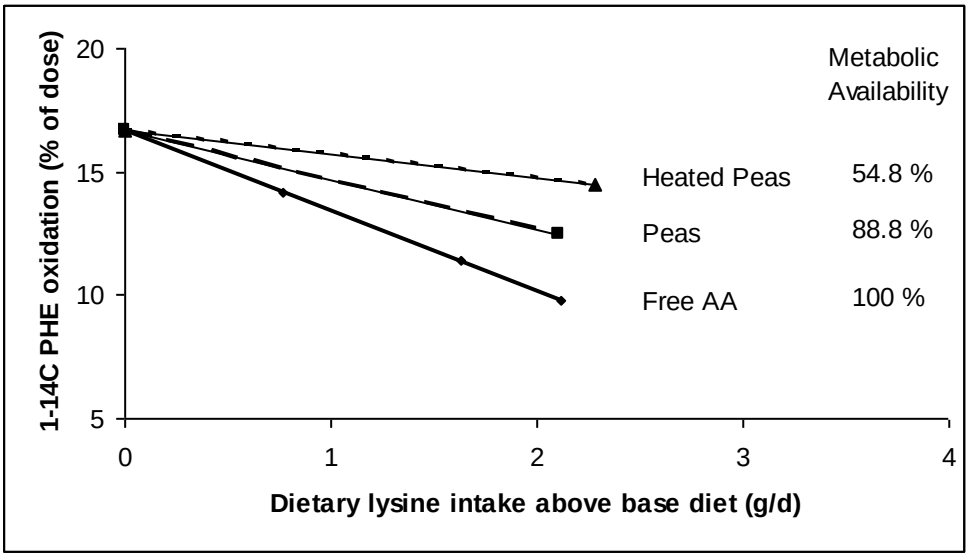
Finally, a dietary Thr deficiency of 50% resulted in a 52% increase in liver lipid deposition (Aoyama and Ashida, 1972). When fed a minimally deficient Thr diet (20% of requirement), total liver protein was reduced with no change in total liver weight in gestating sows (Leonard and Speers, 1983). When total liver weight is similar, a decrease in liver protein is associated with an increase in liver fat (Leonard and Speers, 1983). Greater liver lipid can increase the risk of insulin resistance (Samuel et al., 2004). Sows often become insulin resistant in late gestation as a compensatory mechanism to increase supply of glucose to the gravid uterus (Père et al., 2000); therefore, a deficiency in Thr may exacerbate insulin resistance in sows, particularly in late gestation.

Figure 1.1 Inverse relationship between protein synthesis and oxidation of an indicator amino acid.



Adapted from Ball and Bayley (1986). Oxidation of the indicator AA decreases with increasing intake of the test AA. No further oxidation of the indicator AA occurs once the requirement for the test amino acid has been met. Inversely, at deficient test AA intake, protein synthesis increases with increasing intake of the test AA, followed by no change in protein synthesis at test AA intakes above the requirement. The inflection point represents the AA requirement.

Figure 1.2 Metabolic availability of lysine in peas and heated peas.



Adapted from Moehn et al. (2005). The slope of the response from the addition of free lysine was greater than the slope of the reponse from the addition of protein-bound lysine in peas and heated peas. The metabolic availability (the ratio of the slope from the addition of protein-bound lysine to free lysine) of lysine in peas and heated peas was 88.8 and 54.8%, respectively.

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2.0 Rationale and objectives

2.1 Rationale

Typically, when establishing AA requirements in swine, the lysine requirement is determined first because lysine is commonly first limiting and therefore, AA requirements are often reported as a ratio to lysine (NRC, 1998). However, one of the primary goals of this research project was to evaluate the requirement for AA in early and late gestation independently. We hypothesized that the daily demand for Thr may change to a greater extent from early to late gestation than the requirement for lysine; therefore, the ratio to lysine may be different during early and late gestation. A change in the lysine:Thr ratio will have practical consequences on diet formulation because dietary levels of other essential AA are typically formulated as a ratio to lysine.

As has been previously described, the growth of sows in late gestation shift from maternal body protein gain to mucosal membranes (i.e. intestinal and mammary tissue) which contain a high concentration of Thr in the mucosal protein (Lien et al., 2001). As well, in late gestation, growth rate of the fetal gastrointestinal tract is greater than the rate of fetal BW change (McPherson et al., 2004), thus the requirement for Thr will likely increase to a greater extent in late gestation than lysine. Additionally, the growth of sows in pregnancy leads to an increase in maintenance requirements and the lysine:Thr ratio for maintenance is greater than that for growth or milk production (NRC, 1998). Although the ratios of other essential AA to lysine are also higher in maintenance compared to growth, the increase in essential AA relative to lysine for maintenance is greatest for Thr. Therefore, Thr was most likely to demonstrate the principle of changing

whole body AA requirements in gestation and was chosen as the AA of focus for this research.

Advances in AA requirements must be accompanied by advancements in protein quality evaluation to ensure that AA requirements are being met by the ingested feedstuffs. Mature animals have a greater capacity to digest and absorb AA than growing animals (Noblet and Shi, 1993; Stein et al., 2001); however, in swine production, published digestibility estimates used in diet formulation are based on growing pig data. Thus, under practical conditions using published digestibility estimates, sow diets may be over-formulated for dietary protein and AA. Although the protein source (i.e. soybean meal) makes up only about 15% of the diet (NRC, 1998), it accounts for 35 to 40% of the total diet cost (assuming $450/tonne for soybean meal and $150/tonne for cereal grains; www.canola.ab.ca/price/chart/feedgrains.aspx); therefore, over-formulating dietary protein results in higher sow feed costs.

Determination of standard ileal digestibility estimates requires surgical manipulation for collection of ileal digesta and although the implantation of a simple t-cannula has been successfully applied to pregnant sows the risk of peritoneal infection increases prior to parturition (Stein et al., 1998). Therefore, a new non-invasive method for estimating bioavailability in pregnant sows is necessary.

Selection for higher lean tissue gain and lower age at mature BW may have increased the vulnerability of the modern sow to nutritional deficiencies as evidenced by high sow replacement rates. Improved nutrition during gestation, particularly late gestation, may reduce problems associated with high culling rates such as failure to rebreed or conceive and weak legs, thereby increasing the sow reproductive longevity.

More accurate estimates of AA requirements in gestation along with a direct estimate of the bioavailability of AA in feed ingredients fed to sows will allow formulation of sow diets that more closely meet the changing amino acid demands during pregnancy. In turn this will provide substantial savings in feed costs and potentially increase sow reproductive longevity.

2.2 Specific hypothesis

1. The Thr requirement is significantly greater in late gestation compared to early gestation, measured using IAAO.
2. The IAAO technique can be used to determine the metabolic availability of AA in low protein feed ingredients for sows.
3. The metabolic availability of Thr in feed ingredients fed to adult sows is greater than when fed to growing pigs.
4. Threonine is first limiting in late gestation and the ratio of Lys:essential AA changes in late gestation.

2.3 Objectives

The first objective of this research will be to determine the requirement for Thr in early and late gestation in sows. If differences in Thr exist, this will indicate that current dietary AA recommendations for sows are inappropriate. The second objective will be to evaluate whether the new MA method can be used to evaluate protein quality (i.e. amino acid bioavailability) in low protein feed ingredients. Assuming the MA is applicable to

low protein ingredients and accurately reflects true AA bioavailability, the third objective will be to determine the MA of Thr in cereal grains fed to adult sows. The final objective will be to determine the order of limitation of essential AA and the ideal AA ratio in late gestation.

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3.0 Threonine requirement in early and late gestation in sows

3.1 Introduction

Recommendations for daily amino acid intake during gestation are based on maintenance and growth data from the 1970's and 1980's (NRC, 1998). Genetic selection has increased lean yield 1.5% and litter size by 30% from 1999 to 2006 (CCSI, 2008). These genetic improvements in lean yield and reproductive parameters indicate that the amino acid requirements for sows in gestation are likely greater than current recommendations. Additionally, NRC (1998) recommends a constant value for amino acid requirements during gestation which assumes an equal distribution of nutrient demand throughout gestation. However; the metabolic focus of the sow changes from the recovery of sow body tissue following weaning to the synthesis of fetal tissue in late gestation (McPherson et al., 2004). Fetal weight, fetal protein content and mammary protein content increase 5-, 18- and 27-fold, respectively in the last 45 d of gestation (MacPherson et al., 2004; Ji et al., 2006). These dramatic increases in fetal weight and protein gain indicate that the requirement for amino acids (AA) may also be greater in late gestation compared to early gestation.

The daily demand for threonine (Thr) may change to a greater extent from early to late gestation than the requirement for lysine. Firstly, the growth of sows in pregnancy leads to an increase in maintenance requirements, which are greater for Thr than lysine (NRC, 1998). Secondly, the growth of sows in late gestation shift from maternal body protein gain to mucosal membranes (i.e. intestinal and mammary tissue) with a high concentration of Thr in mucosal protein (Specian and Oliver, 1991). Along with the 27-fold increase in mammary tissue (Ji et al., 2006), the ratio of gastrointestinal tract to total

fetal BW increases 1.6-fold in late gestation (McPherson et al., 2004). Therefore, Thr was most likely to demonstrate the principle of changing whole body AA requirements in gestation. The objective of this study was to determine the requirement for Thr in early and late gestation in sows.

3.2 Materials and methods

Animal care and experimental procedures were the same for each experiment and were approved by the University of Alberta Animal Policy and Welfare Committee.

The study was conducted to determine the response of IAAO, in early (EG) and late (LG) gestation, to increasing levels of dietary Thr, ranging from deficient to excess based on the NRC (1998) recommended Thr requirement of gestating sows (~ 10 g total Thr/d). In Exp. 1, six Large White X Landrace gestating sows (second parity, 172 ± 16 kg BW) were each fed 6 diets of increasing dietary Thr in a 6 X 6 Latin square design. Based on the results of Exp.1 the study was repeated (Exp. 2) where the range of Thr intakes tested in Exp. 2 were adjusted to include lower daily intakes of Thr in EG and higher daily intakes of Thr in LG. In Exp. 2, 8 sows and 6 diets were used in an unbalanced 8 X 6 Latin square design. Four sows from Exp. 1 (fourth parity) plus 4 additional sows (third parity) were used at a mean BW of 211 ± 21 kg.

3.2.1 Experimental diets

The basal diets were based on corn, cornstarch and sucrose (Table 3.1). Corn was included to meet the lowest level of dietary Thr. The inclusion of corn was reduced for

the basal diet in EG in Exp. 2 because dietary Thr was set to meet only 20% of NRC (1998; Table 3.2). Synthetic Thr was used to create diets with incremental increases in dietary Thr of ~ 10% each at the expense of cornstarch and sucrose. All other essential AA were provided at a minimum of 150% (Exp. 1) and 180% (Exp. 2) of their respective NRC (1998) recommended requirement. In Exp. 1, each sow received 3 levels of dietary Thr below (60 to 80%) and 3 levels above (110 to 130%) the NRC (1998) recommendation for second parity sows of similar BW, expected litter size and maternal gain. In Exp. 2, sows received 3 levels of dietary Thr above and 3 below the Thr requirement estimated in Exp. 1 which was ~ 50 and 130% of NRC (1998) in EG and LG, respectively. The vitamin-mineral premix used in Exp. 1 was no longer available when the diets for Exp. 2 were formulated. Separate vitamin and mineral premixes were used for Exp. 2 and the dietary inclusion of NaCl, $NaHCO_3$, KCl and $KHCO_3$ was adjusted to balance dietary electrolytes across all diets.

Dietary phenylalanine (Phe) concentration was kept constant in all diets. To facilitate channeling towards oxidation of any tyrosine synthesized from Phe (Shiman and Gray, 1998) dietary Tyr was set at 180% of requirement. The daily feed allowance was top-dressed with L-Phe equivalent to the amount of Phe provided by the hourly dose of L-[1-^{13}C]Phe. The sows were provided their daily feed allowance in 2 equal daily feedings except on respiration days when half the daily ration was divided into 12 ½-hourly portions based on Moehn et al. (2004; Figure 3.1). Feed allowance was set to achieve the recommended daily energy intake based on BW and backfat at breeding according to Aherne and Foxcroft (2000). Sows were determined to be in a negative energy balance in LG in Exp. 1 thus dietary energy content was increased in LG in Exp. 2 to achieve an

increase in daily energy intake of ~ 6% (Samuel et al., 2007). Cellulose (Solka-Floc®) and canola oil were altered to achieve the desired dietary energy content. All animals were individually housed and acclimated to metabolism pens and the first diet in their rotation for 7 d before the first respiration day. A minimum of 3 d adaptation was used for each successive diet based upon the observations that this was more than adequate adaptation period for respiration experiments using indicator amino acid oxidation (IAAO; Moehn et al., 2004; Elango et al., 2009). Experimental design and statistical analysis were the same for each experiment.

3.2.2 Administration of labeled amino acid

L-[1-^{13}C]Phenylalanine was selected as the indicator AA because the intracellular free Phe pool is tightly regulated (Flaim et al., 1982) and responds rapidly to changes in test AA intake (Neale and Waterlow, 1974). In Exp. 1, sows received an oral dose of 1 mg/(kg BW·h) of L-[1-^{13}C]Phe (99% enrichment, Sigma Aldrich, Mississauga, ON) for 4 h divided into 8 ½-hourly feedings (Figure 3.1). A priming dose equal to 1.75 times the hourly dose was given along with the first ½-h dose. The sows consumed all the feed provided prior to administration of the next ½-h feed allowance. In Exp. 2, L-[1-^{13}C]Phe hourly dose was increased to 2 mg/(kg BW·h) to reduce variation in enrichment during isotopic plateau. In Exp. 1, 44 and 56% of the mean plateau APE values in EG and LG, respectively, had a coefficient of variation > 15%. In Exp. 2, 16 and 8% of the mean plateau APE values in EG and LG, respectively, had a coefficient of variation of > 15%.

3.2.3 Sample collection

Two respiration chambers were used for the study, thus data from 2 sows could be collected each day. Therefore, data were collected from 35 to 53 d and from 92 to 110 d gestation to represent early and late gestation, respectively in Exp. 1. In Exp. 2, the addition of two sows meant that data was collected from 25 to 55 d and from 81 to 111 d gestation to represent early and late gestation, respectively. Prior to initiation of the study, sows were fitted with a surgically implanted Carmeda Bio-Active Surface coated catheter (CBAS® 100 cm catheter, Instech Solomon, Plymouth Meeting, PA) and vascular access titanium injection port (TiSoloPort MAX, Instech Solomon, Plymouth Meeting, PA; Swindle et al., 2005). Sows were allowed a minimum of 21 d recovery time before starting the study.

Changes in oxidation of L-[1-^{13}C]Phe in expired CO_2 was measured in an aliquot of CO_2 from expired air following administration of L-[1-^{13}C]Phe. Two independent airtight respiration chambers (2.0 m^3) were constructed in a temperature-controlled room; each using a standard farrowing crate with a rear door for the sows to enter and exit the chamber and a plexiglass side window. Access to the sows was through a removable piece of plexiglass on top of and near the front of the chamber.

Prior to entering the respiration chambers, the vascular port was accessed using a 22 ga X ¾" SOFTEE right angle Huber needle set (Instech Solomon, Plymouth Meeting, PA) and a 60" extension set (Smiths Medical Canada Ltd., Markham, ON). The extension set was externalized from the chamber to facilitate blood sampling during collection of expired air. Sows were placed in the respiration chambers 30 min before the collection of expired air began to allow the air in the chamber to equilibrate with the ventilating air

stream. Each respiration chamber was fitted with a 10 cm diameter capped PVC tube which allowed feed to be dropped into the feeder and a nipple drinker for *ad libitum* access to fresh water. The chambers were designed with 2 air inlets each consisting of 2.5 cm diameter ABS pipe the length of the chamber with ½ " holes drilled approximately every 30 cm and capped at the opposite end. Ambient air was drawn through the chambers by rotary vane pumps (Gast Model 1023, Gast Manufacturing, Benton Harbor, MI). Air flow was set at 240 L/min to maintain CO_2 concentration below 1.0%.

The CO_2 in ambient air was measured using non-dispersive near infrared analyzers (Qubit Systems, Kingston, ON). Expired air was collected in 30 min intervals into 11 ml 1N NaOH solution. Background $^{13}CO_2$ enrichment was measured for three 30 min periods prior to administration of isotope (Figure 3.1).

3.2.4 Sample analysis

Expired air samples were analyzed for $^{13}CO_2$ according to the following procedure based on El-Khoury et al. (1994). Expired CO_2 which was trapped in 1N NaOH solution in the form of Na_2CO_3 was reacted with H_3PO_4 to release the free CO_2 gas. The $^{13}CO_2$ enrichment in the gas was then measured by a continuous flow, dual inlet isotope ratio mass spectrometer (CF-IRMS 20/20 isotope analyzer, PDZ Europa Ltd, SerCon, Cheshire, UK). Each set of 8 samples (2 baseline, 6 plateau) was separated by reference samples (5% CO_2), which were previously calibrated to an international reference standard (NBS-20; National Institute for Standards and Technology, Gaithersburg, MD). Expired air $^{13}CO_2$ enrichment was calculated as the difference in isotopic abundance at plateau and natural (baseline) abundance and was expressed as atom percent excess

(APE). Plateau $^{13}CO_2$ enrichment value for each sow/diet combination was determined as the data points where the linear regression of enrichment within collection period was not significantly different from zero. This was achieved for all studies within 120 min from the start of isotope administration. The L-[1-^{13}C]Phe oxidation rates were expressed as a percentage of the infused dose. Blood samples (5 ml) were collected in heparinzed vacutainer tubes at 30 min intervals immediately prior to feeding during the 5 ½ h collection period (Figure 3.1). Blood samples were centrifuged at 1500 x g for 15 min for separation of plasma.

Reverse phase HPLC (Waters, Millipore, Mississauga, ON) with the use of phenylisothiocyanate derivatives was used to measure ingredient, diet and plasma AA as previously described (Bidlingmeyer et al., 1984; House et al., 1994). Briefly, 200 g of ingredient or diet were hydrolyzed with 8 ml of 6 *N* HCl, blanketed with nitrogen gas, vortexed and placed at 110ºC for 24 h. Norleucine (0.5 ml of 25 µmol/ml) was added, the hydrolyzate was filtered (Whatman filter paper #1) and a 1 ml aliquot was freeze-dried. The hydrolyzate and AA standards (2.5 µmol/ml, Sigma Diagnostics, St. Loius, MO) were freeze-dried again following addition of 100 µl of 20:60:20 methanol:water:trifluoracetic acid solution. The derivitization reagent (70:10:10:10 methanol:water:trifluoracetic acid:PITC) was added and allowed to derivitize for 35 min. Samples were stored at -20ºC until reconstitution with 200 µl sample dliuent (710 mg anhydrous sodium phosphate in 1 L HPLC water) prior to HPLC analysis. Plasma samples (200 µl) were deproteinated with 1 ml 0.5% trifluoracetic acid (0.67 mol trifluoracetic acid/L methanol). Norleucine (20 µl of 2.5 mol/L) was added as the internal

standard. The protein was removed by centrifugation (3000 x g; 10 min). Amino acid concentrations are expressed as the mean concentration.

3.2.5 Calculations

The L-[1-^{13}C]Phe oxidation rates were calculated according to the equation:

$OX_{Phe} = F^{13}CO_2 / Phe_{inf}$

where Phe_{inf} = rate of L-[1-^{13}C]Phe tracer administered (mmol ^{13}C-Phe/30 min) and $F^{13}CO_2$ = the rate of $^{13}CO_2$ released by Phe tracer oxidation (mmol $^{13}CO_2$/30 min) calculated according to the equation (Matthews et al., 1980):

$F^{13}CO_2 = (FCO_2) (ECO_2) (30) (42.3) / (100) (0.82)$

where FCO_2 = rate of CO_2 production (ml/min), ECO_2 = $^{13}CO_2$ enrichment in expired air at isotopic steady state (APE). The constants 30 min and 42.3 mmol/ml convert FCO_2 to mmol/30 min and the factor 100 changes APE to a fraction. The constant 0.82 accounts for $^{13}CO_2$ retained in the body because of bicarbonate fixation (Moehn et al., 2004).

In Exp. 2, the single pool model was used to study Phe metabolism (Waterlow et al., 1978):

$Q = I + B = S + O$

where Q is the Phe flux (μmol/kg·h^{-1}); I is the rate of Phe intake; B is the rate of Phe released from the body pool; S is the rate of Phe non-oxidative disposal, a measure of the rate of Phe incorporated into body protein; and O is the rate of Phe oxidation.

Plasma Phe kinetics were calculated according to the stochastic model of Matthews et al., (1980). Phe flux (Q) used in the single pool model was calculated from the dilution of

the orally admininistered L-[1-^{13}C]Phe in the body AA pool at isotopic steady state as follows:

$Q = i[(E_i/E_p) - 1]$

where i is the rate of L-[1-^{13}C]Phe administered (μmol/kg·h^{-1}). E_i and E_p are the isotopic enrichments as mole fractions (MPE) of the oral Phe and plasma, respectively at isotopic plateau. The -1 removes the contribution of the administered isotope to the flux.

The formula by Brouwer (1965) was used to calculate heat production from indirect calorimetry:

Heat production = $1.44*[(16.18*V_{O2}) + (5.02*V_{CO2}) - (2.17*V_{CH4})]$

where V_{O2}, V_{CO2} and V_{CH4} are the volumes (L) of O_2, CO_2 and CH_4 produced, respectively. The formula was abbreviated by omitting the urinary nitrogen. The effect of ignoring the urinary nitrogen (i.e. protein metabolism) is 1% for every 12.3% of the total energy that was derived from protein (Weir, 1949).

3.2.6 Statistical analysis

Because daily feed intake was not equal between sows, daily Thr intake was expressed as g Thr/d rather than dietary Thr percent. Data was initially analyzed using linear, quadratic and cubic regression to determine the model of best fit. Non-linear regression analysis was conducted on data where the model of best fit was quadratic or cubic. The NLIN procedure in SAS (SAS Inst. Inc. 2002, Cary, NC) was used to determine the broken-line regression model of best fit (i.e. broken line, quadratic broken line or 2 slope broken line) based on fit statistics and gradient values for each parameter (Robbins et al., 2006). Based on the broken-line model of best fit, the NLMixed

procedure was used to establish the Thr requirement in early and late gestation to account for the random effect of sow (Robbins et al., 2006). The method described by Fadel (2004) was used to determine the starting parameter estimates for the NLMixed analysis.

Data were tested for outliers using the REG procedure (SAS Inst. Inc., 2002, Cary, NC). Correlation analysis, using the CORR procedure (SAS Inst. Inc., 2002, Cary, NC), was used to identify covariables affecting the dependent variable response (i.e. L-[1-^{13}C]Phe oxidation, plasma AA) irrespective of the effect of dietary Thr level. Sow BW, maternal gestation weight gain, parity (Exp. 2), litter weight, litter size and room temperature were tested as potential covariables. Stepwise regression was used to ensure all covariables identified entered the model at no less than $P = 0.10$. In Exp. 1, room temperature was included in the model for L-[1-^{13}C]Phe oxidation for the EG analysis only; room temperature varied during this period because of mechanical problems. In both early and late gestation, BW was included in the model for plasma Thr. No other covariables reached significance. The difference in Thr requirement between early and late gestation was evaluated by a paired *t*-test. The effect of parity was tested in Exp. 2. For all analyses, $P < 0.05$ and $P < 0.10$ were considered significant or tendency, respectively.

3.3 Results

Maternal weight gain was 42.2 ± 7.8 kg, 42.3 ± 2.9 kg and 32.1 ± 1.8 kg in the second, third and fourth parity sows, respectively. Table 3.3 shows reproductive parameters, daily energy intake, heat production and BW gain for both experiments. In Exp. 1, average litter size was 12.6 but ranged from 8 to 18 and the litter weight was 19.3

± 1.0 kg. In Exp. 2, average litter size was 14.0 but ranged from 9 to 17 and the litter weight was 21.3 ± 1.0 kg. Energy intake was greatest in LG in Exp. 2 ($P < 0.01$) and heat production tended to be lowest in EG in Exp. 1 ($P = 0.06$). The increase in energy intake in LG in Exp. 2 appeared to prevent the negative energy balance observed in LG in Exp. 1 where heat production was larger than energy intake.

Plateau in enrichment of expired air was achieved within 120 min of initial isotope administration. Within each sow/diet combination, the APE was not different from collection periods 5 to 8 and hence used to determine a single enrichment value for calculation of % dose oxidized (data not shown).

3.3.1 Experiment 1

Oxidation of excess essential AA, as represented by oxidation of L-[1-^{13}C]Phe, was reduced as daily Thr intake approached requirement (Figure 3.2). There was no further change in oxidation of L-[1-^{13}C]Phe with increased Thr intake after the daily requirement for Thr was achieved as defined by the breakpoint. Breakpoint analysis determined a daily Thr requirement of 6.1 g/d in EG ($R^2 = 0.59$) and 13.6 g/d in LG ($R^2 = 0.60$) based on indicator oxidation (Figure 3.2A&B).

Plasma Thr concentration remained constant then increased rapidly as Thr intake increased above requirement in EG. A breakpoint was determined at 7.0 g/d Thr intake ($R^2 = 0.90$, Figure 3.2C). In LG, plasma Thr concentration increased linearly with increasing daily Thr intake ($R^2 = 0.83$) and did not demonstrate a breakpoint (Figure 3.2D). Plasma Phe was not different across Thr intakes in EG or LG. Plasma concentration of other essential AA were not affected by dietary Thr level (data not

shown). The requirement for Thr based on IAAO tended to be greater in LG compared to EG ($P = 0.06$).

3.3.2 Experiment 2

Similar to the Expt. 1, oxidation of excess L-[1-^{13}C]Phe, was reduced as daily Thr intake approached requirement as defined by the breakpoint (Figure 3.3). A breakpoint analysis determined a daily Thr requirement of 5.0 g/d in EG ($R^2 = 0.71$) and 12.3 g/d in LG ($R^2 = 0.58$) based on indicator oxidation (Figure 3.3A&B). No further reduction in L-[1-^{13}C]Phe oxidation occurred at dietary Thr intakes above requirement.

In both EG and LG, plasma Thr concentrations remained constant followed by a large increase at Thr intakes above requirement. Breakpoint analysis determined a breakpoint at 3.9 g/d ($R^2 = 0.93$) in EG and 10.5 ± 2.8 g/d ($R^2 = 0.67$) in LG (Figure 3.3C&D). Plasma Phe was not different across Thr intakes in EG or LG. There was no effect of dietary Thr intake on plasma levels of other essential AA (data not shown). There was no effect of parity on Thr requirement. The requirement for Thr in LG based on IAAO was greater than the Thr requirement in EG ($P < 0.01$).

Protein turnover kinetics in EG and LG are shown in Table 3.4 and Table 3.5, respectively. Absolute rates of Phe flux, body protein breakdown and protein synthesis (μmol/kg·h) were not affected by changes in Thr intake in EG. Absolute rate of Phe oxidation and Phe oxidation as a proportion of flux was lower at daily Thr intakes above 9.7 g/d compared to Thr intakes at or below 3.0 g/d ($P < 0.05$). A breakpoint was determined at 5.3 and 4.8 g/d, respectively. Protein synthesis as a proportion of flux

increased as Thr intake increased ($P = 0.04$). A breakpoint was determined at 4.8 ± 1.2 g Thr/d ($R^2 = 0.49$).

In LG, Phe flux was highest at the lowest Thr intake (5.8 g/d) but only significantly higher than the measured Phe flux at 15.51 g/d Thr intake ($P < 0.05$). Phenylalanine from body protein breakdown and Phe oxidation was highest at the lowest Thr intake compared to Thr intakes above 15.51 g/d ($P < 0.05$). There was no effect of Thr intake on non-oxidative Phe disposal. Based on protein breakdown and Phe oxidation, a requirement for Thr in LG was estimated to 11.6 ± 4.6 ($R^2 = 0.42$) and 12.3 ± 4.5 ($R^2 = 0.62$) g Thr/d, respectively. Similar trends were observed when protein synthesis and Phe oxidation were expressed as a proportion of flux, where a Thr requirement was estimated to be 12.6 ± 5.0 ($R^2 = 0.12$) and 12.3 ± 4.5 ($R^2 = 0.49$) g Thr/d, respectively.

3.4 Discussion

Based on IAAO, the Thr requirement of gestating sows was in the range of 5.0 to 6.0 g/d in EG and in the range of 12.3 to 13.6 g/d in LG. The greater BW gain in LG compared to EG likely played a role in the greater requirement for Thr in LG compared to EG. The sows were in a negative energy balance in LG in Exp. 1 which may have affected the response to increasing daily Thr intake. The greater daily energy intake in LG in Exp. 2 prevented the negative energy balance observed in Exp. 1; although there was only a minor numerical difference between the estimated Thr requirements in LG in Exp. 1 and 2. The constant plasma Phe concentration observed in the current study at all daily Thr intakes indicated that in the presence of deficient dietary Thr, all excess Phe was oxidized and the isotopic label expired as $^{13}CO_2$. There was no change in the other

plasma free essential AA (except Thr) in response to variations in Thr intake. Zhao et al. (1986) also found no change in plasma concentration of other free AA with increasing dietary Thr intake in adult men.

In Exp. 2, the Thr requirement based on plasma AA was lower than the requirement based on IAAO; whereas in Exp.1, the Thr requirement in EG based on plasma Thr was higher than when based on IAAO. As well, in Exp. 1, the Thr requirement could not be determined in LG due to the linear increase in plasma Thr with increasing Thr intake. Plasma AA have been shown to not correlate to other estimates of requirement or to respond linearly to all treatments (Mitchell et al., 1968; Sohail et al., 1978; Pampuch et al., 2006) and thus are less reliable dependent variables when estimation AA requirements. The quantitative response of plasma AA to changes in dietary AA reflects the complex interaction of the dynamic equilibrium of the plasma AA pool with the tissue AA pool, protein degradation and labile non-protein AA sources (Young and Scrimshaw, 1970). The linear decrease in plasma Thr below requirement observed in LG in Exp. 1 may reflect a conservation of the plasma free Thr pool in response to the increasing fetal demand for nutrients in LG or the increased contribution from protein turnover that occurs with increased protein synthesis in LG.

The Thr requirement based on protein turnover parameters supported the requirement based on IAAO. Wykes et al. (1992) suggest that protein turnover kinetics calculated from a single tracer should be interpreted with caution because the calculation of AA flux is influenced by the route of feeding (oral vs parenteral), route of tracer administration (oral vs parenteral) and AA intake and the impact of each factor is dependent on the tracer AA. Phenylalanine is a good indicator AA for the IAAO technique, as discussed

previously; however, Phe is not a good indicator for estimation of protein turnover kinetics. Protein turnover kinetics requires estimation of the rate of protein breakdown. Plasma enrichment of the tracer ketoacid (i.e. alpha-ketoisocaproate from leucine breakdown) provides an estimate of the rate at which the tracer AA is released from protein (Wolfe and Chinkes, 2005). Phenylalanine does not have a ketoacid thus the rate of appearance of Phe into the plasma pool will underestimate the total rate of protein breakdown because some of the Phe released from protein breakdown will be reincorporated into protein without entering the plasma pool (Wolfe and Chinkes, 2005). However, in the context of the current study, the relationship between the calculated rates of protein turnover with changing dietary Thr, rather than the absolute rates, was of primary interest. Thus protein turnover kinetics could be used to estimate the Thr requirement.

The requirement for Thr was up to 40% lower (~ 6 g/d vs 10 g/d) from 35 to 53 d gestation and up to 30% higher (~ 13 g/d vs 10 g/d) from 92 to 110 d gestation compared to the NRC (1998) recommendation for sows of similar BW, expected growth in gestation and litter size. As this large discrepancy to the NRC (1998) recommendation was not expected, lower and upper limits of Thr intake in Exp. 1 were only marginally below and above the determined requirements. However, the results of Exp. 2, obtained with a wider range of Thr intakes, supported the results of Exp. 1, confirming the 2-fold increase in the Thr requirement in LG compared to EG. This is in contrast to Dourmad and Étienne (2002) who did not find an effect of N-balance periods in gestation on lysine and Thr requirements. It is; however, in agreement with recent results (Srichana, 2006; Samuel et al., 2010) showing that the lysine requirement of sows in early and mid

gestation (30 to 80 d) was lower than in late gestation (90 to 100 d). This experimental evidence is in agreement with recently published recommendations for sow feeding (GfE, 2008; Kim et al., 2009) that suggest increased AA requirements in LG compared to EG based on models of gestational growth.

Preliminary results from our group (Samuel et al., 2010) using the same pig genetics as the current study suggest that the requirement for Lys in early gestation is 13.1 and 8.2 g/d in second and third parity sows, respectively. The Lys requirement in late gestation increased to 18.7 and 13.0 g/d in second and third parity sows, respectively. Calculating a Lys:Thr ratio for early and late gestation using the single NRC (1998) Lys requirement for early and late gestation and separate Thr requirements for early and late gestation based on the current data would be inappropriate. However, using the preliminary results of Samuel et al. (2010) and the current study, the Lys:Thr ratio for second parity sows would be 1:0.47 and 1:0.72 in early and late gestation, respectively and 1:0.61 and 1:0.95 for third parity sows in early and late gestation, respectively. This implies that lean growth of the sow plays a lesser role in the requirement for AA as sow age increases. The greater Thr relative to Lys with increasing parity is in agreement with GfE (2008); although the difference in Thr requirement within parity is not (parity 2: 1:0.70 and 1:0.66 in EG and LG, respectively; parity 3: 1:0.76 and 1:0.68 in EG and LG, respectively).

The GfE (2008) suggested similar Thr requirements for second parity sows in EG (6.6 g/d on a true ileal digestible basis), and LG (9.6 g/d, true ileal digestible basis) compared to the results of the current study. Conversely, Kim et al. (2009) suggested substantially lower Thr requirements for early and late gestation of approximately 3.3 and 6.1 g/d,

respectively on a true ileal digestible basis. Both sets of requirement recommendations (GfE, 2008; Kim et al., 2009) were calculated from the estimated growth of maternal body and conceptus products. However, GfE (2008) included an estimate of efficiency of AA utilization, which was not mentioned by Kim et al. (2009).

The absence of an effect of parity on Thr requirement in Exp. 2 is in contrast to GfE (2008) who suggest decreasing requirements with increasing parity, especially in early gestation. Kim et al. (2009), too, suggest split parity feeding, although they donot provide data concerning the change in requirements. The absence of an effect of parity may be caused by the substantial maternal gestation gain in fourth parity sows as well as the low number of sows per parity (n = 4); however, the use of repeated measures within each sow (each sow received each of the 6 test diets) increased the number of observation per sow, reducing the inter-animal variation (Bertolo et al., 2005). The current results confirm the recent recommendations of GfE (2008) and Kim et al. (2009) in that Thr requirements increase from early to late gestation. However, the present results gave no indication for a decrease in requirements with increasing parity number.

The current study has a number of advantages over other similar studies examining AA requirement of sows during gestation. First, the IAAO method requires only 2 d adaption to test AA levels (Moehn et al., 2004) so that several levels of test AA can be studied in a short time period within the same animal. This reduces the effect of the between-subject variation which is approximately 10% in pigs (Bertolo et al., 2005; Moehn et al., 2008). Furthermore, the IAAO method determines parameters specific to AA metabolism being based on the principle that AA are utilized for protein synthesis and any excess AA must be oxidized (Pencharz and Ball, 2003). As the intake of the

limiting AA increases, the oxidation of the indicator AA decreases linearly until the requirement for the limiting AA is reached. Increases of the test AA beyond the requirement do not increase protein synthesis further, thus oxidation of the indicator AA reaches a plateau. The breakpoint is defined as the requirement for the test AA. The IAAO technique has been extensively developed to determine AA requirements in humans (Elango et al., 2007), chickens (Coleman et al., 2003), piglets (Ball and Bayley, 1984) and growing pigs (Moehn et al., 2008) and was chosen as the gold standard by the World Health Organization for determination of AA requirements of humans (WHO, 2007). Secondly, the requirement was separated into 2 periods, 25 to 55 d to represent early gestation and 81 to 111 d to represent late gestation. The summative results of McPherson et al. (2004), Ji et al (2006) and Kim et al. (2009) clearly indicate that there is an increase in protein accretion from early to late gestation. The current results have further identified the change in daily AA intake required to support the increased protein accretion rate and reduce catabolism of maternal lean tissue in late gestation. Thirdly, the current study utilized 6 levels of dietary Thr. Although 4 levels of dietary nutrient are minimally sufficient, 6 or more allow better fit of the data to a descriptive response curve and thus aids in the objective assessment of requirement (Baker, 1986). Lastly, the greatest animal-to-animal variability occurs in the upper curvilinear area of a growth curve (Baker, 1986), the area also associated with definition of the requirement. Using the IAAO method, the animal-to-animal variation was reduced because each sow received each dietary treatment.

The Thr requirement increased 2-fold in the last third of gestation in multiparous sows (5.0 vs. 12.3 g/d). Therefore, feeding a single level of AA, as recommended by

NRC (1998), throughout gestation results in overfeeding AA in early gestation and underfeeding AA in late gestation. Overfeeding AA will increase feed costs and potentially increase environmental contamination through excretion of excess nitrogen **(**Adeola, 1999**)** whereas underfeeding AA during pregnancy results in breakdown of maternal tissue to support fetal growth and milk production in the following lactation (Clowes et al., 2003). The loss of maternal protein must be reestablished during the subsequent rebreeding and early gestation period. Sow reproductive longevity may be reduced by this continual cycle of protein catabolism and synthesis. Therefore, phase feeding has the potential to improve sow reproductive performance. To develop an appropriate phase feeding program for gestating sows, the requirement for essential AA and the lysine:AA ratios must be established in early and late gestation separately.

Table 3.1 Composition of base diets fed to sows in early (25 to 55 d) and late (81 to 111 d) gestation, as-fed basis[1]

	Exp. 1	Exp. 2	
Item	Early/Late	Early	Late
Ingredients, %			
Corn	70.40	22.80	68.50
Cornstarch	8.56	23.81	8.15
Sucrose	8.56	23.81	8.15
Solka-Floc®[2]	3.00	13.00	-
Canola oil	-	1.00	3.00
Celite	1.00	1.00	1.00
L-Histidine	0.07	0.14	0.12
L-Isoleucine	0.24	0.28	0.30
L-Leucine	-	0.24	-
L-Lysine·HCl	0.79	0.71	0.96
DL-Methionine	0.07	0.11	0.10
L-Cysteine·HCl	0.17	0.28	0.31
L-Phenylalanine	0.10	0.24	0.18
L-Tyrosine	0.13	0.17	0.17
L-Tryptophan	0.10	0.11	0.13
L-Valine	0.23	0.32	0.33
L-Glutamic acid	2.00	6.30	3.00
Vitamin-mineral premix[3]	3.80	-	-

Mineral premix[4]	-	0.50	0.50
Vitamin premix[5]	-	0.70	0.70
Choline chloride[6]	0.10	0.20	0.20
Dicalcium phosphate	0.50	2.40	2.40
NaCl	0.16	0.42	0.10
$KHCO_3$	0.02	-	0.10
Limestone	-	1.10	1.10
KCl	-	0.35	-
$NaHCO_3$	-	-	0.50

[1]Daily feed intake was set to achieve recommended intake based on sow BW and backfat at breeding (Aherne and Foxcroft, 2000). Feed intake was 2.4 ± 0.1 kg.
[2]International Fiber Corporation, Urbana, IL
[3]Provided per kg diet: 8.17 g of Ca; 3.29 g of P; 1.82 g of Na; 0.38 g of Mg; 22.61 mg of Cu; 267 mg of Fe; 60.8 mg of Mn; 0.28 mg of Se; 132 mg of Zn; 0.38 mg of I; 0.23 mg of biotin; 6.65 mg of riboflavin; 33.4 µg of vitamin B-12; 11,400 IU of vitamin A; 58.9 IU of vitamin E; 1.330 IU of vitamin D_3; 1.56 mg of vitamin K; 494 mg of choline; 3.04 mg of folacin; 36.1 mg of niacin; 23.8 mg of pantothenic acid (Consultant Feeds, Calmar, Alberta, Canada**)**.
[4]provided per kg diet: 50 mg of Cu; 75 mg of Fe; 25 mg of Mn; 0.30 mg of Se; 125 mg of Zn; 0.50 mg of I (DSM Nutritional Products, High River, Alberta, Canada).
[5]Provided per kg diet: 10,500 IU of vitamin A; 1,050 IU of vitamin D_3; 7,010 IU of Vitamin E; 5.6 mg of vitamin K; 3.5 mg of thiamin; 7.0 mg of riboflavin; 2.1 mg of pyridoxine; 2.1 µg of vitamin B12; 52.5 mg of niacin; 21.0 mg of pantothenic acid; 3.5 mg of folic acid; 0.35 mg of biotin (DSM Nutritional Products, High River, Alberta, Canada).
[6]Provided per kg diet: 731 mg of choline chloride.

Table 3.2 Nutrient content of base diets fed to sows in early (25 to 55 d) and late (81 to 111 d) gestation, as-fed basis[1]

	Exp. 1	Exp. 2	
Item	Early/Late[2]	Early	Late
Analyzed, g/kg			
Threonine	1.80	0.92	2.61
Lysine	8.10	6.21	9.42
Phenylalanine	4.51	4.13	4.33
Tyrosine	4.55	3.41	4.43
Calculated, %			
Calcium	0.97	0.97	0.97
Crude protein	9.66	8.14	10.67
Available phosphorus	0.45	0.45	0.45
ME[3] (MJ/kg)	13.82	13.96	14.81

[1]Analyses are described in Section 3.2.3. The same base diet was used for early and late gestation in Exp. 1. Dietary energy was increased in the base diet in late gestation in Exp. 2.
[2]The same basal diet was used for early and late gestation.
[3]Based on ME content of ingredients as stated by NRC (1998).

Table 3.3 Sow reproductive performance, heat production and BW gain in early and late gestation[1]

Item	Exp. 1			Exp. 2		
	Early[2]	Late	Pooled SEM	Early	Late	Pooled SEM
Energy intake[3], MJ/d	32.9	32.9	1.3	33.7	35.6	0.6
Heat production, MJ/d	32.0	35.3	1.3	34.6	36.2	1.1
BW gain, kg/d	0.18[a]	1.03[b]	0.06	0.33[a]	0.63[b]	0.06
Feed intake, kg/d	2.4		0.1	2.4		0.04
Litter size[4]	12.6		1.7	14.0		0.9
Litter wt[4], kg	19.3		1.0	21.3		1.0

[a,b,c]Within a row and within experiment, means without a common superscript differ ($P < 0.05$).
[1]Data are means, n = 6 in Exp. 1 and n = 8 in Exp. 2. Data were analyzed separately for Exp. 1 and 2.
[2]Early and late gestation was 35 to 53 and 92 to 110 d, respectively in Exp. 1 and 25 to 55 and 81 to 111 d, respectively in Exp. 2.
[3]Dietary energy concentration was increased in late gestation in Exp. 2.
[4]One sow died at farrowing in Exp. 1 thus n = 5.

Table 3.4 Effect of daily threonine intake on protein turnover based on ^{13}C phenylalanine kinetics in early gestation in Exp. 2 (25 to 55 d)[1]

							Pooled			
Thr intake[2], g/d	1.95	2.95	3.92	7.85	9.82	11.85	SEM	Breakpoint[3]	se	R^2
Protein turnover										
μmol/kg·h										
Flux	75.8	72.3	80.9	69.8	69.5	74.0	5.7			
Breakdown	64.1	61.0	69.2	58.1	58.0	62.6	6.0			
Synthesis (non-oxidative disposal)	55.5	53.8	63.4	54.2	55.8	60.5	6.5			
Oxidation	19.7[a]	18.6[a]	16.7	16.0	14.1[b]	14.2[b]	1.3	5.3	1.3	.42
% of flux										
Breakdown	0.84	0.84	0.85	0.84	0.83	0.84	0.01			
Synthesis	0.73[a]	0.74[a]	0.78	0.77	0.80[b]	0.81[b]	0.02	4.8	1.2	.49
Oxidation	0.27[a]	0.26[a]	0.22	0.23	0.20[b]	0.19[b]	0.01	4.8	1.2	.49

[1]Data are means, n = 6. Means within a row without a common superscript differ: [a,b] $P < 0.05$.
[2]Pooled SEM for Thr intake was 0.13 g/d.
[3]Breakpoint represents daily Thr requirement based on non-linear regression.

Table 3.5 Effect of daily threonine intake on protein turnover on ^{13}C phenylalanine kinetics in late gestation in Exp. 2 (81 to 111 d)[1]

Thr intake[2], g/d	5.80	7.77	9.70	13.56	15.51	17.45	Pooled SEM	Breakpoint[3]	se	R^2
Protein turnover										
µmol/kg·h										
Flux	72.0[a]	67.5[a,b]	63.8[a,b]	65.1[a,b]	61.6[b]	65.8	4.0			
Breakdown	60.1[a,x]	55.4	52.0	52.9	49.9[b]	54.1[y]	4.0	11.6	4.6	.42
Synthesis (non-oxidative disposal	56.6	54.4	51.0	53.0	51.1	54.5	3.7			
Oxidation	15.4[a]	13.2[a]	12.8	12.1	10.4[b]	11.2[b]	1.3	12.3	4.5	.62
% of flux										
Breakdown	0.83[a]	0.82	0.81	0.80[b]	0.81	0.82	0.01			
Synthesis	0.79[x]	0.80	0.80	0.82	0.83[y]	0.83[y]	0.02	12.6	5.0	.12
Oxidation	0.21[x]	0.20	0.20	0.18	0.17[y]	0.17[y]	0.02	12.3	4.5	.49

[1]Data are means, n = 6. Means within a row without a common superscript differ: [a,b] $P < 0.05$, [x,y] $P < 0.10$.
[2]Pooled SEM for Thr intake was 0.26 g/d.
[3]Breakpoint represents daily Thr requirement based on non-linear regression.

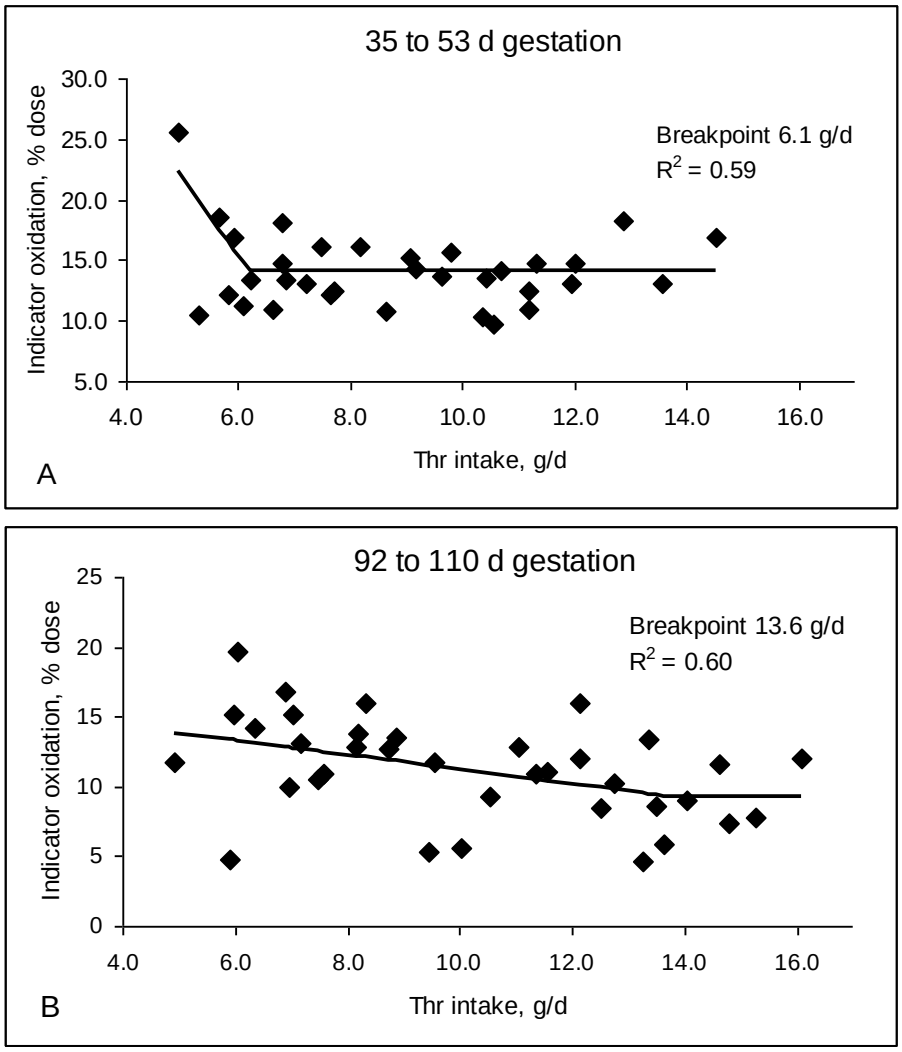
Figure 3.1 Protocol for administration of L-[1-^{13}C] phenylalanine and collection of expired air and blood for determination of $^{13}CO_2$ enrichment and plasma threonine, respectively.

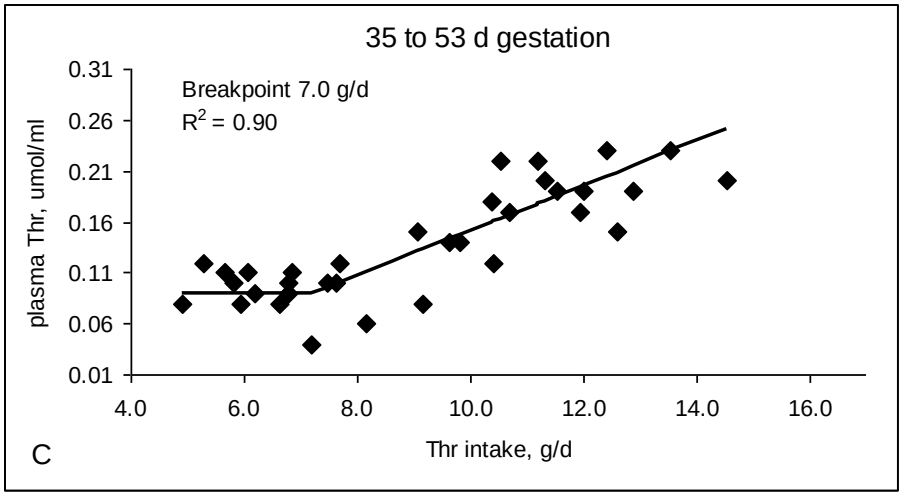
Time (min)	-30	0	30	60	90	120	150	180	210	240	270	300
Period		-3	-2	-1	1	2	3	4	5	6	7	8
Activity												
Feed[1]	*	*	*	*	*	*	*	*	*	*	*	*
Isotope administration[2]					*	*	*	*	*	*	*	*
Collection of expired air[3]		*	*	*	*	*	*	*	*	*	*	*
Blood sampling[4]		*	*	*	*	*	*	*	*	*	*	*

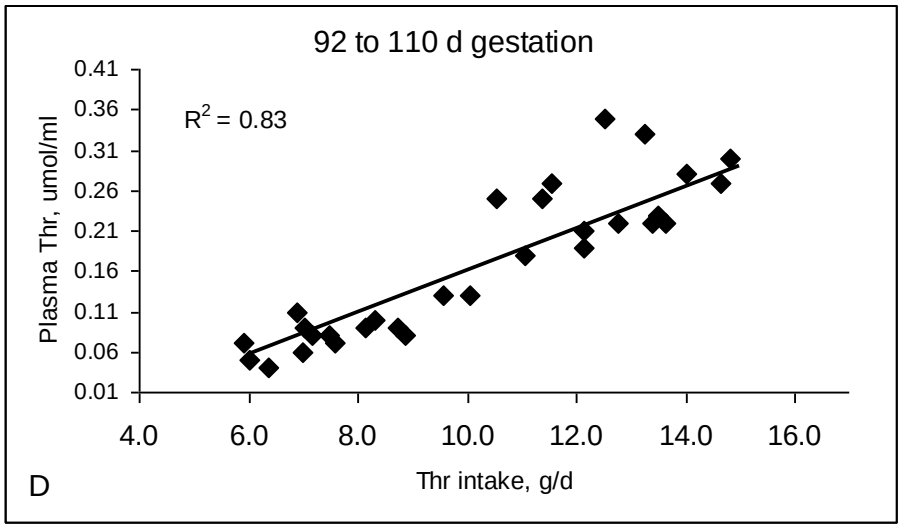
[1]Half the daily ration was divided into 12 ½-hourly portions based on Moehn et al. (2004a).
[2]Isotope was administered orally with the feed. The hourly dose [1 and 2 mg/(kg BW·h) L-[1-^{13}C]Phe in Exp. 1 and 2, respectively; Sigma Aldrich, Mississauga, ON] was divided into 8 ½-hourly doses. Sows received a priming dose of 1.75 X hourly dose along with the first ½-h dose in period 1.
[3]Expired air was collected over each 30 min interval into 11 ml 1N NaOH.

[4]Blood was drawn immediately following feeding via a surgically implanted vascular access port (TiSolo Port MAX, Instech Solomon, Plymouth Meeting, PA) and heparin-coated catheter (Instech Solomon, Plymouth Meeting, PA) into heparinized vacutainers tubes.

Figure 3.2 The response of sows to increasing levels of dietary threonine intake based on L-[1-^{13}C] phenylalanine oxidation (A: early gestation; B: late gestation) and plasma threonine (C: early gestation; D: late gestation) in Exp. 1.

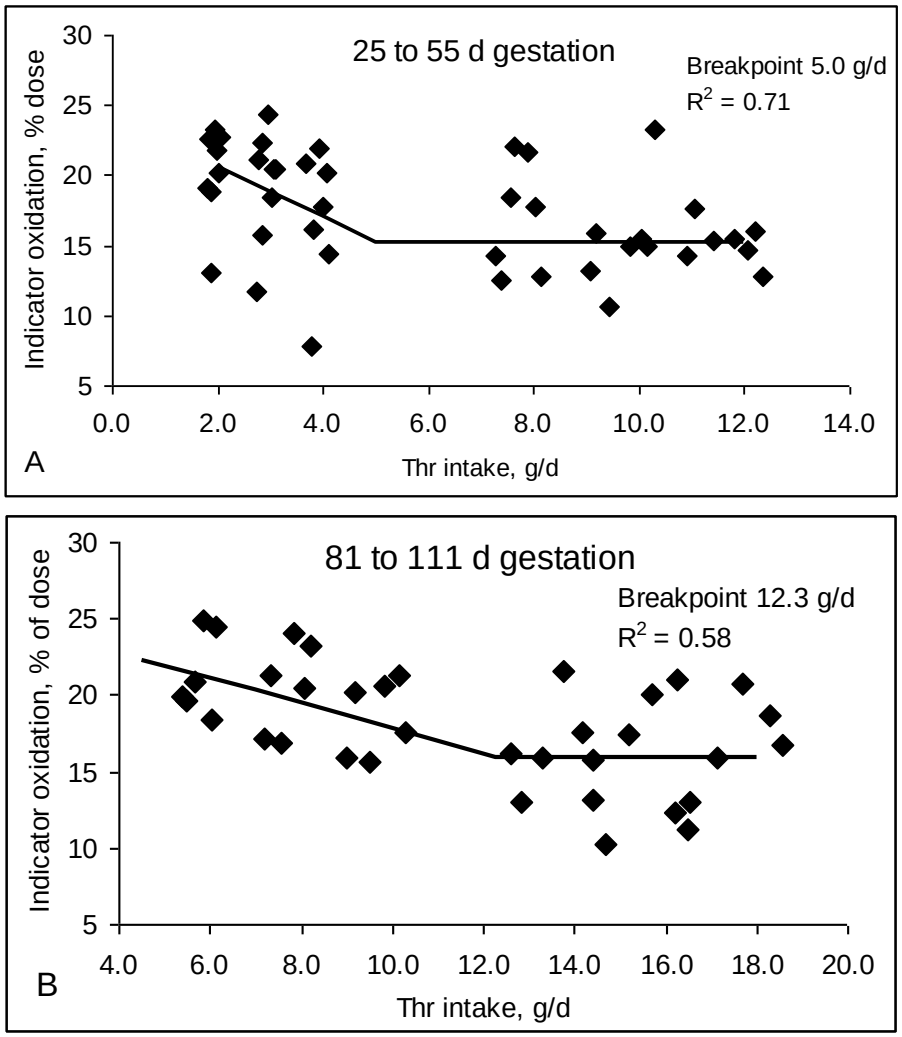


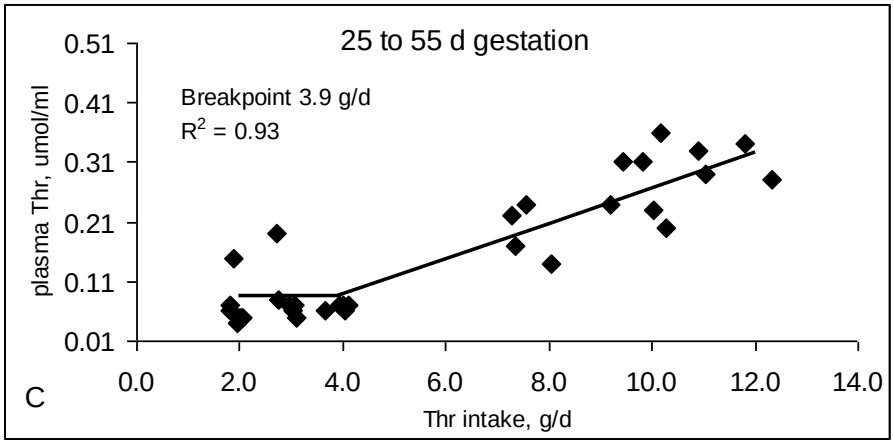


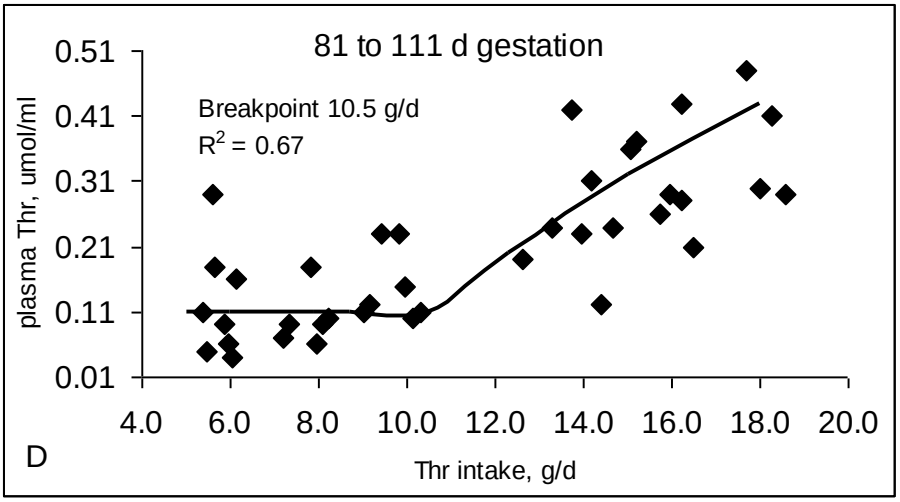


The breakpoint, as determined by broken-line non-linear regression, represents the daily Thr requirement. The Thr requirement was 6.1 g/d (R^2 = 0.59) and 13.6 g/d (R^2 = 0.60) in early and late gestation, respectively, based on indicator oxidation. Based on plasma Thr, the Thr requirement was 7.0 g/d (R^2 = 0.90) in early gestation. The response to plasma Thr in late gestation was linear (R^2 = 0.83) thus no breakpoint was determined.

Figure 3.3 The response of sows to increasing levels of dietary threonine intake based on L-[1-^{13}C] phenylalanine oxidation (A: early gestation; B: late gestation) and plasma threonine (C: early gestation; D: late gestation) in Exp. 2.







The breakpoint, as determined by broken-line non-linear regression, represents the daily Thr requirement. The Thr requirement was 5.0 g/d (R^2 = 0.71) and 12.3 g/d (R^2 = 0.58) in early and late gestation, respectively, based on indicator oxidation. The Thr requirement was 3.9 g/d (R^2 = 0.93) and 10.5 g/d (R^2 = 0.67) in early and late gestation, respectively, based on plasma Thr.

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4.0 The metabolic availability of threonine in corn and barley fed to growing pigs

4.1 Introduction

The development of stable isotope-based methods to assess protein quality of foods provides an opportunity to significantly advance protein quality evaluation in humans (Elango et al., 2009). The metabolic availability (MA) method based on indicator amino acid oxidation (IAAO) was first developed in pigs (Moehn et al., 2005) and adapted for use in humans (Humayun et al., 2007). Metabolic availability reflects the proportion of dietary amino acids (AA) used for whole body protein synthesis and includes all AA losses that occur during digestion, absorption and metabolic utilization and thus can account for the effect of heat processing or anti-nutritional factors on availability (Moehn et al., 2005). For example, the MA of lysine in heated peas was 34 % lower than the MA of lysine in raw peas (Moehn et al., 2005).

The MA of lysine in a variety of high protein ingredients fed to pigs (Moehn et al, 2005; 2007) were similar to published estimates of standard ileal digestibility values (NRC, 1998). The MA of total sulfur AA in casein and soy protein isolate in humans was also similar to published net protein utilization estimates (Humayun et al., 2007) suggesting that MA is an appropriate method for determining the availability of AA in high protein ingredients.

Cereal grains contain up to 30% less crude protein than that contained in high protein ingredients such as soybean meal, peas or canola meal (NRC, 1998) and thus are more sensitive to corrections for endogenous AA losses in the calculation of standard ileal AA digestibility (Stein et al., 2007). Dietary fiber can alter endogenous AA losses through an

increase in mucin production (Myrie, 2004) or through an increase in digestive enzyme secretion (Zebrowska et al., 1983). The high content of threonine (Thr) in gastrointestinal mucus (Lien et al., 2001) means that the digestibility of Thr is particularly sensitive to endogenous AA corrections. The MA method does not rely on an estimate of endogenous AA losses and thus should provide a more accurate measure of AA bioavailability for low protein sources such as cereal grains than ileal digestibility.

This study was conducted to determine the MA of Thr in corn and barley. They are considered to be low protein feed ingredients for pigs (NRC, 1998).

4.2 Materials and methods

Animal care and experimental procedures were the same for both experiments and were approved by the University of Alberta Animal Policy and Welfare Committee. Twelve Large White X Landrace pigs (21.8 ± 0.7 kg BW) were used to determine the MA of Thr in corn and barley relative to a casein-based reference diet (6 pigs per replicate). The experimental design was a standard curve assay as described by Littell et al. (1995) where a standard curve was established by feeding pigs increasing levels of dietary crystalline L-Thr (Ajinomoto, Japan; Thr assumed to be 100% available) and measuring the oxidation of an indicator AA. The indicator oxidation response to diets containing one of 2 test ingredients was compared to the standard curve and MA was determined as previously demonstrated in Figure 1.3.1.

4.2.1 Diets and feeding.

Pigs were fed twice daily except for collection days where half the daily feed allowance was separated into 12, ½-hourly feedings. This protocol was necessary to simulate continuous infusion during the isotope collection period (Moehn et al., 2005). Different batches of test ingredients (corn and barley) were used for each replicate; therefore, the replicates will be referred to as Exp. 1 and 2; however, the feeding regime, diet formulation, and data collection were the same for each replicate. Animals were placed on the next test diet in their rotation following completion of the 6-hr collection period and an amount equivalent to the remaining half daily portion was fed. All daily portions were consumed within 15 min. Feed allowance was set to 95 g/kg $BW^{0.75}$, which is ~ 90% of *ad libitum* intake. Pigs were weighed weekly and feed allowance was adjusted accordingly.

Isonitrogenous, isoenergetic diets were formulated to provide all nutrients, except Thr, at >120% of requirement according to NRC (1998) (Table 4.1 and 4.2). Phenylalanine concentrations were kept constant in all diets. Tyrosine was set at 200% of NRC (1998) requirement to ensure no labeled phenylalanine (Phe) was used to meet the demand for tyrosine and to facilitate the channeling towards oxidation of any tyrosine formed from Phe (Shiman and Gray 1998). The Thr reference diets were based on casein, cornstarch and sucrose. Dietary Thr levels were adjusted by the inclusion of L-Thr at the expense of cornstarch to achieve a range of 50 to 80% of NRC (1998) recommended daily Thr intake (~ 10% incremental increases in Thr), such that each pig received at least 3 levels of dietary Thr. Corn and barley were added, at the expense of sugar and cornstarch, to the 50% Thr base diet to achieve a dietary level of Thr equal to the 80%

Thr diet. A linear response to changes in test AA intake is critical for calculation of MA, thus the highest Thr intake was limited to 80% of the Thr requirement, or 2 SD below the mean Thr requirement (Moehn et al., 2005; 2007).

Animals were initially adapted to the highest Thr reference diet for 5 d prior to the start of collections. Each animal then received each of the 4 reference diets differing only in Thr content in an unbalanced 6 X 4 Latin square design. There were 3 d of adaptation to each reference diet which is more than adequate adaptation for oxidation studies (Möhn et al., 2003; Elango et al., 2009) followed by measurement of Phe oxidation using the IAAO method. Animals then received the barley or corn test diets. Measurement of Phe oxidation followed a 7-d adaptation period for each test ingredient diet (Longland et al., 1993).

4.2.2 Administration of labeled amino acid and recovery of labeled carbon dioxide.

All animals were subjected to repeated, ½-hourly oral application of isotope over 4 h. The animals received of 328.56 kBq (8.88 µCi)/h L-[1-^{14}C]Phe (American Radiolabeled Chemicals, St. Louis, Missouri) divided into 8, ½-hr feedings. A priming dose equal to 1.75 times the hourly dose was given along with the first ½-hr dose (Möhn et al., 2003). The pigs consumed all the feed provided prior to administration of the next ½ hour feed allowance. Background $^{14}CO_2$ enrichment was measured for two 30 min periods prior to administration of isotope.

Animals were placed in respiration chambers 30 min before the breath collection period began to allow the air in the chamber to equilibrate with the ventilating air stream.

Two independent respiration chambers were constructed with a rear door for the animals to enter and exit the chamber. Access to the animals was through a removable plexiglass lid on the top of and near the front of the chamber. Fresh water was provided *ad libitum* via a nipple drinker and feed could be provided directly into a trough through a feed tube which was sealed when not in use. The chambers had a volume of 1.2 m^3 and were air tight, except for two air inlets at the front. Negative pressure was induced in the chambers by rotary vane pumps (Gast Model 1023, Gast Manufacturing, Benton Harbor, MI), thus drawing fresh air (140 L/min) through the inlets. The air was evenly distributed via pipes running the length of the chamber with holes (0.6 cm) drilled approximately every 30 cm and capped at the opposite end.

After passing through a cold water condenser to remove water from the air, the airflow was divided between a series of gas washing bottles for CO_2 collection and a line by-passing the collection. Air volume through each diversion was recorded by two independent AC630 gas meters (Canadian Meter Company Inc., Cambridge, Ontario) and recorded manually every 30 min. The gas washing bottles were changed at 30 min intervals. The CO_2 absorber (ethanolamine:2-methoxyethanol, 1:2, v:v, Caledon, ibid) was weighed, sampled and mixed with scintillation cocktail (Atomlight, Canderra Packard, ibid) for scintillation counting. The samples were counted for 15 min or to an error of 2% in a liquid scintillation counter (Beckman LS3000, Beckman, ibid).

With repeated isotopic tracer administration, there is an accumulation of L-[1-^{14}C] Phe in the pigs' body protein resulting in an increase in radioactive background in expired $^{14}CO_2$ during the experiment (Moehn et al., 2005). The $^{14}CO_2$ plateau values were corrected for radioactive background using the expired $^{14}CO_2$ collected daily prior to

isotope administration. Oxidation rates, expressed as percent dose oxidized, were calculated from these corrected plateau values according to the equation:

$V^{14}CO_2$ (dpm/h)/dose (dpm/h) X % air collected

where $V^{14}CO_2$ is the flow rate of expired $V^{14}CO_2$ at plateau and % air collected is the proportion of the total airflow passing through the gas washing bottles.

A single plateau $^{14}CO_2$ enrichment value for each pig/diet combination was determined as the data points where the linear regression of enrichment within collection period was not significantly different from zero.

4.2.3 Diet AA analysis

Diets were analyzed for AA content by an external laboratory (Experiment Chemical Laboratories, Columbia, MO) due to equipment failure at the Swine Research and Technology Centre, Agricultural, Dept. of Food and Nutritional Sciences, Univeristy of Alberta.

4.2.4 Statistical analysis.

Data for reference and test ingredient diets were tested for linearity, lack of curvature and common intercept (Littell et al., 1995). Threonine intake was expressed as the intake (g/d) above that provided by the base diet (Littell et al., 1995) with the lowest Thr level (50%). The effect of Thr intake on Phe oxidation was estimated using the PROC MIXED procedure in SAS (SAS Inst. Inc., 2002, Cary, NC) with "pig" as random variable. Nesting Thr intake within type of Thr addition (e.g. L-Thr or Thr in corn or barley) gave the change (slope) in Phe oxidation per g of Thr for each type of Thr addition. The MA of Thr in corn and barley was calculated by dividing the slope for Thr from the respective

cereal grain by the slope for L-Thr. Collection day and BW were tested as covariables with main effects but were not significant. Results were expressed as least squares means ± SE and $P < 0.05$ and $P < 0.1$ were considered as significant and tendency, respectively.

4.3 Results

Pig BW gain was not different in Exp. 1 and 2, 11.3 ± 0.72 and 10.8 ± 0.72 kg, respectively. On day of collection, an isotopic steady state was achieved within 120 min of isotope administration. The change in L-[1-^{14}C]Phe oxidation during the 4-hr isotope administration is demonstrated in one pig (Fig. 4.1). Linear regression of indicator oxidation between collection periods 5-8 for each pig/diet combination was not different from zero; therefore, these data were used to calculate the plateau value.

4.3.1 Linearity of response to threonine intake.

There was a linear decrease in Phe oxidation as Thr intake increased from the addition of L-Thr to the diet. The decrease in Phe oxidation was 0.16% (SE 0.06) ($P < 0.01$, $R^2 = 0.22$) and 0.22% (SE 0.02) ($P < 0.001$, $R^2 = 0.62$) for each % increase in dietary Thr in Exp. 1 and 2, respectively.

4.3.2 Metabolic availability of threonine in corn and barley.

In Exp. 1, Phe oxidation decreased 1.79 ± 1.21% for each g of L-Thr added (Table 4.3). With the addition of Thr from corn equivalent to the level of L-Thr supplied by the 80% reference diet, Phe oxidation decreased 1.40 ± 0.92% for each g of protein-bound

Thr added. The addition of Thr from barley equivalent to the level of L-Thr supplied by the 80% reference diet decreased Phe oxidation 2.23 ± 0.92% for each g of protein-bound Thr added. In Exp. 2, Phe oxidation decreased 3.01 ± 0.52% for each g of L-Thr added, 2.63 ± 0.36% for each g of Thr from corn added and 3.69 ± 0.29% for each g of Thr from barley added (Table 4.4).

In Exp. 1, the response to the additional Thr intake from corn or barley, compared to that of L-Thr, indicated a MA of Thr of 78.2% and 125.0%, respectively (Table 4.3). In Exp. 2, the MA of Thr from corn and barley were determined to be 82.2% and 115.3%, respectively (Figure 4.2).

4.4 Discussion

The MA of Thr in corn fed to young pigs was 78.2 and 82.2% in Exp.1 and 2, respectively and was close to published standard ileal digestibility values of Thr in corn (82%; NRC, 1998). The MA of Thr in barley fed to young pigs was > 100% in both experiments. The MA of lysine and sulfur AA in the various high protein ingredients was found to also be similar to published estimates of ileal digestibility (Moehn et al., 2005,2007) and net protein utilization (Humayun et al., 2007).

Calculation of MA > 100% can result if (1) the slope of the reference curve does not represent maximal response to increasing test AA, (2) the L-[1-^{14}C]Phe is retained in the body tyrosine pool, or (3) an increased demand (requirement) for the test AA occurs upon feeding of the test ingredient. Factors affecting the slope of the reference curve can influence the determination of MA because MA is calculated as the ratio of the reference curve slope to the test ingredient slope. In the current study the basal diet was based on

casein and synthetic AA which have been shown to be essentially 100% digestible in growing pigs (Susenbeth, 2001; Chung and Baker, 1992). Therefore, the slope of the IAAO obtained with the addition of L-Thr to the basal diet (i.e. reference curve slope) in the present study represented the maximal unit increase in protein synthesis. Adeola (1996) suggested that bioavailability estimates > 100% may be due to more rapid absorption of the crystalline test AA compared to other essential AA in the reference curve diets resulting in reduced efficiency of utilization of absorbed AA. However, in the current study, a similar proportion of each of the individual essential AA requirements was supplied by the protein source in the basal diet (50 to 65%), with the exception of leucine (72%) and methionine (85%). This means that the proportion of AA supplied by crystalline AA was also similar across essential AA; therefore, it is unlikely that the > 100% MA of Thr in barley observed in the current study was a result of a difference in absorption rate of the dietary essential AA.

Retention of the the label (L-[1-^{13}C]Phe) in the body tyrosine pool will result in a lower expiration of $^{14}CO_2$; however, the measured tyrosine content of the barley diet was > 150% of the tyrosine requirement (NRC, 1998). Thus it is unlikely that the > 100% MA of Thr in barley was due to retention of the label in the body tyrosine pool.

Changes in oxidation of the indicator AA reflect whole body utilization of the test AA; therefore, an increase in demand for the test AA upon feeding of the test ingredient diet would result in a reduction in oxidation of the indicator. The greater slope with the addition of barley is likely a reflection of the higher requirement for Thr for synthesis of mucin and digestive enzymes because barley increases digestive enzyme secretion (Zebrowska et al., 1983) and endogenous protein secretions (Leterme et al., 2000; Myrie,

2004). Soluble fibers have been shown to increase cell proliferation in the small intestine as evidenced by increased crypt depth, villus height and the rate of fractional protein synthesis in the rat (Pirman et al., 2007). The increased protein synthesis and cell proliferation would result in a greater requirement for essential AA, particularly Thr, thus oxidation of the indicator AA would be reduced. Using the equation of the line for the reference curve and a Thr intake equal to the barley diet (3.44 g Thr above base/d), Phe oxidation on the reference diet would be 1.68% greater than the Phe oxidation measured on the barley diet (15.8%). This equates to an additional 0.53 g/d Thr utilized when pigs were fed the barley diet. Leterme et al. (2000) determined that Thr secretion in endogenous losses increased 0.55 g/kg dry matter intake per kg barley added to the diet. Based on the diet inclusion level of barley and the dry matter intake in the current study 0.28 g of additional Thr would be expected in the endogenous losses when pigs were fed the barley diet. Therefore, > 50% of the observed decrease in Phe oxidation when fed the barley diet can be attributed to the increased demand for Thr in endogenous losses. The pigs were 8 to 10 kg heavier when fed the barley diets, although this difference in BW was unlikely to affect digestive capability. Little difference was observed in protein and carbohydrate enzyme activities from 7 to 8 wks of age (~ 20 to 30 kg BW) in pigs (Hartma et al., 1961). The increased BW may also have increased the demand for Thr thus reducing oxidation of the indicator AA. Accounting for the increased requirement for Thr when fed the barley diets, the results suggest that the availability of Thr in barley fed to growing pigs is greater than published standard ileal digestibility estimates (NRC, 1998). Myrie (2004) also found the standard ileal digestibility of Thr in barley fed to growing pigs (87.1%) to be higher than NRC (1998) estimates (81 %). As well, the ‘real’

ileal digestibility of nitrogen (accounting for basal and diet specific endogenous nitrogen losses) in barley fed to young pigs (~ 17 kg BW) was ~ 95% regardless of barley variety or dietary inclusion level (Leterme et al., 2000).

Four conditions must be met for the determination of MA: (1) the test AA must be first limiting, (2) the response to changes in test AA intake must be predictable, preferably linear, (3) an isotopic steady state must be achieved when applying the IAAO in a slope-ratio assay, and (4) the observed response must not be influenced by other dietary nutrients in the test feed ingredient (Batterham, 1992; Littell et al., 1995; Levesque et al., 2010).

Based on the observed responses the first three conditions were met. The linear decrease in L-[1-^{14}C]Phe oxidation to increasing dietary Thr demonstrated that the test AA was first limiting and that the response to the test AA was linear. The IAAO method is based on the principle that AA are either utilized for protein synthesis or must be oxidized. The change in oxidation of the indicator AA is inversely proportional to protein synthesis (Ball and Bayley, 1986), thus the oxidation of the indicator AA indicated that the rate of whole body protein synthesis was driven by the limiting AA. The addition of crystalline AA to the casein reference diet ensured the basal diet contained all AA in slight excess, except the AA of interest, to ensure the test AA was first limiting. The non-significant linear regression of indicator oxidation between collection periods 5-8 indicated that an isotopic steady state was achieved.

The test ingredients were incorporated at the expense of a non-protein energy source (cornstarch) at a level to supply a similar quantity of test AA as the highest level of Thr in the reference curve diets (i.e. 80% Thr), as suggested by Batterham (1992), to ensure that

the observed response was due to the test AA and not influenced by other nutrients from the test protein. The dietary profile of essential AA and crude protein were equalized across all diets with the use of crystalline AA. However, the level of functional nutrients (i.e. fiber) between diets was not constant. The difference in added cellulose and the inherent difference of insoluble:soluble fiber content of corn and barley (NRC, 1998; Baik and Ullrich, 2008) resulted in decreasing relative levels of insoluble:soluble fiber (basal>corn>barley); although, the difference was minimal between the basal and corn diets. The difference in fiber content between the basal and barley diets may also have played a role in the higher than expected MA of Thr from barley when fed to growing pigs. Higher levels of dietary soluble fiber can increase digesta viscosity thus slowing digesta transit time through the small intestine (Dikeman and Fahey, 2006) allowing increased contact time between digestive enzymes and digesta resulting in greater digestion and absorption of dietary protein. Hooda et al. (2010) found that digesta viscosity was higher in diets containing 5% β-glucan (soluble fiber) compared to diets containing 5% cellulose (insoluble fiber). The higher digesta viscosity resulted in an increase in apparent ileal AA digestibility in pigs fed the 5% β-glucan diet compared to the 5% cellulose diet.

The results of the current study for Thr availability in corn and previous studies of the lysine availability in high protein ingredients (Moehn et al., 2005; 2007; Humayun et al., 2007) demonstrate the suitability of the MA method for protein quality evaluation. However, potential functional properties of the test ingredient (i.e. effects on digesta viscosity, gut retention time or endogenous secretions) should be considered when formulating the basal diet. Further work is needed to assess the effects of functional

nutrients (i.e. soluble fiber) on digesta passage rate and viscosity, protein digestion and the availability of AA, particularly Thr, for metabolic processes. When determining MA in ingredients containing functional nutrients the basal diet should contain similar levels of the identified functional nutrients.

Table 4.1 Composition of reference and test ingredient diets fed to growing pigs, as-fed basis[1]

	Exp. 1 and 2		
	Reference	Corn	Barley
% of NRC (1998)[2]	50	80	80
Diet composition (%)			
Casein	9.20	9.20	9.20
Corn	-	50.00	-
Barley	-	-	47.30
Cornstarch	29.30	10.82	16.21
Sucrose	29.30	10.82	16.21
Sulkafloc[3]	15.00	8.00	-
Canola oil	3.00	3.00	3.00
Celite	1.00	1.00	1.00
L-Arginine	0.25	0.03	-
L-Histidine	0.21	0.07	0.09
L-Isoleucine	0.31	0.15	0.13
L-Leucine	0.49	-	0.12
L-Lysine·HCl	0.91	0.73	0.68
L-Methionine	0.10	-	0.01
L-Cysteine·HCl	0.52	0.38	0.35
L-Phenylalanine	0.37	0.14	0.12
L-Tyrosine	0.13	0.01	-

L-Tryptophan	0.12	0.09	0.06
L-Valine	0.39	0.16	0.12
L-Glutamic acid	4.00	-	-
Mineral premix[4]	0.40	0.40	0.40
Vitamin premix[5]	0.50	0.50	0.50
Choline chloride[6]	0.10	0.10	0.10
Dicalcium phosphate	1.90	1.90	1.90
$KHCO_3$	0.80	0.80	0.80
Limestone	1.00	1.00	1.00
$NaHCO_3$	0.30	0.30	0.30
$MgSO_4$	0.40	0.40	0.40

[1]Diets were fed to meet daily energy requirement according to NRC (1998). Daily feed allowance was supplied at 0.95 kg/$BW^{0.75}$.
[2]% of NRC (1998) based on NRC (1998) daily Thr requirement for growing pigs 20 kg BW.
[3]International Fiber Corporation, Urbana, IL.
[4]provided per kg diet: 40 mg of Cu; 60 mg of Fe; 20 mg of Mn; 0.24 mg of Se; 100 mg of Zn; 0.4 mg of I (DSM Nutritional Products, High River, Alberta, Canada).
[5]Provided per kg diet: 3,750 IU of vitamin A; 750 IU of vitamin D3; 7,010 IU of Vitamin E; 4.0 mg of vitamin K; 2.5 mg of thiamin; 5 mg of riboflavin; 1.5 mg of pyridoxine; 15 µg of vitamin B12; 37.5 mg of niacin; 15 mg of pantothenic acid; 2.5 mg of folic acid; 0.25 g of biotin (DSM Nutritional Products, High River, Alberta, Canada).
[6]Provided per kg diet: 731 mg of choline chloride.

Table 4.2 Nutrient content of reference and test ingredient diets fed to growing pigs, as-fed basis[1]

	Exp. 1 and 2		
	Reference	Corn	Barley
% of NRC (1998)[2]	50	80	80
Analyzed, g/kg			
Threonine	0.34	0.51	0.40
Lysine	1.25	1.43	1.32
Phenylalanine	0.75	0.78	0.90
Tyrosine	0.48	0.56	0.56
Crude protein	14.52	14.13	14.92
Calculated			
Calcium, %	0.86	0.87	0.88
Available phosphorus, %	0.41	0.43	0.46
ME (MJ/kg)	14.31	13.92	13.89

[1]Analyses conducted by Experiment Station Chemical Laboratories, Columbia, MO.
[2]% of NRC (1998) based on NRC (1998) daily Thr requirement for growing pigs 20 kg BW.
[3]Based on ME content of ingredients as stated by NRC (1998).

Table 4.3 The metabolic availability of threonine from corn and barley in growing pigs based on indicator amino acid oxidation (Exp. 1)[1]

Diet	Threonine intake above base diet[2]	Indicator oxidation	Oxidation response[3]	Metabolic availability
	g/d	% of dose	% of dose per g THR intake	%
THR reference	0.00 ± 0.11	23.8 ± 1.39		
	0.51 ± 0.11	21.8 ± 1.39		
	0.82 ± 0.11	26.7 ± 1.39		
	1.12 ± 0.11	20.3 ± 1.39		
	1.52 ± 0.11	22.0 ± 1.39		
	1.70 ± 0.11	19.6 ± 1.39	-1.79 ± 1.21	100.0[4]
Corn	1.89 ± 0.08	21.4 ± 0.98	-1.40 ± 0.92	78.2[5]
Barley	1.89 ± 0.08	19.8 ± 0.98	-2.23 ± 0.92	125.3[5]

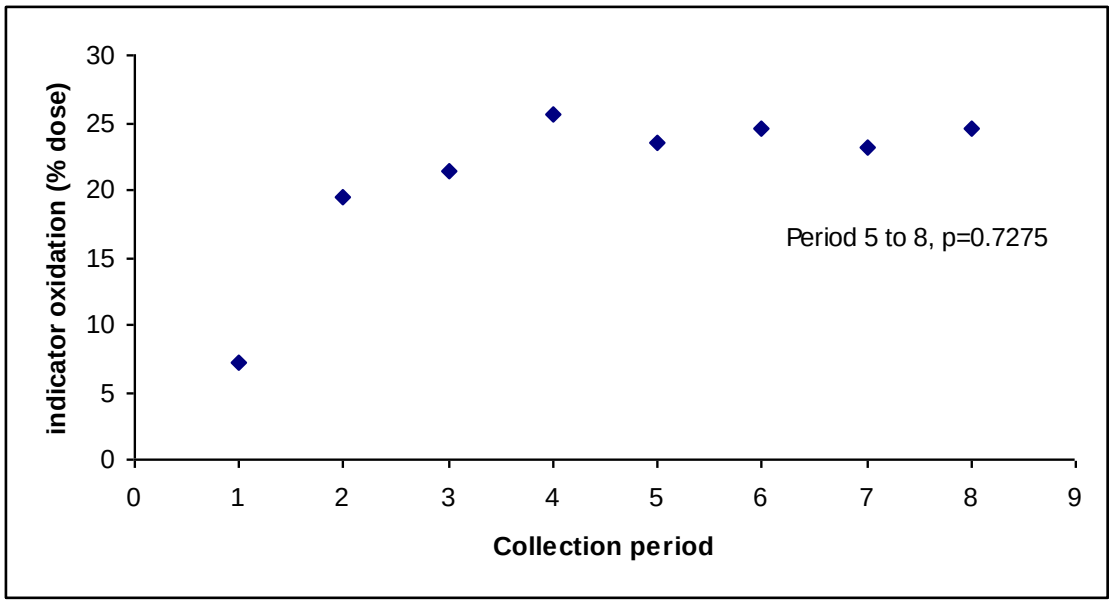
[1]Values are means ± SEM, n=6.
[2]Base diet (50% THR) provided 3.5 g/d.
[3]Change of indicator oxidation (% of dose) per g additional THR intake. Estimated from mixed model analysis.
[4]Susenbeth et al., 2001.
[5]Oxidation response for corn (or barley) divided by oxidation response for free THR.

Table 4.4 The metabolic availability of threonine from corn and barley in growing pigs based on indicator amino acid oxidation (Exp. 2)[1]

Diet	Threonine intake above base diet[2]	Indicator oxidation	Oxidation response[3]	Metabolic availability
	g/d	% of dose	% of dose per g THR intake	%
THR reference	0.00 ± 0.06	27.8 ± 0.83		
	0.87 ± 0.02	26.0 ± 0.82		
	1.58 ± 0.04	23.8 ± 0.82		
	2.29 ± 0.06	20.7 ± 0.88	-3.20 ± 0.48	100.0[4]
Corn	2.73 ± 0.06	21.3 ± 0.82	-2.63 ± 0.36	82.2[5]
Barley	3.44 ± 0.06	15.8 ± 0.82	-3.69 ± 0.29	115.3[5]

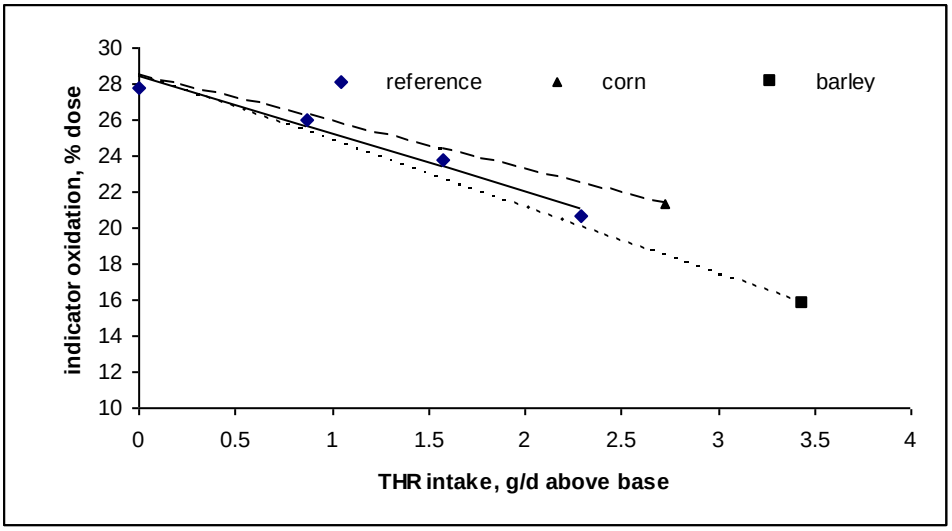
[1]Values are means ± SEM, n=6.
[2]Base diet (50% Thr) provided 3.5 g/d.
[3]Change of indicator oxidation (% of dose) per g additional Thr intake. Estimated from mixed model analysis.
[4]Susenbeth et al., 2001.
[5]Oxidation response for corn (or barley) divided by oxidation response for crystalline Thr.

Figure 4.1 The change in indicator amino acid oxidation following L-[1-^{14}C] phenylalanine administration during isotope administration.



Linear regression was NS (P = 0.7275) between period 5 to 8. Period 1 represents administration of priming dose (1.5 times the hourly dose) and the first ½ hourly dose. Animals received 328.56 kBq of [1-^{14}C] Phe per hr divided into 8, ½-hourly feedings.

Figure 4.2 Oxidation of indicator amino acid in response to changes in threonine intake above that provided in the base diet in growing pigs.



Regression lines show the response from crystalline Thr compared to Thr provided by the feedstuff. Results from Exp. 2.

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5.0. The metabolic availability of threonine in corn and barley fed to sows

5.1 Introduction

Nutritional quality of protein foods can be defined as the ability of a protein source to meet the individual's (animal or human) requirement for nitrogen and AA (Schaafsma, 2005). This is particularly important in human nutrition when daily protein intake is low, as in developing countries or in hospitalized or chronically-ill individuals (Rowan et al., 1994). Standard ileal digestibility and protein digestibility-corrected amino acid score (PDCAAS) are the most commonly used methods to estimate protein quality in pigs and humans, respectively. However, these values only represent the disappearance of amino acids (AA) from the gut, with estimated corrections to attempt to account for the contribution of endogenous AA from intestinal secretions (ileal digestibility) and fecal AA utilization (PDCAAS) based on feeding protein-free diets (Stein et al., 2007; Elango et al., 2009). However, endogenous AA losses are affected by many dietary factors such as type and quantity of fiber, antinutritional factors and protein content. Correction for dietary factors that affect the determination of standard or true ileal digestibility of AA in feedstuffs requires surgical intervention (Stein et al., 2007), and PDCAAS utilizes rats to determine fecal AA contributions (Schaafsma, 2005).

The indicator amino acid oxidation (IAAO) technique was developed as a non-invasive, rapid method to determine the MA of AA in feeds and foods (Moehn et al., 2005; Humayun et al., 2007; Elango et al., 2009). Metabolic availability reflects the proportion

of dietary AA used for protein synthesis and includes all AA losses that occur during digestion, absorption and metabolic utilization (Moehn et al., 2005).

Recent evidence suggests that standard ileal AA digestibility in pregnant sows is higher than in growing pigs (Stein et al., 2001). However, current AA digestibility values for feed ingredients are based on growing pig data (NRC, 1998). In human nutrition research, it is also recognized that ileal digestibility measurements are superior to fecal or total tract digestibility measurements. Individuals with established ileostomies can be utilized for collection of ileal digesta and determination of ileal digestibility of foods; however, collection of ileal digesta in uncompromised persons is challenging (Rowan et al., 1994). The surgical intervention necessary for determination of standard or true ileal digestibility makes its use as a routine method impractical in sows and unethical in humans; therefore, the new metabolic availability (MA) technique is the method of choice in these situations (Levesque et al., 2010). The IAAO technique was used to determine the MA of threonine (Thr) in corn and barley fed to pregnant adult sows which was compared to the large body of data on growing pigs (NRC, 1998).

5.2 Materials and methods

Animal care and experimental procedures were the same for the Exp. 1 and 2 and were approved by the University of Alberta Animal Policy and Welfare Committee.

A dose response study (Exp. 1) was conducted to determine the range of limiting Thr intakes that gave a linear response to IAAO necessary to determine MA using 6 Large White X Landrace sows (170 ± 17 kg BW, parity 2) in mid gestation (60 to 75 d). Mid gestation was selected to allow the same sows to be used for both the Thr requirement

study (Chapter 3.0, Exp. 1) and the current metabolic availability study. An additional 6 sows (145 ± 6 kg BW, parity 1) were used to determine the MA of Thr in corn and barley relative to a highly digestible casein-based reference diet (Exp. 2) according to the standard curve assay described by Littell et al. (1995).

5.2.1. Diets and feeding.

Daily feeding protocol was the same as that used previously in Chapter 3.0. See Section 3.2.1 for details.

In both experiments, isonitrogenous, isoenergetic diets were formulated to provide all nutrients, except Thr, at >120% of requirement according to NRC (1998) (Table 5.1 and 5.2). Phenylalanine (Phe) concentrations were formulated to be constant in all diets; although, there were slight differences in analyzed Phe content between diets. Tyrosine was set at 200% of NRC (1998) requirement to ensure no labeled Phe was used to meet the demand for tyrosine and to facilitate the channeling towards oxidation of any tyrosine formed from Phe (Shiman and Gray, 1998). The Thr reference diets were based on casein, cornstarch and sucrose. Threonine levels were adjusted by the inclusion of L-Thr at the expense of cornstarch to achieve a range of Thr concentrations from 55 to 82% of NRC (1998) estimated requirement in the Exp. 1 and from 20 to 45% of NRC (1998) in Exp. 2. The dietary level of Thr for Exp. 2 were determined based on the results of Exp. 1. In Exp. 2, corn and barley were added to the lowest reference diet (20% Thr) at the expense of sugar and cornstarch to achieve a dietary Thr level equivalent to the 45% Thr reference diet. Animals were adapted to the highest Thr reference diet within each study for 5 d each prior to collection days. Adaptation to each successive reference diet was 3 d

(Möhn et al., 2003) and a 7-d adaptation period was used for each test ingredient diet (Longland et al., 1993).

5.2.2 Administration of labeled amino acid and recovery of labeled carbon dioxide.

Administration of labeled AA and recovery of labeled CO_2 was the same as that described in Chapter 3.0. See Section 3.2.2 for details.

5.2.3 Sample collection.

Sample collection and analysis of expired air for total CO_2 and $^{13}CO_2$ and calculations were the same as that described in Chapter 3.0. See Section 3.2.3 for details.

5.2.4 Diet AA analysis

Diets were analyzed for AA content by an external laboratory (Experiment Chemical Laboratories, Columbia, MO) due to equipment failure at the Swine Research and Technology Centre, Agricultural, Dept. of Food and Nutritional Sciences, Univeristy of Alberta.

5.2.5 Statistical analysis.

The linearity of response to Thr intake was analyzed using linear, quadratic and cubic regression to determine the model of best fit. Where a quadratic or cubic regression was the model of best fit, the NLMixed procedure in SAS (SAS Inst. Inc., 2002, Cary, NC)

was used for broken-line regression analysis to determine a breakpoint (Robbins et al., 2006). The method described by Fadel (2004) was used to determine the starting parameter estimates for the NLMixed analysis.

Calculation of MA was the same as that described previously in Chapter 4.0. See Section 4.3.2 for details.

5.3 Results

Plateau in enrichment of expired air was achieved within 120 min of initial isotope administration. Figure 5.1 demonstrates the enrichment plateau of $^{13}CO_2$ in expired air in 1 sow during the study. Within each sow/diet combination, the atom percent excess (APE) was not different from collection periods 5 to 8 and hence used to determine a single enrichment value for calculation of % dose oxidized.

5.3.1 Linearity of response to threonine intake.

Sows responded to changing daily Thr intakes. In Exp. 1, oxidation of excess 1-^{13}C Phe decreased as daily Thr intake approached 7 g/d with no further decline as intakes increased to 9 g/d. Broken-line regression analysis determined a breakpoint at 6.3 g/d (R^2 = 0.85) with a SD of 1.1 g/d and 95% confidence limit of 5.4 to 7.3 g/d (Figure 5.2). These data demonstrated that the daily Thr requirement of sows from 63 to 73 d pregnancy was 6.3 g/d and that the Thr intake used to determine the MA of corn and barley must be no greater than 5.0 g/d (80% of requirement; Moehn et al., 2005) to ensure a linear response to increasing Thr intake. In Exp. 2, when Thr intake ranged from

2 to 5 g/d, there was a linear decrease in Phe oxidation as Thr intake increased (Figure 5.3, $P < 0.05$, $R^2 = 0.92$) and the quadratic model was not significant.

5.3.2 Metabolic availability of Thr in corn and barley.

Threonine intake (g above base) had a significant effect ($P < 0.01$) on the oxidation response to different sources of dietary Thr. Phenylalanine oxidation decreased 1.59 ± 0.53% for each g of crystalline Thr added (Table 5.3). With the addition of Thr from corn or barley, equivalent to the level of Thr supplied by the 45% dietary Thr reference diet, Phe oxidation decreased 1.40 ± 0.62% and 1.42 ± 0.81%, respectively, for each g of protein-bound Thr added. The ratio of the response to additional Thr intake from corn or barley, compared to that of crystalline Thr, indicated a MA of Thr of 88.0% and 89.3%, respectively (Figure 5.4).

5.4 Discussion

The increase in indicator oxidation when protein-bound Thr from corn and barley was substituted for an equivalent amount of crystalline L-Thr indicated that the Thr in corn and barley was less metabolically available than the crystalline L-Thr. The MA of Thr in corn (88.0%) and barley (89.2%) fed to pregnant sows in the current study were 7 and 9% greater than the published standard ileal digestibility of Thr in corn (82%) and barley (81%), respectively, based on growing pig data (NRC, 1998). Previous authors have also suggested that pregnant sows have a greater capacity to digest and utilize protein-bound AA compared to growing pigs. (Noblet and Shi, 1993; Stein et al., 2001; Le Goff and

Noblet, 2001). Noblet and Shi (1993) and LeGoff and Noblet (2001) found the total tract digestibility of crude protein a complete diet was 14 and 6% greater, respectively, in pregnant sows than growing pigs. The standard ileal digestibility of crude protein and AA of individual feed grains averaged 10% greater in pregnant sows but ranged from 1.7 to 16% (Stein et al., 2001). The current results also suggest about a 10% greater availability of AA in feedstuffs fed to adult sows compared to growing pigs. Noblet and Shi (1993) developed predictive equations to estimate nutritional value of complete feeds for sows based on growing pig data and suggested that the positive intercept for all equations indicated that the relative difference between growing pigs and pregnant sows was reduced as nutrient digestibility increased.

The results of the current study were similar to the standardized ileal digestible Thr content of corn (84.7%) determined in pregnant sows but were higher than standard ileal digestibility estimates for barley (78.3%) as reported by Stein et al. (2001). In the study by Stein et al. (2001) endogenous AA losses were determined using a protein-free diet; however, AA composition of endogenous protein changes with the protein status of the pig (i.e. protein-free vs. protein source diet; de Lange et al., 1989). When the change in AA composition of endogenous protein was accounted for, the true ileal digestibility of AA in barley increased, with the greatest increase in Thr digestibility by 7.4% (de Lange et al., 1989). Therefore, reassessing the results of Stein et al. (2001) using the results of de Lange et al. (1989), the standard ileal digestibility of Thr in barley would increase to 85.9%, close to the MA determined in the current study. Thus, it can be concluded that ileal AA digestibility coefficients determined in growing pigs underestimate the true availability of AA in feedstuffs for adult pigs.

The greater availability of feed AA in pregnant sows compared to growing pigs may be due to differences in dry matter intake and digesta retention time. Standard ileal digestibility was greatest in restricted fed sows compared to *ad libitum* fed growing pigs or lactating sows but not different from that determined in growing pigs when pregnant sows were fed *ad libitum* ileal digestibility was (Stein et al., 2001). However, Rowan et al. (1994) found the ileal digestibility of Thr was greater in adult humans fed at close to normal intake compared to growing pigs fed at 100 g/kg $BW^{0.75}$ (close to *ad libitum* intake). Ileal digestibility of Phe and methionine were also greater in adult humans but there was no difference for all other AA (Rowan et al., 1994).

Digestibility studies, using growing pigs, tend to be conducted at restricted feed intake to reduce errors associated with feed spillage and hence estimation of nutrient intake. When feed intake was supplied at > 70 g/kg $BW^{0.75}$, endogenous losses were proportional to feed intake (Hess and Seve, 99). At feed intakes lower than 70 g/kg $BW^{0.75}$, endogenous losses were reduced disproportional to the reduction in feed intake (Hess and Sève, 1999). A commonly used intake level for growing pig digestibility studies is ~ 90 g/kg $BW^{0.75}$ or 3X maintenance energy requirement. However, pregnant sows are typically restricted to 2.0 to 2.5 kg/d. In a first parity sow (~ 145 kg), this relates to ~ 60 g/kg $BW^{0.75}$ (1.2 X maintenance energy requirement) suggesting that endogenous losses measured in growing pigs are not reflective of those in sows partly due to the much lower intake level. Since the calculation of standard ileal digestibility relies on an accurate estimate of endogenous AA losses, endogenous losses measured in growing pigs at typical intake levels would be higher than that observed in sows. Thus the digestibility of AA in sows will be underestimated if endogenous losses determined in growing pigs

are applied. This is supported by the observation that endogenous losses of protein and AA were significantly higher for restrictively fed pregnant sows compared to *ad libitum* fed pregnant or lactating sows (Stein et al., 1999).

The increased diet availability of Thr in pregnant sows observed in the current study and in adult humans (Rowan et al., 1994) compared to growing pigs may also be due to an increase in digestion and absorption and physiological state. There is greater bacterial colonization of the lower small intestine in mature animals thus an increase in digestion and absorption may occur due to bacterial digestion activity (Shi and Noblet, 1994). Bacterial digestion of both dietary and endogenous protein may increase AA recycling in the gut, particularly Thr due to the high Thr content in gut secretions such as mucin (Stoll, 2006). In order to support the increased demand for nutrients and changes in nutrient metabolism during pregnancy, utilization of nutrients from the diet may be altered by increasing intestinal absorption (King, 2000). This is supported by observations in rats that the specific activity of digestive enzymes was increased during late pregnancy and lactation (Burdett and Reek, 1979); although no data was found in humans or pigs.

The four 4 conditions necessary for determination of MA (Chapter 4.0, Section 4.4) were met in the current study. The results of Exp. 1 indicated that the upper limit of daily Thr intake of sows in mid-pregnancy should not exceed 5 g/d. This is lower than current NRC (1998) estimates of the Thr requirement for pregnant sows but close to that reported previously in early gestation (Chapter 3.0). In Exp. 2, when Thr intake ranged from 2 to 5 g/d a significant linear response in oxidation of indicator AA was observed and the quadratic model was not significant thus both the condition of linearity of response to

changes in test AA intake and the condition that the tests AA intake was first limiting were satisfied. The plateau in APE from collection periods 5 to 8 demonstrated that an isotopic steady state was achieved. The dietary profile of crude protein, essential AA and dietary energy were equalized across diets thus the final condition that the observed response was not influenced by other nutrients in the feed was met. Finally, as with the previous study in growing pigs (Chapter 4.0) the basal diet was based on casein with incremental increases in crystalline Thr; therefore, the slope of the IAAO obtained with the crystalline form of the test AA represents the maximal unit increase in protein synthesis and is equivalent to 100% MA of the test AA.

The current results suggest that mature animals have a greater capacity to digest and utilize AA in common feed ingredients than growing pigs. Therefore, sow diets based on growing pig ileal digestibility coefficients are overformulated with respect to AA content. Sow feed accounts for 30% of the total production feed cost in a farrow-finish operation (Moehn et al., 2009), thus overfeeding protein has a direct negative effect on overall production efficiency. Given the current recommendation to utilize phase feeding of sows during pregnancy (Moehn et al., 2009) formulating diets based on accurate estimates of nutrient availability becomes critical. Metabolic availability is a valuable estimate of the true availability of AA in foodstuffs. Continued development of the MA method in pigs will also assist in expanding the practical application of MA for evaluating the quality of protein sources in humans.

Table 5.1 Composition of reference and test ingredient diets fed to pregnant sows, as-fed basis[1]

	Exp. 1	Exp. 2		
		Reference	Corn	Barley
% of NRC (1998)[2]	55	20	45	45
Diet composition (%)				
Casein	5.96	2.10	2.10	2.10
Corn	-	-	28.70	-
Barley	-	-	-	27.00
Cornstarch	35.14	32.52	23.64	27.31
Sucrose	35.14	32.52	23.64	27.31
Sulkafloc®[3]	11.00	15.00	8.50	3.30
Canola oil	0.60	1.00	1.00	1.00
Celite	1.00	1.00	1.00	1.00
L-Arginine	-	0.01	-	-
L-Histidine	0.08	0.16	0.08	0.09
L-Isoleucine	0.12	0.26	0.16	0.15
L-Leucine	0.07	0.36	-	0.15
L-Lysine·HCl	0.35	0.63	0.53	0.50
L-Methionine	0.01	0.10	0.04	0.05
L-Cysteine·HCl	0.22	0.39	0.30	0.29
L-Phenylalanine	0.15	0.26	0.13	0.12
L-Tyrosine	-	0.15	0.09	0.08

L-Tryptophan	0.05		0.10	0.08	0.06
L-Valine	0.11		0.31	0.18	0.16
L-Asparagine	-		0.50	0.50	0.50
L-Glutamic acid	5.20		6.30	3.00	2.50
Mineral premix[4]	-		0.50	0.50	0.50
Vitamin premix[5]	-		0.70	0.70	0.70
Choline chloride[6]	0.14		0.20	0.20	0.20
Vitamin-mineral premix[7]	3.80		-	-	-
Dicalcium phosphate	0.34		2.40	2.40	2.40
NaCl	-		0.05	0.05	0.05
$KHCO_3$	0.46		0.65	0.65	0.65
Limestone	-		1.10	1.10	1.10
$MgSO_4$	0.06		0.13	0.13	0.13
$NaHCO_3$		-	0.60	0.60	0.60

[1]Daily feed intake was set to achieve recommended intake based on sow BW and backfat at breeding (Aherne and Foxcroft, 2000). Feed intake was 2.5 ± 0.1 kg/d.
[2]% of NRC (1998) based on NRC (1998) daily Thr requirement for sows of similar BW, gestation weight gain and expected litter size.
[3]International Fiber Corporation, Urbana, IL
[4]provided per kg mineral premix: diet: 50 mg of Cu; 75 mg of Fe; 25 mg of Mn; 0.30 mg of Se; 125 mg of Zn; 0.50 mg of I (DSM Nutritional Products, High River, Alberta, Canada).
[5]Provided per kg diet: 10,500 IU of vitamin A; 1,050 IU of vitamin D_3; 7,010 IU of Vitamin E; 5.6 mg of vitamin K; 3.5 mg of thiamin; 7.0 mg of riboflavin; 2.1 mg of pyridoxine; 2.1 µg of vitamin B12; 52.5 mg of niacin; 21.0 mg of pantothenic acid; 3.5 mg of folic acid; 0.35 mg of biotin (DSM Nutritional Products, High River, Alberta, Canada).
[6]Provided per kg diet: 1,023 and 1,462 mg of choline chloride, Exp. 1 and 2, respectively.
[7]Provided per kg diet: 8.17 g of Ca; 3.29 g of P; 1.82 g of Na; 0.38 g of Mg; 22.61 mg of Cu; 267 mg of Fe; 60.8 mg of Mn; 0.28 mg of Se; 132 mg of Zn; 0.38 mg of I; 0.23 mg of biotin; 6.65 mg of riboflavin; 33.4 µg of vitamin B-12; 11,400 IU of vitamin A; 58.9 IU of vitamin E; 1.330 IU of vitamin D_3; 1.56 mg of vitamin K; 494 mg of choline; 3.04

mg of folacin; 36.1 mg of niacin; 23.8 mg of pantothenic acid (Consultant Feeds, Calmar, Alberta, Canada**)**.

Table 5.2 Nutrient content of reference and test ingredient diets fed to pregnant sows, as-fed basis[1]

	Exp. 1	Exp. 2		
		Reference	Corn	Barley
% of NRC (1998)[2]	55	20	45	45
Analyzed, g/kg				
Threonine	1.17	0.90	1.61	2.01
Lysine	6.48	6.20	6.50	6.10
Phenylalanine	5.58	5.10	4.90	5.70
Tyrosine	2.84	1.90	2.00	2.60
Crude protein, %	9.32	9.30	8.31	9.56
Calculated				
Calcium, %	0.93	0.97	0.97	0.98
Available phosphorus, %	0.42	0.46	0.47	0.49
Metabolizable energy[3], MJ/kg	14.00	14.00	14.10	14.10

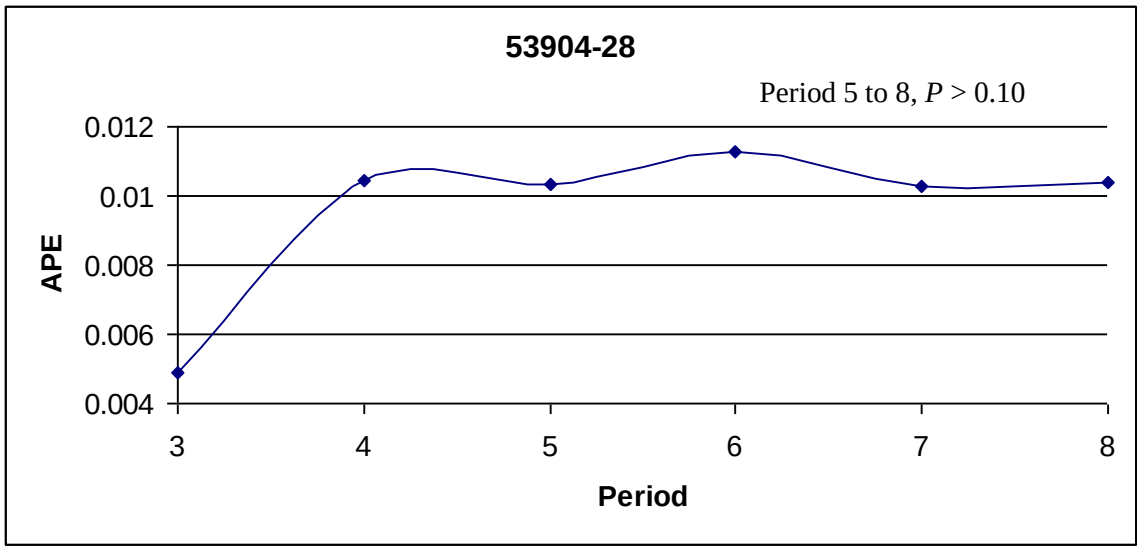
[1]Analyses for Exp.1 described in Section 3.2.2. Analyses for Exp. 2 conducted by Experiment Station Chemical Laboratories, Columbia, MO.
[2]% of NRC (1998) based on NRC (1998) daily Thr requirement for growing pigs 20 kg BW.
[3]Based on ME content of ingredients as stated by NRC (1998).

Table 5.3 The metabolic availability of threonine from corn and barley in pregnant sows based on indicator amino acid oxidation[1]

Item	Threonine intake above base diet[2]	Indicator oxidation	Oxidation response[3]	Metabolic availability
	g/d	% of dose	% of dose per g Thr intake	%
Thr-20%	0.00 ± 0.05	23.8 ± 3.6		
Thr-28%	0.82 ± 0.02	21.4 ± 2.0		
Thr-26%	1.61 ± 0.04	21.2 ± 3.7		
Thr-45%	2.49 ± 0.06	19.4 ± 3.2	-1.59 ± 0.53	100.0[4]
Corn	2.47 ± 0.06	21.6 ± 1.4	-1.40 ± 0.62	88.0[5]
Barley	2.47 ± 0.06	20.8 ± 2.2	-1.42 ± 0.81	89.3[5]

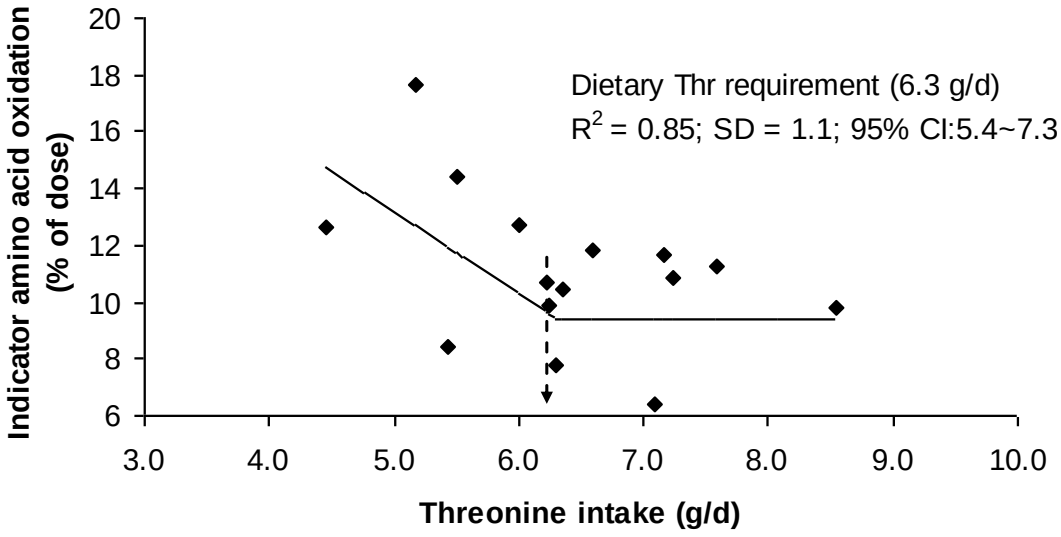
[1]Values are means ± SEM, n = 6.
[2]Base diet (20% Thr) provided 2.0 g/d.
[3]Change of indicator oxidation (% of dose) per g additional Thr intake. Estimated from mixed model analysis.
[4]Susenbeth et al., 2001.
[5]Oxidation response for corn (or barley) divided by oxidation response for crystalline Thr.

Figure 5.1 Isotopic enrichment of expired air ($^{13}CO_2$) in one sow following administration of 2 mg L-[1-^{13}C] phenylalanine/kg BW per hr.



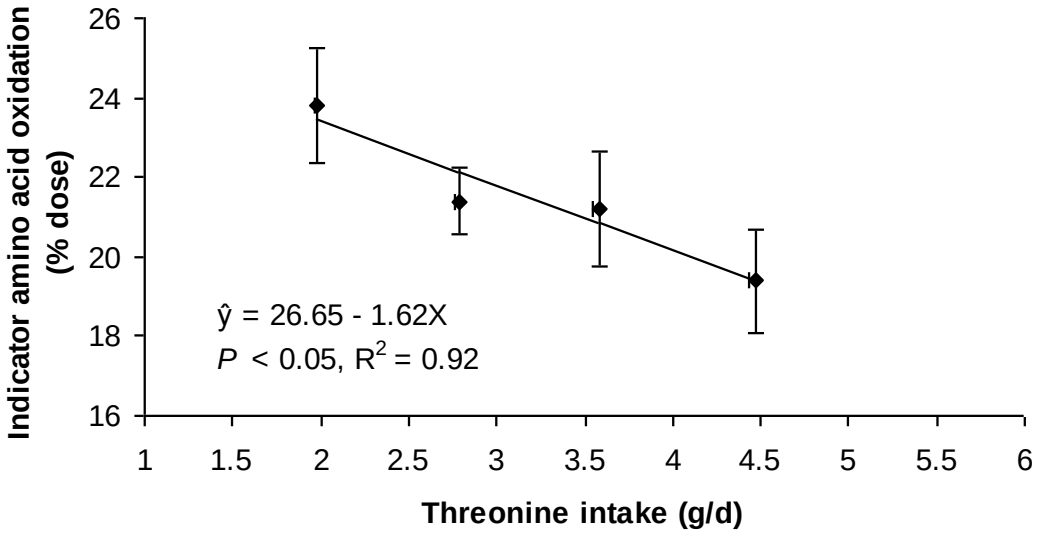
A priming dose (1.75 X hourly dose was given at period 1. Each period represents 30 min. No difference in APE from period 5 to 8.

Figure 5.2 The relation between indicator amino acid oxidation and daily threonine intake in pregnant sows (63 to 73 d gestation).



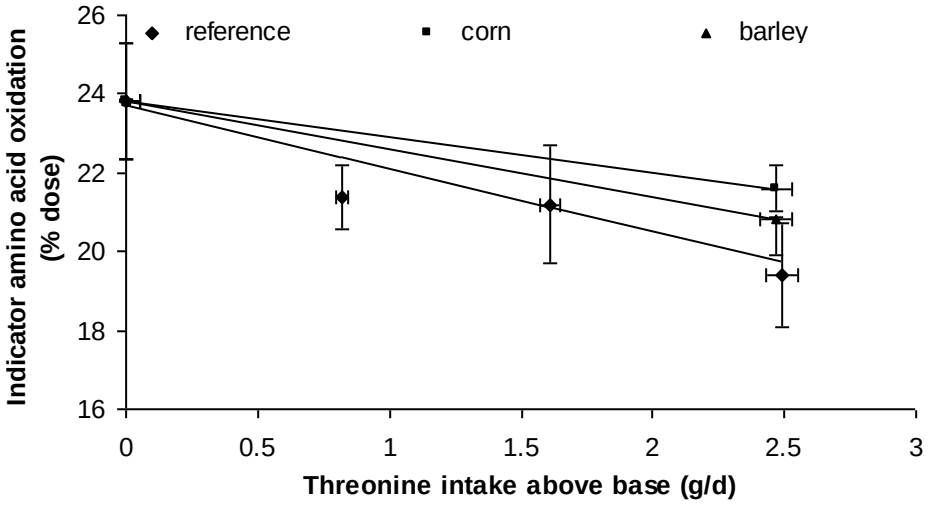
A breakpoint was determined indicating a daily Thr requirement of 6.3 g/d (P = 0.05, R^2 = 0.85).

Figure 5.3 The relation between indicator amino acid oxidation (ŷ) and daily intake of threonine (X) in pregnant sows.



The response best fit a linear model: $\hat{y} = 26.65 – 1.62X$ ($P = 0.05$, $R^2 = 0.92$). Data points are mean ± SE of 6 sows fed the reference diet with incremental increases in crystalline Thr in Exp. 2.

Figure 5.4 Oxidation of indicator amino acid in response to changes in threonine intake above that provided in the base diet in pregnant sows.



Regression lines show the response from crystalline Thr compared to Thr provided by the feedstuff.

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Chapter 6.0 The order of limitation of lysine, threonine, tryptophan and branched-chain amino acids in sows during late gestation

6.1 Introduction

An ideal amino acid pattern is one in which the pattern among essential amino acids (AA) corresponds to the AA requirements of the animal with neither excesses nor deficiencies (Wang and Fuller, 1989). Kim et al. (2009) suggested that the change in rate and composition of tissue gain in late gestation may affect the requirement for AA and potentially alter the ideal protein ratio for sows. The data reported previously (Chapter 3.0) indicated that the requirement for threonine (Thr) increased 2.5 times in late gestation compared to early gestation; however, other work from our group indicated that the requirement for lysine was only 1.5 times higher in late compared to early gestation (Samuel et al., 2010). These data suggest that the first limiting AA in late gestation may be Thr rather than lysine (Lys) and that the ratio of Lys:essential AA may change in late gestation.

Wang and Fuller (1989) describe a method for determining the ideal AA pattern for growth. The method assumes that the optimum dietary AA profile is considered to be that which, for a given AA intake, results in the greatest response of the dependent variable. Partial removal of a non-limiting AA has no effect on the dependent variable response; whereas, the degree of change in the dependent variable response from the partial removal of a limiting AA is used to determine the ideal AA pattern (i.e. greatest response = first limiting AA, next greatest response = second limiting AA, and so on). The method

utilizes an AA deletion study to determine the order of limitation and then calculate a regression equation for each essential AA. Using the regression equations, an ideal AA pattern, relative to lysine, is calculated in which each essential AA would be equally limiting. The ideal AA pattern for young pigs determined by Wang and Fuller (1989) was compared against the ideal AA pattern from ARC (1981) and a chemically defined ideal AA pattern developed by Chung and Baker (1991) and NRC (1998). The Wang and Fuller ideal AA pattern resulted in superior nitrogen retention compared to the ARC pattern, superior growth and feed efficiency compared to the NRC pattern and equal daily gain and feed efficiency compared to the Chung and Baker pattern (Wang and Fuller, 1989; Chung and Baker, 1992). The method has also been used to determine ideal AA ratios for maintenance and protein accretion in young pigs (Fuller et al., 1989).

An AA deletion study, examining Lys, Thr, tryptophan (Trp) and total branched-chain AA (BCAA), was conducted in gestating sows in the last 30 d of gestation based on the method described by Wang and Fuller (1989). The indicator amino acid oxidation (IAAO) technique was utilized as the dependent variable thus the most limiting AA occurs when oxidation of the indicator AA is greatest. Based on our previous results it was hypothesized that Thr would be first limiting in late gestation in sows.

6.2 Materials and methods

6.2.1 Animals and experimental diets

Animal care and experimental procedures were approved by the University of Alberta Animal Policy and Welfare Committee. Eight multiparous sows (170.2 ± 9.1 kg BW)

were used to assess the order of first through fourth limiting AA in gestation in an unbalanced latin square design (8 sows X 6 diets). Positive and negative control diets were based on casein, cornstarch and sucrose (Table 6.1). The positive control (PC) diet was formulated to contain all essential AA at 160% of NRC (1998) recommendations for sows of similar BW, maternal gain and expected litter size. This percent of NRC (1998) was previously determined to exceed the requirement for Thr and Lys in late gestation (Chapter 3.0; Samuel et al., 2010) and thus assumed to exceed the requirement for all other essential AA. The negative control (NC) diet was formulated to contain lysine (Lys), Thr, tryptophan (Trp) and total branched-chain AA (BCAA) at 60% of NRC (1998). Four additional diets were developed by removing each of the test AA from the PC diet to match the level in the NC diet (Table 6.1). The diets were isoenergetic and isonitrogenous, accomplished by altering the level of dietary glutamate, with all other nutrients formulated at 160% of NRC (1998) recommendations. Each animal received each diet in random order. Dietary phenylalanine (Phe) concentration was kept constant in all diets. To facilitate channeling towards oxidation of any tyrosine synthesized from Phe (Shiman and Gray, 1998) dietary tyrosine intake was set at 200% of NRC (1998) requirement. The daily feed allowance was top-dressed with L-Phe equivalent to the amount of Phe provided by the hourly dose of L-[1-^{13}C]Phe.

The sows were provided their daily feed allowance in two equal daily feedings except on oxidation days when half the daily ration was divided into 12 ½-hourly portions based on Moehn et al. (2004). Feed allowance (2.32 kg/d) was set to achieve the recommended daily energy intake based on body weight (BW) and backfat at breeding according to Aherne and Foxcroft (2000). All animals were individually housed and acclimated to

metabolism pens and the first diet in their rotation for 7 d before the first oxidation day. A minimum of 3 d acclimation was used for each successive diet based upon the observations that this was more than adequate adaptation period for oxidation experiments (Moehn et al., 2004; Elango et al., 2009).

6.2.2 Administration of labeled amino acid and recovery of labeled carbon dioxide

Administration of labeled AA and recovery of labeled CO_2 was the same as that described in Chapter 3.0. See Section 3.2.2 for details.

6.2.3 Sample collection

Data were collected during 92 – 110 d of gestation to ensure a period of maximum AA requirement in late gestation. The previously reported data (Chapter 3.0) demonstrated a significant increase in Thr requirement above NRC (1998) from 80 – 111 d of gestation.

Pigs were fed at 8:00 and 14:00 h each day then placed in open-circuit respiration chambers with Plexiglass windows at 18:00 h on the day prior to isotope administration to acclimate the sows to the chambers. This reduced sow activity prior to start of isotope administration the following morning and thus reduced variability in background $^{13}CO_2$. Chamber design and air flow details were described previously in Chapter 3.0. See Section 3.2.3 for details.

6.2.4 Sample analysis

Diets were analyzed for AA content by an external laboratory (Experiment Chemical Laboratories, Columbia, MO) due to the lack of an internal protocol for base hydrolysis for analysis of Trp in ingredients and diets. Tryptophan is particularly sensitive to redox reactions in strong acids necessary for hydrolysis of proteins (Molnár-Perl, 1997); therefore, the standard protocol for AA analysis could not be used.

Calculations and analyses total CO_2 and $^{13}CO_2$ in expired air, plasma AA concentration and plasma 1-^{13}C Phe enrichment were the same as that described in Chapter 3.0. See Section 3.2.4 for details. Approximately 80 to 90% of circulating Trp is bound to albumin (McMenamy et al., 1957); therefore, measured plasma Trp concentrations were divided by 0.85 to account for bound Trp. Plasma samples from period 5 were used to determine plasma urea nitrogen (PUN) according to Marsh et al. (1965). Plasma samples from period 5 (2.5 hrs after start of isotope administration) were used because previous data demonstrated an isotopic steady state during periods 5 to 8 (2.5 to 4 hr after start of isotope administration) indicating a steady state in AA metabolism. During the study, 5 out of 8 indwelling catheters lost patency thus; there were only 3 observations from each dietary treatment for the PUN, plasma AA and protein turnover dependent variables.

6.2.5 Statistical analysis

Data were analyzed using the PROC Mixed procedure (SAS Inst. Inc., 2002, Cary, NC). Sow was used as the random variable. The NC diet was included to demonstrate the response to an AA deficient diet for each dependent variable. Amino acid analysis

revealed that an error occurred in formulation of the NC diet. Although the test AA were formulated to 60% of NRC (1998) as intended, the 3 remaining essential AA (Phe, methionine and histidine) were formulated at 100% of NRC (1998) rather than 160% as in the PC and PC-test AA diets. As a result, the dietary Phe was content was lower in the NC diet than the other test diets (Table 6.2). Therefore, oxidation of the indicator AA (% of dose), PUN, plasma AA and protein turnover data for each AA deletion diet were compared to the response from the PC diet only. The Dunnett's test (SAS Inst. Inc., 2002, Cary, NC) was used to compare the least squares means for treatments (PC-Lys, PC-Thr, PC-Trp and PC-BCAA) to the PC diet where $P < 0.05$ and $P < 0.10$ were considered significant and trend, respectively.

6.3 Results

Sow performance was close to that previously observed in late gestation (Chapter 3.0). Sow BW increased 15.9 ± 1.6 kg over the study period (92 to 110 d of gestation) for an average of 0.55 kg/d. Mean litter size was 12.9 but ranged from 8 to 19 and piglet birth weight was 1.5 ± 0.1 kg.

6.3.1 IAAO, PUN and plasma AA

Oxidation of the indicator AA (Table 6.3) was lower when sows were fed the NC diet compared to the PC diet ($P < 0.05$); however, this may be due to the lower dietary Phe content in the NC diet compared to the PC diet. Oxidation of indicator AA (Table 6.3) was not affected by reduced Lys, Thr, Trp or BCAA intake. However, there was a

numerical increase in indicator oxidation (% of dose) when Thr (18.8%; $P = 0.20$) or Trp (18.4%; $P = 0.28$) was removed from the PC diet (16.4%). Reducing Lys, Thr, Trp and/or BCAA intake had no effect on PUN concentrations. The lack of effect on PUN was likely due to the low numbers of sows from which blood could be sampled (n = 3). Plasma Lys, Thr and BCAA were lower when sows were fed the NC diet compared to the PC diet ($P < 0.01$; Table 6.4). Removal of Lys, Thr, or BCAA from the PC diet reduced plasma levels of Lys ($P < 0.01$), Thr ($P < 0.001$) and BCAA ($P < 0.01$) concentrations, respectively, compared to the PC diet. There was no effect of diet on plasma Trp concentrations. Plasma Lys, Thr and BCAA were reduced 52, 83 and 56%, respectively, when the respective AA was removed from the PC diet (Figure 6.1). Reducing dietary Lys, Thr, Trp or BCAA had no effect on plasma concentrations of Phe, histidine, arginine, proline, or alanine (Table 6.3.4); however, plasma glutamate was higher when pigs were fed the NC diet compared to the PC diet ($P < 0.05$).

6.3.2 Protein turnover

Amino acid flux, protein breakdown and non-oxidative disposal (protein synthesis) were affected by dietary amino acid level ($P < 0.05$).There was a trend to lower amino acid flux when Lys or BCAA were limiting in the diet. Amino acid flux was lower when Trp was limiting in the diet compared to the PC diet ($P < 0.05$). Similarly, protein breakdown and synthesis were reduced when Trp ($P < 0.05$) or BCAA ($P < 0.05$) was limiting in the diet. However, when expressed as a proportion of flux, only protein breakdown was reduced ($P = 0.01$) when sows were fed the Trp limiting diet.

6.4 Discussion

The results of the current study suggest that in late gestation in sows the first limiting AA was most likely Thr followed by Lys or Trp; however, the order of limiting AA was dependent on the response variable used. When based on IAAO or PUN, Thr was first limiting in late gestation and the greater change in plasma AA with limiting dietary Thr supports this conclusion. However, there was little change in protein turnover when sows were fed the Thr limiting diet. The greatest response in protein turnover was observed when sows were fed the Trp limiting diet suggesting that Trp was first limiting.

The level of L-[1-^{13}C]Phe oxidation (% of dose) when all essential AA requirements were met (16.4%) was similar to that observed in Chapter 3.0 when the requirement for Thr had been met (15.5%). However, the low L-[1-^{13}C]Phe oxidation (% of dose) when dietary Thr was set at 60% of NRC (1998) requirement was surprising given the indicator oxidation response observed in Chapter 3.0 (Exp. 2) at a similar dietary Thr level (18.8 vs 22%). As well, separate analysis of the results from Chapter 3.0 (data not reported) indicated that a dietary Thr deficiency of 60% of NRC (1998) resulted in a highly significant increase in indicator oxidation, thus the level of AA deficiency was set at 60% of NRC (1998). The difference between the formulated and analyzed dietary Phe content of the PC and PC-test AA diets (~ 2.4 g/kg) was due to the Phe top-dress. The minimal response when Lys or BCAA were reduced in the diet may have been due to the slightly lower dietary Phe content in the PC-Lys and PC-BCAA diets compared to the PC, PC-Thr and PC-Trp diets. However, the variation across diets in analyzed Phe content was < 10% which is within the analytical error of AA analyses of feed ingredients and complete diets (Cromwell et al., 1999; 2003). The lower than expected response in indicator

oxidation for all AA deletion diets may account for the lack of significance observed in the current study.

The lack of significant response in PUN to limiting essential AA may have been due to the low number of observations along with the time of sampling. It was assumed that collection periods 5 to 8 would represent a steady state in PUN because the plateau in enrichment during collection periods 5 to 8 had consistently demonstrated an isotopic steady state (Chapter 3.0, Section 3.3; Chapter 5.0, Section 5.3). As well, Cai et al. (1994) found that PUN concentrations were constant from 2 to 6 hr after start of feeding in pigs when allowed *ad libitum* access to feed. On collection days in the current study, animals were provided a portion of feed every half hour representing a steady intake. However, analyzed PUN concentrations for period 6 were 20 to 87% higher than those for period 5 suggesting a steady state in PUN was not achieved. Only the PUN concentrations for period 5 were reported in the results because the same trend in order of AA limitation was observed despite the difference in absolute values. Plasma urea nitrogen concentrations have also been shown to be affected by restricted water intake (Utley et al., 1970; Cai and Zimmerman, 1995); however, PUN concentration was not affected by a free choice water treatment (Utley et al., 1970). The sows in the current study had free access to water, thus the PUN response was not likely due to water intake.

In the current study, plasma samples were taken 20 to 25 min post feeding, just prior to the next ½-hr feed allocation and thus the change in PUN with feeding may not have reached a plateau. Plasma urea nitrogen increased for the first 2 hr after initial access to feed in pigs with free access to feed; however, no measurements were taken at less than 2 hr (Cai et al., 1994). In meal-fed pigs PUN reached a peak only after four hr post feeding

(Eggum, 1970). Numerous other studies have reported significant differences in PUN with changes in dietary AA level when fasted or post-absorptive blood samples were used (Eggum, 1970; Coma et al., 1995a,b; 1996); therefore, fasting or post-absorptive (4 hr post feeding) blood samples may have been more appropriate for analysis of PUN.

The decrease in plasma Lys, Thr and BCAA, when each AA was removed from the PC diet, indicated a decrease in the amount of each respective AA available for metabolism. Although the decrease in plasma AA concentration cannot be used to determine the first limiting AA, the greater degree of change in the PC-Thr diet suggests that Thr was most limiting. The increased plasma glutamate when pigs were fed the NC diet compared to the PC diet was likely due to the higher inclusion of L-glutamate in the NC diet to balance crude protein across experimental diets. Dietary addition of L-glutamate was similar between the PC and PC-test AA diets and thus no effect of dietary treatment on plasma glutamate concentration was observed.

In the method of Wang and Fuller (1989) to determine the ideal AA ratio, individual regression equations are determined for each limiting essential AA based on the measured response to its respective deletion from the control diet (Figure 6.2). Using the regression equations, an ideal AA pattern, relative to lysine, is then calculated in which each essential AA would be equally limiting. In the current study, a regression equation for the PC-Lys diet could not be determined due to the lack of response in indicator oxidation when pigs were fed the PC-Lys diet. Therefore, a ratio to Lys could not be determined. However, as discussed in Chapter 3.0 the Lys:Thr ratio can be extrapolated. The requirement for Lys in early gestation was 13.1 and 8.2 g/d in second and third parity sows, respectively. The Lys requirement in late gestation increased to 18.7 and 13.0 g/d

in second and third parity sows, respectively (Samuel et al., 2010). Thus, the Lys:Thr ratio for second parity sows would be 1:0.47 and 1:0.72 in early and late gestation, respectively and 1:0.61 and 1:0.95 for third parity sows in early and late gestation, respectively. This is in contrast to Kim et al. (2009) where the Lys:Thr ratio decreased from 0.79 in early gestation to 0.71 in late gestation; although no indication of an effect of parity was discussed. The greater Lys:Thr ratio with increasing parity is; however, in agreement with GfE (2008). The estimate of ideal protein ratio by Kim et al. (2009) was based on changes in tissue AA accretion only, thus it does not account for AA utilization for all metabolic processes (i.e. tissue growth, immunoglobulin synthesis, digestive enzyme and mucosal protein production). The IAAO technique measures whole body AA utilization; therefore, all AA utilization was accounted for in the current study and thus may explain the difference in ideal AA ratio in late gestation between the current results and those of Kim et al. (2009).

Although calculation of the complete ideal protein ratio requires an estimate of the requirement for all essential AA, only Lys, Thr, Trp and total BCAA were examined in the current study. Common swine feed ingredients (i.e. corn, soybean meal) are high in methionine, histidine, isoleucine, arginine and phenylalanine, relative to the other essential AA (NRC, 1998). For example, based on the NRC (1998) amino acid composition of corn, a diet containing only 50% corn would supply methionine, histidine, isoleucine, arginine and phenylalanine at 71, 79, 137, 730 and 83% of NRC (1998) requirement, respectively. The level of the other essential AA would be supplied at $\leq$ 50%, thus under practical conditions a deficiency in methionine, histidine, isoleucine, arginine or phenylalanine are unlikely.

The lack of significant response to dietary AA deficiency in the current study means that the order of limitation of essential AA in late gestation in sows cannot be determined with confidence from this study. Contributing to the lack of significance was the low number of observations for all plasma variables (n = 3). Additional observations are necessary to appropriately determine the order of limiting AA and the ideal AA ratio in late gestation in sows. However, based on the observed trends it is likely that the Lys:AA ratios change, and that Thr is first limiting, in late gestation.

Table 6.1 Composition of control and partial amino acid deletion diets fed to sows in late gestation, as-fed basis[1]

Item (%)	NC	PC	PC-Lys	PC-Thr	PC-Trp	PC-BCAA
Casein	3.40	3.40	3.40	3.40	3.40	3.40
Cornstarch	32.76	33.80	33.35	33.76	34.05	33.72
Sugar	32.76	33.80	33.35	33.76	34.05	33.72
Canola oil	1.00	1.00	1.00	1.00	1.00	1.00
Celite	1.00	1.00	1.00	1.00	1.00	1.00
Sulkafloc®[2]	10.00	9.00	9.50	9.00	8.50	9.00
L-Histidine	0.10	0.20	0.20	0.20	0.20	0.20
L-Isoleucine	0.04	0.35	0.35	0.35	0.35	0.04
L-Lysine·HCl	1.38	8.31	1.38	8.31	8.31	8.31
L-Leucine	0.00	0.47	0.47	0.47	0.47	0.00
L-Cysteine·HCl	0.28	0.46	0.46	0.46	0.46	0.46
L-Methionine	0.05	0.14	0.14	0.14	0.14	0.14
L-Phenylalanine	0.16	0.34	0.34	0.34	0.34	0.34
L-Tyrosine	0.04	0.16	0.16	0.16	0.16	0.16
L-Threonine	0.12	0.53	0.53	0.12	0.53	0.53
L-Tryptophan	0.02	0.12	0.12	0.12	0.02	0.12
L-Valine	0.03	0.39	0.39	0.39	0.39	0.03
L-Glutamate	12.00	7.90	9.00	8.40	8.00	9.20
Choline chloride[3]	0.25	0.25	0.25	0.25	0.25	0.25

Mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[5]	0.60	0.60	0.60	0.60	0.60	0.60
Dicalcium phosphate	2.30	2.30	2.30	2.30	2.30	2.30
Limestone	1.10	1.10	1.10	1.10	1.10	1.10
$MgSO_4$	0.14	0.14	0.14	0.14	0.14	0.14
$NaHCO_3$	0.48	0.48	0.48	0.48	0.48	0.48
NaCl	0.08	0.08	0.08	0.08	0.08	0.08
$KHCO_3$	0.65	0.65	0.65	0.65	0.65	0.65

[1]Daily feed intake set to achieve recommended intake based on sow BW and backfat at breeding (Aherne and Foxcroft, 2000). Feed intake was 2.32 kg/d. NC = negative control; PC = positive control; PC-Lys, PC-Thr, PC-Trp, PC-BCAA = positive control minus lysine, threonine, tryptophan, branched-chain AA, respectively to match level of NC (60 % of NRC, 1998 requirement).
[2]International Fiber Corporation, Urbana, IL
[3]Provided per kg diet: 1,828 mg of choline chloride.
[4]provided per kg diet: 50 mg of Cu; 75 mg of Fe; 25 mg of Mn; 0.3 mg of Se; 125 mg of Zn; 0.25 mg of I (DSM Nutritional Products, High River, Alberta, Canada).
[5]Provided per kg diet: 9,000 IU of vitamin A; 900 IU of vitamin D_3; 60 IU of Vitamin E; 4.8 mg of vitamin K; 3 mg of thiamin; 6 mg of riboflavin; 1.8 mg of pyridoxine; 18 µg of vitamin B12; 45 mg of niacin; 18 mg of pantothenic acid; 3 mg of folic acid; 0.3 g of biotin (DSM Nutritional Products, High River, Alberta, Canada).

Table 6.2 Analyzed amino acid content of control and partial amino acid deletion diets fed to sows in late gestation, as-fed basis[1]

	NC	PC	PC-Lys	PC-Thr	PC-Trp	PC-BCAA
Essential amino acids, g/kg						
Lysine	3.44	8.87	3.45	8.82	8.20	8.30
Threonine	2.65	6.27	5.92	2.43	6.28	6.12
Tryptophan[2]	0.24	1.11	1.60[3]	1.60[3]	0.43	1.60[3]
Total BCAA (analysed)	8.07	17.46	18.03	17.91	17.15	8.76
Isoleucine	1.97	5.07	6.11	6.52	4.93	3.63
Leucine	2.85	6.12	5.30	5.29	5.93	2.24
Valine	3.25	6.27	6.62	6.10	6.29	2.89
Phenylalanine	4.17	7.11	6.44	7.78	7.72	6.45
Crude protein, % (calculated)	10.98	10.93	10.94	10.93	10.91	10.92

[1] NC = negative control; PC = positive control; PC-Lys, PC-Thr, PC-Trp, PC-BCAA = positive control minus lysine, threonine, tryptophan, branched-chain AA, respectively to match level of NC (60 % of NRC, 1998 requirement). Diets were formulated to provide 0.95 % Ca and 0.45 % available P and 14.8 MJ/kg metabolizable energy, based on ME content of ingredients as stated in NRC (1998). Analyses are described in Section 3.2.3
[2]Dietary tryptophan content was analyzed by Ajinomoto Heartland, Chicago, Illinois, USA.
[3]Formulated Trp content, only the NC, PC and PC-Trp diets were analysed for actual Trp content.

Table 6.3 Effect of partial deletion of lysine, threonine, tryptophan or branched-chain amino acids on indices of protein metabolism compared to more than adequate amino acid intake during late gestation in sows[1]

	Dietary amino acid level[2]							
Variable	NC	PC	PC-Lys	PC-Thr	PC-Trp	PC-BCAA	Pooled SEM	*P* =
L-[1-^{13}C] Phe oxidation, % of dose	13.2^{b}	16.4^{a}	16.9^{a}	18.8^{a}	18.4^{a}	17.0^{a}	1.3	0.05
PUN, mg/dL	10.7	7.3	8.0	8.5	6.9	6.6	1.7	NS
Protein turnover, μmol/kg·h								
Flux	90.4^{a}	99.6^{a,x}	86.9^{y}	94.7^{a}	70.6^{b}	85.6^{y}	4.7	0.02
Breakdown	60.9^{a}	69.0^{a}	57.2^{a}	65.3^{a}	40.5^{b}	54.4^{b}	4.7	0.02
Non-oxidative disposal (synthesis)	74.2^{a}	78.4^{a}	70.9^{a}	70.4^{a}	53.2^{b}	63.7^{b}	3.6	0.008
Oxidation	16.1	21.2	16.1	24.2	17.4	21.9	2.7	NS
Protein turnover, % of flux								
Breakdown	0.67^{a}	0.69^{a,x}	0.65^{a}	0.69^{a}	0.57^{b}	0.63^{y}	0.02	0.01
Non-oxidative disposal	0.82	0.79	0.82	0.74	0.76	0.74	0.03	NS

(synthesis)								
Oxidation	0.18	0.21	0.18	0.26	0.24	0.26	0.03	NS

[1]Data are means, n = 8 for L-[1-[13]C]Phe and n = 3 for PUN and protein turnover. Within a row, treatment means (NC, PC-Lys, PC-Thr, PC-Trp, PC-BCAA) compared to the PC diet differ: [a,b] $P < 0.05$, [x,y] $P < 0.10$.
[2]Dietary level of test AA differ by treatment: NC = all test AA set to 60% of NRC (1998); PC = all test AA set to 160% NRC (1998); Lys, Thr, Trp, BCAA = lysine, threonine, tryptophan, branched-chain AA set to 60% of NRC (1998), respectively.

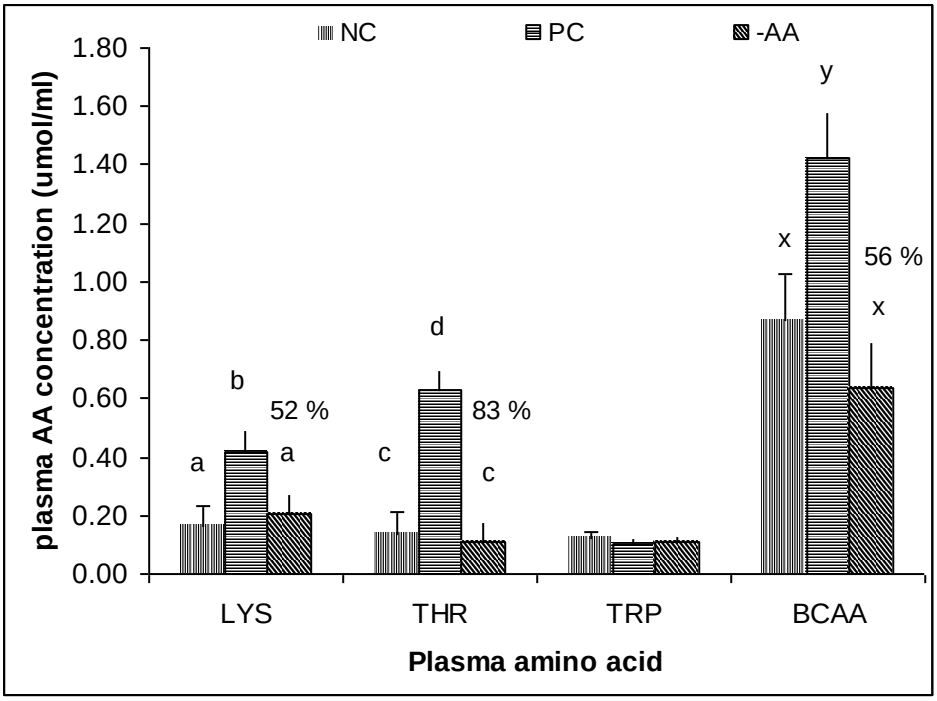
Table 6.4 Effect of partial deletion of lysine, threonine, tryptophan or branched-chain amino acids on plasma amino acid concentration compared to more than adequate amino acid intake during late gestation in sows[1]

	Dietary amino acid level[2]							
Plasma AA, μmol/g	NC	PC	PC-Lys	PC-Thr	PC-Trp	PC-BCAA	SEM	*P* =
Lysine	0.16[b]	0.42[a]	0.20[b]	0.50[a]	0.53[a]	0.51[a]	0.07	0.002
Threonine	0.14[b]	0.63[a]	0.66[a]	0.11[b]	0.55[a]	0.61[a]	0.01	0.0004
Tryptophan	0.82	0.69	0.93	0.80	0.73	0.67	0.15	NS
BCAA	0.87[b]	1.42[a]	1.57[a]	1.44[a]	1.26[a]	0.63[b]	0.16	0.007
Phenylalanine	0.20	0.21	0.25	0.22	0.18	0.19	0.03	NS
Histidine	0.14	0.17	0.15	0.15	0.12	0.12	0.03	NS
Glutamate	1.94[b]	0.87[a]	1.16[a]	0.87[a]	0.65[a]	0.87[a]	0.19	0.01
Alanine	2.30	1.78	1.44	1.18	1.17	1.51	0.33	NS
Arginine	0.14	0.13	0.13	0.10	0.10	0.11	0.02	NS
Proline	0.71	0.56	0.60	0.54	0.45	0.45	0.09	NS

[1]Data are means, n = 3. Within a row, treatment means (NC, PC-Lys, PC-Thr, PC-Trp, PC-BCAA) compared to the PC diet differ: [a,b] $P < 0.05$, [x,y] $P < 0.10$.

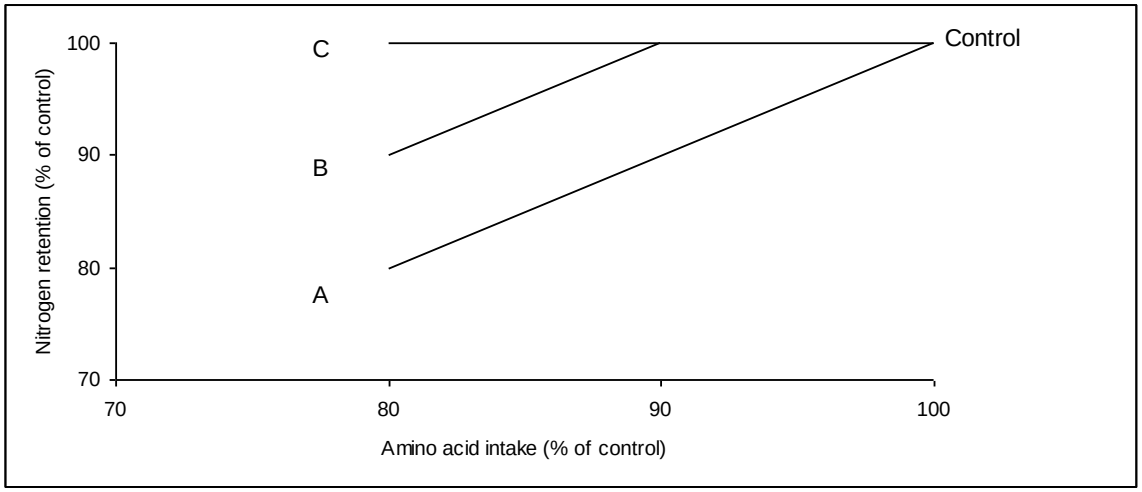
[2]Dietary level of test AA differ by treatment: NC = all test AA set to 60% of NRC (1998); PC = all test AA set to 160% NRC (1998); Lys, Thr, Trp, BCAA = lysine, threonine, tryptophan, branched-chain AA set to 60% of NRC (1998), respectively.

Figure 6.1 Changes in plasma amino acid concentration in sows given diets with partial deletion of lysine, threonine, tryptophan and branched-chain amino acids.



NC = negative control diet; PC = positive control diet; -AA = partial deletion of Lys, Thr, Trp or BCAA from the PC diet. Within dietary treatment, bars with superscript differ a,b $P < 0.05$. Plasma Lys, Thr and BCAA concentrations were reduced 52, 83 and 56%, when the respective AA concentration was removed from the PC diet.

Figure 6.2 Principle of the method of determining amino acid requirements by deletion.



A is the first limiting AA; B and C are AA which are 10 and >20% in excess relative to A, respectively. Adapted from Wang and Fuller (1989).

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Chapter 7.0 General discussion and conclusions

The first objective of this research was to determine the Thr requirement of pregnant sows in early and late gestation. The requirements for Thr in early and late gestation, based on IAAO, were determined to be at least 40% below and 30% above (Chapter 3.0), the current NRC (1998) recommendations and the requirement for Thr remained 30% below NRC (1998) recommendations up to 75 d of gestation (Chapter 5.0, Exp. 1). The increased demand for Thr in late gestation is supported by recent evidence that the requirement for Lys is also ~ 30% higher than NRC (1998) recommendations in late gestation (Srichana, 2006; Samuel et al., 2010). Changes in the rate of AA accretion in maternal tissue, including the mammary gland and conceptus, and fetal tissue also support the higher demand for AA in late gestation (Kim et al., 2009).

Another objective was to determine the order of limiting AA and the Lys:AA ratio in late gestation. The Lys:AA ratio could not be determined due to the lack of significant response to partial deletion of essential AA from the adequate AA diet; however, the observed trends suggest that Thr and Trp may be first and second limiting in late gestation. Kim et al. (2009) calculated a decrease in the ratio of Thr, valine and isoleucine relative to Lys but an increase in the ratio of leucine and arginine relative to Lys from early to late gestation. Wu et al. (1999) found that, although the accretion rate of essential AA in the fetus throughout gestation was similar, the concentration of Thr, Trp and leucine (mg/g piglet) increase 29, 54 and 34%, respectively, but the concentration of Lys increased only 12%. The calculated Lys:Thr ratio based on the reported research (Chapter 3.0) and Samuel et al. (2010) and the response trends observed in Chapter 6.0 would suggest that the ratios of Thr and Trp relative to Lys, increase in late gestation.

This research project clearly identified the higher demand for AA in late compared to early gestation; however, there were a number of limitations of the results. First, the lack of an effect of parity on the Thr requirement (Chapter 3.0) must be verified, given that an effect of parity was observed for Lys (Samuel et al., 2010). The GfE (2008) also estimated that the requirement for AA in gestation were parity dependent. A chronic problem with sow research is the lack of continuance through successive parities. Secondly, although there is clear evidence that the requirements for AA increase as gestation progresses, a change in the ideal AA ratio during gestation could not be conclusively determined. Finally, the determined requirement may have been influenced by the method used. Cuaron et al. (1984) found that when based on nitrogen balance and PUN, Lys was first limiting, before Thr, in late gestation. However, a decrease in maternal plasma IgG concentration was reversed with the addition of Thr, and not Lys, suggesting that in late gestation, Thr was first limiting for the production of IgG. A higher Thr requirement for immunoglobulin synthesis compared to the requirement for lean tissue protein synthesis was also reported by Defa et al. (1999) in growing pigs. In relation to sow AA requirements, the current Thr requirements and the Lys requirement determined by Samuel et al. (2010) were based on IAAO whereas; the Lys requirement determined by Srichana (2006) was based on nitrogen balance. The IAAO represents whole body protein synthesis and thus accounts for the AA demand for all metabolic processes (Ball and Bayley, 1986). Therefore, the IAAO technique would account for AA utilization for the synthesis of immune proteins, as well as lean tissue, and is likely the best response criterion to determine AA requirements during pregnancy.

Another objective of this research was to validate the use of the MA method to determine the availability of AA in low protein ingredients (corn and barley), then apply the MA method in sows. The MA method provided a good estimate of the bioavailability of AA in corn fed to growing pigs but the > 100% MA of Thr from barley was unexpected (Chapter 4.0). One of the assumptions of the slope-ratio assay for estimating AA bioavailability is that the slope of the response to the test AA is due to the addition of the test AA alone and not influenced by other nutrients contributed by the feed ingredient (Batterham, 1992). The use of semi-synthetic diets and the substitution of the test ingredient for a non-protein energy source allow the formulation of diets with similar concentrations of major nutrients (Batterham, 1992). However, differences in anti-nutritional factors, such as fiber, between the basal and test ingredient diets are not accounted for with this method. The influence of fiber on digestive processes has been extensively studied (Zebrowska et al., 1983; Jensen and Jørgensen, 1994; Johansen et al., 1996; Leterme et al., 2000; Myrie et al., 2008; Hooda et al., 2010) but the effect of fiber on whole body AA utilization (i.e. IAAO) has not been examined. Observations from Hooda et al. (2010), Leterme et al. (2000) and Zebrowska et al. (1983) suggest that the > 100% MA of Thr from barley fed to growing pigs was due to an increase in the demand for AA for mucin and mucous protein production, hence whole body AA utilization, in the presence of dietary fiber from barley. However, further work is necessary to understand the impact of fiber and other antinutritional factors on whole body AA utilization and thus IAAO. One reference diet may not be suitable to compare the slope of the response from the reference diet to the slope of response from the protein source in the MA method.

In spite of the aberrant results from barley in growing pigs, the MA method was deemed adequate to assess the availability of Thr from feed ingredients fed to sows (Chapter 5.0). The MA of Thr in corn and barley fed to sows was found to be ~ 10% higher than when fed to growing pigs. The higher availability of Thr from corn fed to sows in gestation (Chapter 5.0) is supported by Stein et al. (2001) who found a 10% increase in standard ileal digestibilty of Thr from corn in sows compared to growing pigs. Stein et al. (2001) conclude that the higher availability of AA in feeds observed in gestating sows is due to the difference in feed intake (gestating sows: restricted; growing pigs: *ad libitum*) rather than an effect of age. Regardless, in commercial swine production gestating sows are restrictively fed to minimize housing and farrowing difficulties associated with overconsumption of energy (NRC, 1998). The increase in AA digestibility from feed ingredients when fed to sows compared to growing pigs reported by Stein et al. (2001) was dependent on the feed ingredient and the AA. The standard ileal digestibilities of all essential AA were not different from barley, wheat and canola meal but higher from corn and soybean meal when fed to sows in gestation compared to growing pigs. However, the standard ileal digestibility of only arginine, isoleucine, methionine and Phe from meat and bone meal were higher when fed to sows compared to growing pigs. The difference in AA digestibility observed between feed ingredients may be due to the method used for estimating endogenous AA losses (a protein-free diet). Endogenous losses are influenced by ingredient characteristics, such as fiber (Leterme et al., 2000) and feed ingredients differ in their level and type of fiber (NRC, 1998). Myrie et al. (2008) found endogenous protein excretion varied up to 25% among common feed ingredients. The error associated with feeding a protein-free diet to estimate endogenous

AA losses is dependent on the AA, the error associated with Thr is the highest (7.4 percentage units), and is greater for individual ingredients than for complete diets (de Lange et al., 1989). Therefore, as with the reference curve for the MA method, a single correction for endogenous losses for all protein sources is inadequate.

There are a number of practical implications stemming from the current research: 1) a phase feeding program should be utilized for sows during gestation, 2) the higher AA availability from feed ingredients masks the AA deficiency in late gestation under current sow feeding protocols, 3) new empirical data is necessary to improve factorial requirement equations for sow AA requirements and 4) the change in AA requirements during gestation can be applied to human pregnancy AA requirements.

A phase feeding program could be implemented by increasing feed intake or top dressing with a high protein source (i.e. soybean meal) in late gestation, eliminating the need for additional feed storage. However, it would also increase total protein and energy intake and may result in a negative impact on the environment through excess nitrogen excretion. As well, excess energy intake in late gestation can result in increased BW gain and fat deposition and reduced voluntary feed intake in lactation which reduces sow milk production and increases sow BW loss in lactation (Baker et al., 1969; NRC, 1998). Assuming a typical diet formulation and daily feed intake (diet: Thr, 4.1 g/kg and metabolizable energy, 14.0 MJ/kg; feed intake: 2.4 kg/d), a feed intake increase of 33% (0.8 kg/d) would be necessary to reach the Thr requirement in late gestation as reported in this research. Energy intake would increase 11.2 MJ/d, 6 times greater than that needed to reach energy balance in late gestation (Chapter 3.0, Table 3.2). Implementation of a phase feeding program for maximum benefit would require usage of 2 separate diets

during gestation and allow dietary energy and Lys:AA ratios to be adjusted accordingly. Using the determined requirement for Thr in early and late gestation and assuming a similar change in the lysine requirement, Moehn et al. (2009) estimated a decrease in sows feed costs of up to $5/sow per gestation through the incorporation of a 2-diet phase feeding program.

A deficient dietary AA intake in late gestation may be expected to reduce fetal growth or litter parameters (litter size, litter weight); however, litter parameters are consistently high in commercial swine production and continue to increase (CCSI, 2008). The lack of clinical manifestation of symptoms from deficient dietary AA intake may be due, in part, to the buffering capacity of the sow to increase maternal body tissue mobilization in the face of dietary deficiency (Shields et al., 1985). The higher availability of AA from feed ingredients when fed to sows compared to growing pigs (Chapter 5.0) may also help to buffer the negative impact of deficient AA intake in late gestation under current recommended sow feeding programs.

This research project demonstrated the importance of the concept that factorial estimates be based on solid empirical data because the absolute values for the Thr requirement in early and late gestation determined in the current study (Chapter 3.0) are higher than the factorial estimates of Kim et al. (2009). The factorial estimates calculated by Kim et al. (2009) and the AA requirement equations from NRC (1998) are based on the AA composition of end products of metabolism (i.e. lean tissue, major organs, fetal tissue, milk) and do not account for the inefficiency of protein and AA anabolism and catabolism. The GfE (2008) included an estimate of the efficiency of AA utilization in

their model of AA requirements during gestation and recommended dietary Thr intakes in early and late gestation similar to that observed in the current research.

The current research program was conducted in growing and adult pigs; however, the results can be applied to human AA requirements and to protein quality evaluation of protein sources for humans. Previous work has shown that the requirement for AA in piglets is ~ 5 times that in human neonates (Roberts et al., 2001; Courtney-Martin et al., 2008; Chapman et al., 2009) suggesting that a ratio of 5:1 (pig:human) for daily AA requirements may be applicable at other stages of growth. Daily growth of the fetus and the fetal AA composition is similar between pigs and humans. Assuming a birth weight of 1.5 and 3.4 kg for the piglet and human, respectively, fetal growth rate over the gestation length is 13 g/d for the pig and 12 g/d for the human fetus. The essential AA composition of the pig and human fetus in late gestation vary < 10% (Wu et al., 1999). Thus, the increase in AA requirements during late pregnancy observed in sows (Chapter 3.0) and the maternal buffering capacity in the presence of nutritional deficiency may also apply to humans and thus have particular importance for nutritional aid programs in undernourished women and in teenage pregnancies (Higgins, 1976; Wallace et al., 2006). Higgins (1976) found that, under adequate nourishment, fetal weight increased with the second pregnancy and the size was maintained with successive pregnancies. However, fetal weight declined after the first pregnancy and remained low in undernourished, poor urban women. Similarly, in sows under current nutritional recommendations, there is a drop in litter weight in the second litter (Shelton et al., 2009) suggesting a compromised ability to buffer nutritional deficiencies.

Similarities in morphology and physiology of the gastrointestinal tract, as well as comparable digesta transit time, digestive efficiencies and post-absorptive metabolism make the omnivorous pig one of the best animal models for the study of nutrition in the omnivorous human (Miller and Ullrey, 1987). Rowan et al. (1994) demonstrated that the pig was a good animal model to assess the digestion of food proteins in humans. Humayun et al. (2007) applied the MA method in adult males and found that MA provided a good indication of the availability of total sulfur AA from soy protein or casein. Thus further development of the MA method in pigs could significantly advance protein quality evaluation of foods in humans beyond that obtainable with rodents because MA is determined directly in the subject of interest.

Observations in rats during pregnancy and energy restriction on nutrient absorption may also be important in sows during gestation. Nutrient absorption in the small intestine increased in late pregnancy (Burdett and Reek, 1979) and prolonged energy restriction (defined as 60 to 70% of *ad libitum* intake for 270 d) resulted in increased energy and AA uptake per unit metabolic BW despite a decrease in total intestinal weight (Ferraris et al., 2001). Sows are restricted to ~ 60% of *ad libitum* intake during gestation, thus a period of marginal energy intake in late pregnancy maybe advantageous for the gestating sow to improve nutrient absorption prior to parturition. Further work is necessary to determine whether similar changes in nutrient absorption occur in sows. The portal vein and carotid artery catheterization model from Hooda et al. (2009) may be used to demonstrate an increase in nutrient absorption in late gestation in the presence or absence of marginal energy intake. Evaluation of morphological changes in the gastrointestinal tract with gestation in sows will also help to understand the changing nutrient demands of sows

prior to parturition. Kim et al (2009) found no change in gut length during gestation in sows; however, no changes in intestinal wall characteristics (mucosal epithelium weight, crypt depth, villus height, mucosal epithelium protein content, etc) were reported.

Although nutrition may influence reproduction at one or more stages of a single breeding and lactation cycle, strategies for sow nutrition must include attention to longer term reproduction because of the 'carry-over' consequences of poor nutrition (Close and Cole, 2000). Further studies determining the requirement for all essential AA across multiple parities and long term studies evaluating the full economic cost (i.e. sow reproductive longevity, litter performance in nursery and to market, feed cost) of dietary AA deficiency, and adequacy, in gestation are necessary for full translation of the current observations into practical sow feeding programs. For the sow, understanding the effect of AA deficiencies on her susceptibility to pathogen challenge and immune status is also important to ensuring optimal reproductive performance and longevity. Comparison of traditional methods for determining AA requirements (i.e. nitrogen balance or PUN) and the IAAO method will also help to establish optimal dietary AA recommendations during gestation.

In conclusion, the results of the current research suggest that current feeding programs for gestating sows do not meet the changing demands for nutrients with advancing pregnancy and that the requirement for all essential AA during gestation must be re-evaluated. Re-evaluation of all the essential AA requirements in early and late gestation will allow the establishment of ideal AA ratios and development of more accurate factorial equations for estimating AA requirement in gestation. Further work is

necessary to fully understand the importance of periparturition nutrition in the sow and its impact on overall pig production efficiency.

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