Science is facts; just as houses are made of stones, so is science made of facts; but a pile of stones is not a house and a collection of facts is not necessarily science. - Henri Poincare

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Simultaneous measurement of protein and energy metabolism and application to determine lysine requirements in sows

by

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To my family: past, present, and future.

ABSTRACT

Simultaneous measurements of energy and protein metabolism can provide valuable information about their interactions. Dietary lysine is limiting in typical feedstuffs fed to swine and, therefore, limits protein synthesis. Current recommendations for dietary amino acid and energy intakes may not be reflective of the requirements for modern, highly productive sows and, therefore, invalidate requirement estimates determined according to the factorial approach. Current feeding recommendations suggest a constant amino acid intake throughout gestation. However, the demands for amino acids changes from maternal tissue accretion in early-gestation to fetal, conceptus, and mammary tissue development in late-gestation. This thesis reports the method development associated with simultaneous measurements of energy and protein metabolism and its application to determine dietary lysine requirements in non-pregnant and pregnant sows using the indicator amino acid oxidation method.

Two indirect calorimetry systems and an experimental feeding regimen were tested and validated for use in studies of amino acid requirements by stable isotope dilution. Protein and energy balance studies were performed in non-pregnant sows fed two distinct levels of energy and protein intake. The systems reacted appropriately to changes in gas concentrations induced by sow respiration. Protein and energy balance studies were also performed in pregnant and lactating sows fed typical diets. Sows appeared more anabolic during mid-gestation and were catabolic by late-gestation and through lactation, where additional energy intake provided by *ad libitum* feed intake increased milk energy output.

The dietary lysine requirement in non-pregnant sows at maintenance was determined as 49 mg/kg$^{0.75}$, 30% greater than current recommendations. The dietary lysine requirement was determined to be 10.1 g/d and 16.5 g/d, in early- and late-gestation, respectively. These results suggest that a constant diet formulation for the entirety of gestation is not appropriate.

In conclusion, simultaneous measurements of energy and protein metabolism combining indirect calorimetry and stable isotope techniques may be used to define requirements for dietary amino acids in sows. Basic assumptions of the factorial approach to estimate requirements require further investigation, including the dietary lysine requirement. Application of phase feeding for sows during gestation can more correctly meet the demands for amino acids and energy, improving sow longevity.

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ADF – acid detergent fibre
AIA – acid insoluble ash
AP – atom percent
APE – atom percent excess
B – protein breakdown
BW – body weight
cal – calorie
CBAS – Carmeda BioActive Surface
CF – crude fat
CF-IRMS – continuous flow isotope ratio mass spectrometer
CH_4 – methane
CO_2 – carbon dioxide
CP – crude protein
CTAB – cetyl trimethylammonium bromide
CV – coefficient of variation
d – day
DE – digestible energy
EE – ether extract
EI – electron ionization
ER – energy retention
GC – gas chromatography
GCMS – gas chromatography mass spectrometry
GE – gross energy
h – hour
H_2SO_4 – sulfuric acid
HCl – hydrochloric acid
HP – heat production
I – intake
IAAO – indicator amino acid oxidation
IV - intravenous
J – joules
k – kilo
α-KIC – alpha-ketoisocaproate
k_m – efficiency of energy utilization for maintenance
k_{pf} – efficiency of energy utilization for protein and fat deposition
L – litres
m – milli
M – mega
ME – metabolizable energy
ME_m – metabolizable energy requirement for maintenance
min - minute
mol – mole
MPE – mole percent excess
MS – mass spectrometry

N – normal
NaOH – sodium hydroxide
$NaHCO_3$ – sodium bicarbonate
Na_2CO_3 – sodium carbonate
NDF – neutral detergent fibre
NE – net energy
NO_x – nitrous oxide compounds
O – oxidation
O_2 – oxygen
Q - flux
RPM – rotations per minute
RQ – respiratory quotient
S – protein synthesis
SEM – standard error of the mean
USP – United States Pharmacopeia
WBPT – whole-body protein turnover
WSW – weigh-suckle-weigh
μ - micro

1.0 LITERATURE REVIEW

This literature review will include a discussion of the state of current knowledge in sow nutrition. The discussion will focus on the underlying assumptions that are included in the factorial approach defining nutrient requirements of breeding swine utilized by NRC (1998). Leucine as a metabolic tracer of protein turnover, the method development associated with the use of stable isotopes in large animal research, and the indicator amino acid oxidation experimental technique will be discussed. Finally, previous investigations of protein and energy metabolism in non-pregnant, pregnant, and lactating sows and the current knowledge about the dietary lysine requirements of non-pregnant and pregnant sows will be explored.

1.1 Introduction

Domestic swine are raised commercially to produce pork for human consumption. Sows are, ultimately, the productive unit of pork production. Maximal reproductive capacity and growth rate are, theoretically, determined by genetics but, practically, limited by nutrition (Boyd et al, 2000a). Pigs regulate their feed intake and metabolic pattern according to the nutritional content of the diet (Bereskin et al, 1975). Inadequate nutrition during late-pregnancy limits mammary tissue development (Beyer et al, 1994; Kim et al, 1999; Ji et al, 2005) and impacts milk production during lactation (Mahan and Mangan, 1975; Kim and Wu, 2009). Culling rates are typically high in commercial production and most commonly associated with absent or poor reproductive performance

(Foxcroft et al, 2005). Therefore, reproductive performance is synonymous with longevity (Mahan, 1977; Mahan, 1981; Aherne and Kirkwood, 1985; Dourmad et al, 1994a,b; Gaughan et al, 1995; O'Dowd et al, 1997; Young and Aherne, 2005; Anil et al, 2006; Tvrdoň and Marková, 2007) and reduced by insufficient nutritional status (Boyd et al , 2002), especially during lactation (Vinsky et al, 2006) and negative effects on body composition (Clowes et al, 1998; Clowes et al, 2003a,b; Clowes et al, 2005).

Sows are litter producing animals with relatively short time to sexual maturation. A gilt selected, bred, and properly managed will have produced at least seven female pigs to her equivalent weight at initial selection within one years time. Therefore, herd sizes can increase in number rapidly (Luscombe, 1972). Sows are the basis of pork production because they are required to produce and rear new piglets into the pork production systems (Elsley, 1971). Proper management of the sow herd results in increased pork production through greater numbers of pigs born, weaned, and eventually slaughtered for pork (Thornton, 1973).

Increased demand for pork led to an emphasis on pork production and increased specialization of the farms and management techniques (Thornton, 1988). Pork production was moved entirely indoors and, therefore, sows rely solely on the correct nutrition being provided as mixed rations. Thus, nutrient requirements and the nutrients provided from feedstuffs have been investigated and characterized. The Nutrient Requirements of Swine summarized both the nutrient requirements of sows and the chemical composition of feedstuffs, thus

allowing nutrient requirements to be matched with nutrients available from feedstuffs (NRC, 1998).

The most recent review of sow nutrient requirements was the NRC (1998) Nutrient Requirements of Swine. However, certain weaknesses exist in the data used to estimate sow nutrient requirements and the underlying assumptions of the factorial approach. For example, the data used for energy requirements was, in part, based on data from young and early-weaned piglets (Samuel, 2008). Similarly, protein (i.e. amino acid) requirements were based on data from growing pigs (Wang and Fuller, 1989; Pettigrew, 1993) and do not necessarily represent the nutrient requirements of sows. There is considerably more data available on the nutrient requirements of growing-finishing animals than is available for sows (Ball et al, 2008).

Significant improvements in sow productivity, including increased litter size and offspring growth rates and improved rebreeding success, have changed the nutrient requirements of the sow (Ball et al, 2008). O'Dowd et al (1997) correlated reduced body fatness of sows with reduced longevity due to failure to conceive or reduced milk production resulting in removal from the herd. Modern breeding has resulted in sows that are larger and leaner than their predecessors (Whittemore, 1996). Therefore, it is necessary to investigate the values utilized within the factorial approach in determining sow nutrient requirements for energy and protein in modern sows.

1.2 Concepts in protein and amino acid nutrition

Early research considered the crude protein content of the diet to be the criteria of its ability to provide the nutrients necessary for maintenance of body protein. Crude protein is defined as the nitrogen (N) content times 6.25, correcting for the fact that there is, on average, 16 g of N in 100 g of protein. However, because protein is composed of amino acids, the dietary protein requirement is more correctly defined as individual requirements for each of the 20 amino acids that make up protein. All of the constituent amino acids required to synthesize new protein must be present at the time of synthesis, or else the protein cannot be produced. Therefore, the amino acid available in the smallest amount (relatively) limits protein synthesis and is, consequently, termed the limiting amino acid (Block, 1956). Of the 20 amino acids that compose protein, only 12 are considered dietary indispensable amino acids for adult swine. The remaining eight amino acids can be synthesized by the body in sufficient quantities, as long as adequate amino nitrogen is available. More recently, estimates of the dietary protein requirement have been more correctly determined as requirements for the individual amino acids (NRC, 1998). Defining the requirements of individual amino acids and their bioavailability from feedstuffs (Reeds, 2001) has expanded the inclusion of dietary ingredients to include non-traditional ingredients because the amino acid contributions of all the ingredients can be balanced with the requirements of the animals. Correctly defined requirements facilitate targeted nutrition for specific production objectives and reduce excretion of nitrogen into the environment (McMillan, 2003).

Investigations of protein metabolism changed the early belief that dietary protein was not incorporated into body tissue, except during growth or repair. In fact, dietary protein is readily incorporated into body tissue through the continuous processes of protein synthesis and breakdown (Sprinson and Rittenberg, 1949). The original 'wear-and-tear' theory of Folin (1905) was dismissed and replaced with the notion of a continuous process of protein synthesis and breakdown proposed by Borsook and Keighley (1935) and Schoenheimer (1946) (Block, 1956).

Dietary amino acids are not stored in the body in any appreciable quantity, except as protein. Therefore, dietary amino acids have two fates following ingestion and absorption – oxidation or incorporation into protein. Short-term storage of amino acids can occur as labile proteins are synthesized from recently absorbed dietary amino acids. These labile proteins include enzymes that are excreted into the gastrointestinal tract, hydrolyzed, and the amino acids reabsorbed (Young et al, 1968). By extension, the body generally uptakes amino acids in a familiar pattern as amino acids are released from the breakdown of previously synthesized labile proteins in the gastrointestinal tract (Block, 1956). Minimal oxidation of a particular amino acid occurs at dietary intakes below its requirement and oxidation of the indispensible amino acids is increased in proportion to their intake above the limiting amino acid or individual requirement (Benevenga et al, 1993).

1.2.1 The factorial approach to determining nutrient requirements

The Nutrient Requirements of Swine (NRC, 1998) uses the factorial

approach to define nutrient requirements of swine. In this approach, the quantity of a particular nutrient associated with different metabolic processes (e.g. maintenance, protein accretion, or milk production) is summed to define the entire requirement for that nutrient. For example, a sow during gestation will require a certain quantity of lysine for maintenance plus a quantity for growth, which, summed, represents the total daily lysine requirement for that sow (NRC, 1998).

Table 1.1 – Factors in the factorial approach to defining nutrient requirements in sows

		Maintenance of body tissue	Protein and fat accretion	Milk production
Maintenance	Lysine	36 mg/kg$^{0.75}$	-	-
	Energy	444 kJ/kg$^{0.75}$	-	-
Gestation	Lysine	36 mg/kg$^{0.75}$	44.4 kJ/g protein	-
	Energy	444 kJ/kg$^{0.75}$	52.3 kJ/g fat	-
Lactation	Lysine	36 mg/kg$^{0.75}$	-	22 g lysine/kg litter weight gain
	Energy	444 kJ/kg$^{0.75}$	-	(4.92 x litter gain) – (90 x piglets) kJ

1.2.2 Ideal protein ratio

The British Agriculture Research Council (ARC, 1981) summarized the amino acid requirements for swine and reported them in relation to lysine, based on the suggestion of Cole (1980). The ratios are based on the concept that there is an ideal pattern of the indispensable amino acids that provide an optimal balance of amino acids exactly in proportions with the requirements of the animal, more commonly known as the “ideal protein” ratio (NRC, 1998). One advantage of using the ideal protein ratio concept is that requirements for individual amino

acids can be predicted from the requirement for lysine, which is the amino acid that most is known about. Also, lysine is utilized mostly for protein synthesis, so its requirement is mostly dependent on protein accretion rate. Therefore, the requirements for dietary amino acids can be adjusted for levels of production, based on the requirement for lysine (Baker, 2000). Application of the ideal protein ratio is universal across all species, with remarkable similarities between species (Block, 1956). NRC (1998) reported four distinct ideal protein patterns for sows: maintenance, protein accretion, milk synthesis, and body tissue. Each of these patterns differs slightly in the relative requirements for specific amino acids. For example, the sulfur amino acids and threonine requirements are higher for maintenance than for protein accretion (Baker, 2000).

1.2.3 Importance of lysine for sow nutrition

Lysine is the first limiting amino acid in diets typically fed to swine (NRC, 1998). Therefore, most of the research on amino acid requirements has focused on lysine as the limiting amino acid (Baker, 2000). However, there are few reports of the lysine requirement for maintenance in sows, seemingly due to the apparent unimportance of nutrient requirements for sows at maintenance. Inaccurate requirement values for lysine result in inaccurate requirement values for the other amino acids because of the reliance on lysine as the basis for the ideal protein ratio concept. Also, when using the factorial approach to define total nutrient requirements, the maintenance requirements are included in the total to which requirements for growth during gestation and milk production during lactation are added. Sufficient dietary lysine intake is highly correlated with optimal

reproductive and lactation performance and, ultimately, longevity. Therefore, the lysine requirement for modern sows must be determined to optimize sow productivity.

1.3 Requirements for energy and protein during maintenance

1.3.1 Definition

Within the bodies of living organisms, there are a number of regulatory and metabolic processes which consume energy and nutrients for maintenance of the body tissues. The intake of metabolizable energy (ME) leading to zero energy retention (ER) is defined as the maintenance energy requirement (ME_m) (ARC, 1981). Specifically, ME_m is the energy required by the body for the obligatory metabolic processes that maintain bodily functions and body temperature and allows for moderate physical activity. Obligatory metabolism includes digestion, assimilation, and transport of nutrients, and the production and release of wastes (Wenk et al, 1980). The ME_m is further defined as the energy intake required to maintain constant body weight and body composition (NRC, 1998). Similarly, the intake of protein that leads to zero net nitrogen retention is the maintenance protein requirement. The maintenance intake of protein must provide the indispensable amino acids, plus a supply of amino nitrogen for synthesis of the dispensable amino acids, in order to maintain lean-tissue mass.

1.3.2 Nutrient requirement estimates

Current nutrient requirement estimates have been summarized by ARC (1981) and NRC (1998). However, modern sows are much leaner and larger than sows

used in previous research (Whittemore, 1996). Therefore, energy requirements for the maintenance of body tissue have increased (Tess et al, 1984). Protein turnover has been reported to have increased in modern breeds of swine (Wenk et al, 1980) and in breeds with greater lean-tissue deposition rates (Rivera-Ferre et al, 2006). Biologically, this is necessarily true to support the remarkable lean-tissue growth of modern pigs because protein breakdown concomitantly increases as protein synthesis increases. The result is a greater flux of amino acids through the process of protein turnover and, therefore, a greater requirement for dietary protein intake (Baker, 2000).

1.3.2.1 Energy

Current estimates of the maintenance energy requirement for swine were summarized by the NRC (1998). The reported mean for the daily maintenance energy requirement (ME_m) of 106 kcal ME/kg $BW^{0.75}$ was previously discussed by Samuel (2008) to have been based on ten literature values ranging from 92 to 160 kcal ME/kg $BW^{0.75}$ and included early and conventionally weaned piglets and growing pigs from two to 180 kg BW. The suggested ME_m for sows, based on the reviews of ARC (1981) and NRC (1998) are 458 kJ/$BW^{0.75}$ and 106 kcal/kg $BW^{0.75}$ (equivalent to 444 kJ/kg $BW^{0.75}$), respectively.

The eight other literature estimates of sow ME_m shown in Table 1.2 are all estimates from primiparous animals and of animals not housed in thermoneutral environments and, therefore, should be interpreted with caution (Samuel, 2008). Increased ME_m was previously discussed by Samuel (2008) to result from increased heat production associated with increased lean percentage (Tess et al,

1984; Brown-Brandl et al, 2004) and lean tissue turnover (Wenk et al, 1980) of modern sows.

Table 1.2 – Literature estimates of ME_m for non-pregnant sows

ME_m ($kJ/BW^{0.75}$)	Weight (kg)	Comments	Reference
420	105 – 203	Calorimetry	Close et al, 1985
502	115 – ?	applied energy coefficients of Thorbek (1975); comparative slaughter	De Wilde, 1980
409	114 – 154	average from early (40-60 d), middle (60-80 d), and late (90-110 d) gestation; calorimetry	Noblet and Close, 1980b
452	130 – 161	average from d 56 and d 112 gestation; comparative slaughter; summer	Lodge et al., 1979
640	120 – 132	average from d 56 and d 112 gestation; comparative slaughter; winter	Lodge et al., 1979
753		housed at 5 ºC; comparative slaughter	Hovell et al, 1977
476		housed at 5 ºC; comparative slaughter	Hovell et al, 1977
385	128 – 202	housed at 23 ºC; calorimetry	Holmes and McLean, 1974

1.3.2.2 Protein & amino acid requirement estimates for sows at maintenance

Amino acid requirement estimates for maintenance are based on the concept of the ideal protein ratio and, therefore, expressed in relation to the requirement for lysine. The mean of the maintenance requirements for each of the other indispensible amino acids determined by Baker et al (1966a,b), Baker and Allee (1970), and Fuller et al (1989) were divided by the maintenance requirement for lysine (NRC, 1998).

1.3.2.3 Lysine requirement estimates for maintenance in sows

The National Research Council (NRC, 1998) reported the maintenance lysine requirement to be 36 mg/kg$^{0.75}$ for all ages of swine. The requirement estimate was based on data from Wang and Fuller (1989) from experiments on growing pigs and, therefore, does not necessarily reflect the requirement of sows. Rippel et al (1965) indicated that NRC (1959) estimates of the dietary lysine requirement for maintenance were also extrapolated from data from growing-finishing pigs. Model calculations of the GfE (2008) suggested 38 mg/kg$^{0.75}$ as the digestible lysine requirement for maintenance. Pettigrew (1993) applied a, seemingly, arbitrary apparent digestibility coefficient and calculated a dietary lysine requirement for maintenance of 49 mg/kg$^{0.75}$ for sows from the data of Fuller et al (1989). Consequently, the available estimates for the dietary maintenance requirement for lysine in sows are highly questionable and require further experimentation to obtain reliable estimates for application within the factorial and ideal protein ratio approaches to determine nutrient requirements of modern sows.

1.4 Nutrient metabolism during gestation

The goal of nutrition during gestation is to provide sufficient nutrients for development of the maternal, fetal, and conceptus tissues (NRC, 1998). Maternal gain is especially important for adolescent sows (i.e. parity ≤ four) because they have not achieved adult body weight (Close and Mullan, 1996; Cooper et al, 2001). Therefore, maternal growth must be considered in diet formulations for adolescent animals. Sows which have recently been rebred following lactation must be provided the necessary nutrients to allow for maternal regrowth of tissues mobilized in support of the previous lactation (Etienne et al, 1991). The weaned, and subsequently rebred, sow may obtain some nutrients from the involution of the mammary gland and this may reduce the dietary nutrient requirements. Nutrient requirements during gestation are dependent on the period of gestation (McPherson et al, 2004; Mahan et al, 2009) and tissue mobilization during the previous lactation (Etienne et al, 1991).

1.4.1 Feeding of sows during gestation

Gestating sows are limit fed to avoid excessive weight gain and adiposity because energy intake during gestation is negatively related to feed intake during lactation (Xue et al, 1997). This results in sows not being satiated and therefore eating more than their share of feed when given the opportunity, as in group feeding (Csermely and Wood-Gush, 1990). Different feeding strategies have different impacts on diet formulations. Therefore, energy and protein density of the diet may need to be adjusted to ensure proper nutrition, depending on the feeding strategy (Wenk et al, 1980).

Gestation stalls and electronic feed stations (Leonard, 1988) have a common goal for management of the breeding sow; these systems allow feeding allowances directed toward an individual animal. This is very important for proper body conditioning to maximize the production and longevity of each sow. Consider the extremes of energy intake on sow performance: high energy intakes are associated with increased embryonic mortality, greater body fat deposition leading to decreased conception rate, reduced mobility of the sows, and poor lactation feed intake; low energy intakes are associated with causing reduced numbers of piglets born, weak and very small piglets, and poor lactation performance of the sow exacerbating these problems (Elsley, 1971). Gestation feeding strategy has implications for the formulation of diets; sows fed more frequently may require more energy to compensate for the increased energy expenditure associated with eating compared to less active sows fed larger meals less frequently (Wenk et al, 1980).

1.4.2 Growth of conceptus

Ji et al (2005) dissected whole carcasses at various stages of gestation and determined the chemical content of the maternal and conceptus tissues. Measured accretion of protein in the products of conception (i.e. piglets, placenta, and associated growth of the uterus and fluid accumulation (NRC, 1998)) was greater after day 70 of gestation. Protein accretion for maternal and fetal tissues combined was 40 and 103 g/d, before and after day 70 of gestation, respectively. Mahan (1990) reviewed the mineral requirements of sows and determined that mineral requirements are highest during late-gestation and lactation. Ji et al

(2005) reported that the ether extract content of the whole carcass increased linearly during gestation. Noblet et al (1997) reported that the concentration of energy increased in the products of conception during gestation up to 4,8 MJ/kg. Therefore, rates of tissue deposition are different depending on the tissue of interest, which has implications for the optimum feeding of sows.

1.4.2.1 Placenta

The development of the fetus occurs within the maternal body and, therefore, requires a means by which gases can be exchanged, nutrients delivered, and metabolic wastes removed. The placenta establishes the necessary contact between the chorion of the fetus and the uterus to facilitate the transfer of metabolites to and from the fetus (Ferrell, 1989).

The four common classifications of placental types were originally defined by Grosser in the 1900s based upon the number of interhemal layers between the maternal and fetal circulation. Epitheliochorial type placentae of the pig have the most interhemal layers. Maternal and fetal blood supplies are separated by maternal endothelium, connective tissue, uterine epithelium, chorionic epithelium, connective tissue and endothelium (Ferrell, 1989).

The weight of placenta increases much faster than the fetus early in pregnancy and then the growth rate of the placenta slows down as the fetal weight increases. The placenta continues to grow until late-gestation in the pig and then declines near term (Anderson, 1975; Ji et al, 2005). Placenta weight tends to decrease as parity increases, which can reduce the efficiency of nutrient transport and impact fetal growth rate (Ferrell, 1989).

The efficiency of nutrient transfer across the placenta is a function of its size, structure, and expression of nutrient specific transporters. Vallet and Freking (2007) identified differences in placental structure associated with large and small pig fetuses and the effect on efficiency of nutrient transport. Small fetuses were associated with smaller weight placentae. In a, seemingly, compensatory response, the width of placental microscopic folds of the trophoblast/endometrial epithelial bilayer were increased in these smaller placentae. In the smallest living fetus at day 105 of the experiment, these microscopic folds had almost completely penetrated the stroma. Vallet and Freking (2007) concluded that these changes "may compensate for the crowded intrauterine environment experience by these fetuses". Vallet and Freking (2007) further hypothesized that when this compensatory mechanism becomes exceeded, (i.e. the microscopic folds exceed the availability of the stroma) late pregnancy fetal losses occur. Growth and development of the placentae is essential for the growth and development of the fetuses because it is the exchange system for nutrients and wastes. Therefore, it is important to formulate diets which encourage proper development of the placentae for proper development of the litter.

1.4.2.2 Fetuses

MacPherson et al (2004) employed the comparative slaughter technique to measure fetal growth on various days of gestation. Piglet weight, and the weight of the major internal organs, increased cubically as gestation progressed. Fetal protein gain was relatively limited at 0.25 g/d until after day 69, when protein mass began to increase linearly up to parturition, at a rate of 4.63 g/d. Fetal fat

accretion also showed a bi-phasic linear response, increasing slowly before and much faster after day 69 of gestation. This is consistent with previous observations that the growth of porcine fetuses accelerates during the second half of pregnancy (Knight et al, 1977). Mahan et al (2009) reported that mineral deposition rates within fetal tissues similarly increased more rapidly after day 70 of gestation. Therefore, based on the accretion of protein, fat, and mineral of growing swine fetuses, it seems illogical to provide constant feed intake during the entirety of gestation.

1.4.3 Placental nutrient transport

Nutrient transfer across the placenta is aided by a number of mechanisms, including passive and active transport and passive and facilitated diffusion. Nutrient transfer mechanisms include differences in maternal and fetal hemoglobin affinity for oxygen, placental glucose transporters, endometrial uteroferrin and retinol-binding protein, and amino acid transporters. Free fatty acid transporters have not been identified in the placenta of swine, which is consistent with low body fat content at birth (Père, 2003). Transport of oxygen and other gases and nutrients such as glucose, free fatty-acids and steroids are dependent on blood flow (Reynolds et al, 1985).

Active amino acid transport is the main determinate of amino acid uptake by the fetus. Fetal plasma amino acid concentrations are, on average, twice that of the maternal plasma, indicating active transport in most farm animal species (Ferrell, 1989; Père, 2003). Interestingly, maternal plasma amino acid concentrations have been reported to decrease by 25% very early in human

pregnancy and return to normal levels shortly after parturition (Bonsnes, 1947). Further evidence of active transport comes from experiments demonstrating competitive inhibition and saturation in the transfer of amino acids from the same group (Young, 1971). Neutral amino acids typically have 1 to 1.3 times greater concentration in the fetal plasma compared to the basic amino acids which are typically found at 2.7 times greater concentration (Young, 1971). There have been different transfer rates observed for the different amino acids, but the fetal:maternal ratios of amino acids are consistently high. The 'A-preferring' neutral and basic amino acids are transferred slowly compared to the branch-chain amino acids. Amino acid transport is dependent on maternal blood flow (Young, 1971), which changes during gestation (Reynolds et al, 1985; Père and Etienne, 2007). Infusions of some of the amino acids cause fetal amino acid concentrations to similarly rise, while other amino acids do not have this effect. For example, an infusion of leucine increases fetal plasma concentration of leucine, whereas an infusion of ornithine does not have the same effect in pregnant ewes. Lysine and the branched chain amino acids are typically readily transferred across the placenta, increasing fetal plasma concentrations (Young, 1971). Therefore, transport of amino acids across the placenta is the major determinant of amino acid nutrition of the fetuses.

There is no lipid transport across the swine placenta to the developing fetus, so lipids provided in the diet to the sow or through *de novo* synthesis cannot be transferred to the developing fetuses (Père, 2003). Instead, glucose is the main energy source of the developing fetuses and its' transport is facilitated (Ferrell,

1989). Sows, like other species, gradually develop insulin resistance during gestation which partitions nutrients towards the gravid uterus (Père, 2003). Active amino acid transport provides the necessary amino acids for protein accretion and, when necessary, can deplete the protein reserves of the sow to ensure adequate nutrition of the developing conceptus products (Mahan, 1981). Clearly, the porcine placenta has developed nutrient transport systems and coordinated processes to ensure adequate development of the growing fetuses.

1.4.4 Nutrient requirement estimates for gestation

Pregnancy is the most common condition of sows in modern pork production. Sows are bred for maximum reproductive capacity in both the number and size of the litters (Foxcroft et al, 2005). Sows are capable of producing 2.5 litters per year based on 115 days of gestation and 21 days of lactation. Therefore, nearly 80% of the year sows are pregnant (Lucia et al, 1999). Sows are kept in production for an average of 4 litters in US breeding herds (Rodriguez-Zas et al, 2006) and 3.8 litters in top Canadian herds (Foxcroft et al, 2005). There are a number of reasons to increase sow longevity including: 1) the opportunity to recover the initial economic cost of breeding stock, 2) improved productivity (i.e. litter size and weight), and 3) improved disease resistance (Rodriguez-Zas et al, 2006). Therefore, feeding pregnant sows for optimal maternal and fetal development and longevity is essential for efficient pork production (Mahan, 1977; Mahan, 1981; Aherne and Kirkwood, 1985; Dourmad et al, 1994a,b; Gaughan et al, 1995; O'Dowd et al, 1997; Young and Aherne, 2005; Anil et al, 2006; Tvrdoň and Marková, 2007). Gestation requires a significant input of

nutrients in excess of the maintenance requirements for growth and development of the placenta and fetuses. Approximately 20 to 40% of the energy and amino acids consumed by sows are used for the growth of the uterus, placenta, and fetuses (NRC, 1998). Significant changes to maternal and fetal metabolism occur throughout gestation (McPherson et al, 2004; Ji et al, 2005; Kim et al, 2009; Mahan et al, 2009). Early in gestation, nutrients are required for the development of the placenta. By late-gestation, placental weight begins to decline in sows (Ji et al, 2005). Nutrients are directed to the rapidly developing fetuses, especially in the last one-third of pregnancy. Traditionally, energy and protein required to sustain pregnancy are provided by a constant diet during the entire gestation. Birth weights are minimally affected by maternal nutrient restriction in swine (Mahan, 1981; Ferrell, 1989). However, the short-term and long-term effects of nutrient restriction over multiple parities on the overall reproductive efficiency are unknown (Ball et al, 2008).

Sow nutrient requirements for gestation have been reviewed in the past for energy (ARC, 1981; Noblet and Etienne, 1987; Verstegen et al, 1987; NRC, 1998) and protein (ARC, 1981; Speer et al, 1990; Whittemore and Morgan, 1990; Pettigrew, 1993; NRC, 1998). However, recent reviews of nutrient requirements for pregnant sows appear to be limited to Dourmad et al (2008) and GfE (2008).

1.4.4.1 Energy

Only nine reports were found in the literature that specifically measured the ME_m for pregnant sows (Table 1.3). Reports by Lodge et al (1979) and Noblet and Close (1980a,b) were investigations comparing the partition of ME intake at

several stages of pregnancy to non-pregnant animals. Body weight gain was greater for pregnant animals, in both studies, due to the growth of products of conception. Noblet and Close (1980b) indicated that, by separation of total gain into maternal and reproductive portions, weight gain of the pregnant animals was not different in early (i.e. 47 and 49 days) and mid (75 and 68 days) gestation compared to non-pregnant animals at equal energy and protein intakes. They did report, however, that weight gain of pregnant animals in late (98 days) gestation was lower than occurred in non-pregnant animals of similar age. Further, Noblet and Close (1980b) reported little difference in the efficiency of energy utilization for maintenance (k_m) between the pregnant and non-pregnant animals. Lodge et al. (1979) also reported no differences in efficiency of energy utilization for maintenance for pregnant animals compared to non-pregnant animals. The energy requirement of pregnant sows is predicted by the factorial approach of NRC (1998), and therefore, requires reliable estimates of ME_m to which additional energy requirements for pregnancy (i.e. maternal and conceptus growth) can be added. The results of Noblet and Close (1980a) indicate that pregnant sows require 27 to 30 MJ ME of energy supplied daily during gestation.

Pregnant sows require energy intake above maintenance to support growth of maternal, placental, and fetal tissues during gestation (Millward et al, 1976; Reeds et al, 1982). The ME_m reported by ARC (1981) of 458 $kJ/BW^{0.75}$ equates to 24 MJ of energy required for maintenance for a 200 kg sow. NRC (1998) indicated that energy intake above 25 MJ ME/d will increase maternal weight gain. Energy intake predicted by NRC (1998) software suggests that pregnant

sows should receive at least 125% of ME_m, equivalent to 30 MJ, to support associated tissue gains during gestation. Additional energy intake should also be provided to replace lean and lipid tissues losses from the previous lactation (Etienne et al, 1991). Therefore, recommendations of Dourmad et al (2008) were stratified by parity and show an energy requirement of 33.5 MJ ME/d for gilts and suggest an increase of 3.5 MJ ME/d for the second and third parities. The recommended weight gain of sows is 25 kg of maternal tissue for at least the first three or four parities. Additional weight gain, associated with the placentae and products of conception, of approximately 20 kg should be expected (NRC, 1998). However, as modern sows typically have larger litters, conceptus weight gain should be expected to be greater. For example, the weight gain in the products of conception is assumed to be 2.28 kg/pig (NRC, 1998). Therefore, a litter size of 12 piglets equates to 27 kg of weight gain associated with the products of conception alone. Ultimately, energy intake limits the growth and development of maternal tissue, however, fetal tissue growth is not dependent on dietary intake (Anderson, 1975).

Table 1.3 – Literature estimates of ME_m from pregnant sows

ME_m ($kJ/BW^{0.75}$)	Weight (kg)	Parity[1]	Comments	Reference
418	133 – 219	P	average from early (30-50 d), middle (50-80 d), and late (95-110 d) gestation; calorimetry	Noblet and Etienne, 1987
422	105 – 203	P	Calorimetry	Close et al, 1985
427	100 – 162	P	Calorimetry	Burlacu et al, 1983
513	115 – ?	P	applied energy coefficients of Thorbek (1975); comparative slaughter	De Wilde, 1980
407	116 – 200	P	average from early (40-60 d), middle (60-80 d), and late (90-110 d) gestation; calorimetry	Noblet and Close, 1980b
452	130 – 180	P	average from d 56 and d 112 gestation; comparative slaughter; summer	Lodge et al., 1979
661	118 – 146	P	average from d 56 and d 112 gestation; comparative slaughter; winter	Lodge et al., 1979
530[2]		P	comparative slaughter	Hovell et al, 1977
444	128 – 202	P	housed at 18 ºC; calorimetry	Holmes and McLean, 1974
418	168 – 227	M	Assumed ME_m of 100 kcal	Verstegen et al, 1971

[1]: P = primiparous; M = multiparous;

[2]: $BW^{0.85}$

1.4.4.2 Protein and amino acids

Early research focused on the crude protein content of the diet as the indication of its nutritional value (Duee, 1976; Young et al, 1976; Haught et al, 1977; Mahan, 1977; Wahlstrom and Libal, 1977; Greenhalgh et al, 1980). It was concluded that increasing protein intake during gestation increased the body weight gain of the gilt or sow. However, it was noted that increased gain in gestation was correlated with increased body weight loss during lactation. None of the studies reported an effect of protein intake on litter size. In two studies (Young et al, 1976; Mahan, 1977) birth weight of the piglets was increased by increasing the protein content of the gestation diet and the difference was maintained to day 21 of lactation before it declined. The increased crude protein contents of the diets were achieved by the incorporation of soybean meal into the previously cereal-only diet. However, because cereal grains are low in lysine the inclusion of a complementary protein source such as soybean meal better meets the protein requirement of swine (NRC, 1998). Anon (1978), using corn-soybean meal diets, and Greenhalgh et al (1977, 1980), using a barley-based diet with added soybean and fish meal, determined that a 9% and 11% were the minimum crude protein contents for an adequate gestation diet, respectively.

The amino acid requirements of sows, for amino acids other than lysine, during gestation have been predicted using the ideal protein ratio concept by ARC (1981), NRC (1998), and GfE (2008). However, utilizing the ideal protein ratio concept may not correctly predict the amino acid requirements due to the differences in amino acid requirements for maintenance and growth (Kim et al,

2009). Whereas, during the first third of pregnancy, the demand for nutrients is the deposition of body stores lost during the previous lactation (Jackson et al, 1977; Etienne et al, 1991) in late-gestation, the demand for nutrients is maintenance of the maternal tissue and growth of the fetal tissues (McPherson et al, 2004). Thus, a combination of the ideal protein ratio for maintenance and growth may reflect the actual dietary requirement, but is difficult to predict without investigations determining the actual dietary amino acid requirements.

1.4.4.3 Lysine

Previous estimates of the lysine requirement for reproduction (Rippel et al, 1965; NRC, 1973; Woerman and Speer, 1976) were determined during late pregnancy and, therefore, do not represent the requirements for the entirety of gestation. Current recommendations (NRC, 1998) report the lysine requirement as a mean value over the entirety of gestation. Dourmad and Etienne (2002) also found that lysine requirement was not affected by period of gestation. Alternatively, Dourmad et al, (1994b, 1999) and Kim et al (2009) reported that the late-gestation requirement for lysine is greater than during early-gestation. Srichana (2006) and GfE (2008) have more recently reported that the dietary lysine requirement was greater than predicted by NRC (1998) and greater during late-gestation than during early-gestation for adolescent sows. Srichana (2006) determined that N retention was maximized when 13 and 17 g/d of standard ileal digestible lysine was provided in early- and late-gestation, respectively, to primiparous sows.

1.5 Nutrient metabolism during lactation

1.5.1 Feeding of sows during lactation

It has been well established that *ad libitum* feeding of lactating sows is required to maximize productivity through milk production, reduced wean to estrus interval, and increased subsequent litter size (Koketsu et al, 1996a). However, it has also been shown that allowing sows to consume feed *ad libitum* within 4 days after parturition can reduce feed intake over the total of the lactation period (Neill and Williams, 2010), thus reducing milk production and body condition (Koketsu et al, 1996a). Consequently, it is recommended that producers properly manage sow feed intake during gestation and lactation to avoid drops or persistent low feed intakes. Koketsu et al (1996a,b) and Neill and Williams (2010) recommend that an artificially imposed gradual increase in lactation feed intake is beneficial to increase overall lactation feed intake.

1.5.2 Nutritional factors affecting milk production

Modern sows are capable of producing 10 to 15 litres of milk per day or 1 kg per piglet (Auldist et al, 1998). First litter milk production is generally lower than during subsequent lactations (Boyd et al, 2000b). Greater dietary lysine intake is correlated with increased milk production (Pettigrew, 1993). Additional valine has been shown to increase milk production, although the effect is controversial (Gaines et al, 2006). Excess energy intake during gestation negatively impacts milk production during lactation (Mahan and Mangan, 1975; Kim and Wu, 2009), decreases lactation feed intake, and increases body weight

loss (Clowes et al, 2003a,b).

1.5.2.1 Nutritional factors affecting mammary gland development

Inadequate nutrition during late-pregnancy limits mammary tissue development (Head and Williams, 1991; Beyer et al, 1994; Kim et al, 1999; Ji et al, 2005). There is a high requirement for threonine in development of the mammary gland secretory tissue (Ji et al, 2005). The demand for nutrients by the developing fetuses is so great that, when nutrient intake is limited, nutrients are not available for mammary gland development. This reduces milk production and, therefore, negatively impacts litter growth. Therefore, it is imperative that sows receive sufficient nutrition during late-gestation to encourage growth of the mammary gland.

1.5.3 Nutrient requirement estimates for lactation

1.5.3.1 Protein, amino acids & energy

It has been shown that increasing the dietary crude protein and energy content of the lactation diet increases milk production in sows. It is important to note that the two main amino acids affecting milk production are lysine and valine. The dietary lysine requirement of sows was investigated most recently by Srichana et al (2007). Total lysine intake ranged from 53.5 to 70.0 g/d resulting in a linear increase in litter growth rate, weaning weight, and average daily gain of the piglet. Litter growth rate was highest at the highest dietary lysine intake (1.35% total lysine) meaning that the requirement was not less than 70 g/d. Coma et al (1996) determined the lysine requirement of lactating sows by measuring plasma urea nitrogen. They determined that, for sows nursing 10 piglets gaining

2.22 kg/d, the dietary lysine requirement was 55.3 g/d.

The second most studied amino acid for lactating sows, after lysine, is valine. Dietary valine intake equal to (Tokach et al, 1993) or above 100% of lysine intake (Richert et al, 1996; Richert et al, 1997a,b; Guan et al, 1998; Moser et al, 2000) has been reported to improve lactation productivity through increased litter weaning weight and growth rates due to increased milk production. Alternatively, no effects of additional dietary valine intake were observed by Feng et al (1996) or Boyd et al (1995). Gaines et al (2006) concluded, based on measures of maternal tissue loss and piglet growth rates, that the dietary valine requirement was not greater than the NRC (1998) value of 85% of lysine, which supported earlier work by Carter et al (2000) and Southern et al (2000). However, Gaines et al (2006) also noted that dietary valine of 125% of lysine improved litter gain in sows fed a diet containing 0.275% L-lysine·HCl. The lysine intakes ranged from 52.1 to 55.3 g/d and the valine intakes ranged from 40.0 to 66.1 g/d. The value of 85% of lysine intake was modified from Pettigrew (1993) by NRC (1998) to reflect the uptake of valine by the mammary gland in excess of that present in milk (Boyd et al, 1995; Richert et al, 1996; Trottier et al, 1994; Trottier et al, 1997). Finally, Gaines et al (2006) concluded that further experiments are required to determine if valine is the second limiting amino acid for lactating sows and the appropriate ratio to lysine. Results from experiments to determine amino acid requirements of sows during lactation may be difficult to interpret due to amino acids mobilized from body tissue (Lewis and Speer, 1973; Kim et al, 2001).

1.6 Application of the concepts of homeorhesis versus homeostasis to gestation and lactation

1.6.1 Definitions

Homeostasis is defined as the "maintenance of physiological equilibrium or constant conditions in the internal environment" of an organism (Bauman and Currie, 1980) "through a series of interacting physiological processes" (Webster English Dictionary, 1993). The most common example of homeostasis is the regulation of body temperature in animals despite varying external environmental temperatures. This is in contrast to the definition of homeorhesis, which is defined as the coordinated metabolic processes necessary for the maintenance of a physiological state. Homeorhesis specifically relates to pregnancy and lactation where the maternal body tends to prioritize nutrients to growth and development of the offspring.

1.6.2 Partitioning of nutrients during gestation and lactation

Homeostatic control serves to regulate ongoing metabolic processes of the body in order to achieve and maintain a stable internal environment. Because of the importance of reproduction to the long term survival of a species, high priority is given to the flow of nutrients to the offspring *in utero* and through milk production. Homeorhesis is the term applied to the prioritization of nutrients to support the physiological state of reproduction. The priority of nutrients to the development and growth of the offspring is so important that it is possible for a disease state to be induced in the mother during pregnancy and lactation when

metabolic processes essential for homeostatic control do not receive sufficient nutrients (Bauman and Currie, 1980).

1.6.3 Insulin resistance during gestation and lactation

Insulin resistance has been observed in humans, rats, guinea pigs, rabbits, sheep, and pigs to develop progressively as an adaptive response to increasing nutrient demands of the gravid uterus. Maternal nutrient intake is commonly inadequate in sows during the final 1/3 of gestation because feed intake is controlled in gestating sows and is not typically adjusted during gestation. Maternal tissues instead utilize other energy substrates, including free fatty acids, thus sparing glucose for the gravid uterus (Père, 2003). Insulin resistance has also been observed to continue into lactation from late gestation (Mosnier et al, 2010a,b), again as an adaptive response to prioritize nutrients to the mammary glands. Insulin resistance is reversed once the metabolic demands of pregnancy and lactation are removed (Père and Etienne, 2007). Diabetes mellitus is not observed in swine, however poor and varying glucose tolerances have been reported for adult swine (Père, 2003).

Glucose is the main energy substrate of the developing fetuses. Ultimately, the growth rate and development of the litter is regulated by the availability of glucose. Plasma glucose concentration in the maternal circulation is 2.5 times greater than in the fetal circulation, owing, in part, to the inefficiency of the porcine placenta. In fact, fetal plasma glucose concentration increases very little in response to changes in maternal plasma concentration.

Insulin resistance is the inability of normal amounts of insulin to induce

effects on target cells due to reduced insulin receptor response (Paz et al, 1997). A serine/threonine kinase cascade can induce serine/threonine phosphorylation of critical IRS-1 sites, thereby inhibiting IRS-1 binding and activation of phosphoinositol 3-kinase, resulting in reduced insulin-stimulated glucose transport (Paz et al, 1997).

1.6.4 Ketosis

Ketosis is the metabolic state characterized by elevated levels of ketone bodies in the blood. Ketone bodies are produced through ketogenesis when liver glycogen stores are depleted. Glycogen stores can become depleted because dietary carbohydrate intake is insufficient (Alsop et al, 1994). The ketone bodies acetoacetate and ß-hydroxybutyrate are used for energy, especially by the brain, because the brain cannot utilize fatty acids for energy. Ketone bodies can produce acetyl-CoA, which can be utilized for lipogenesis in the brain during short-term starvation or they can be oxidized to provide energy through the Krebs cycle (Garrett and Grisham, 1999).

Alsop et al (1994) reported that ketosis is rarely diagnosed in commercial swine production, making it difficult to evaluate its prevalence and importance to sow health. The authors provide a case report of a sow which presented with no signs of systemic illness. However, the sow exhibited severe inappetence, which is a clinical symptom of ketosis when ketones accumulate in extracellular fluid. Urinalysis detected moderate levels of ketone bodies, which subsequently doubled before parturition. After parturition, the levels of ketone bodies gradually decreased to normal as energy intake became sufficient. Ketosis is an adaptive

response of the body to provide energy to the most vital organ, the brain. However, the liver is prone to fat accumulation during ketosis (Alsop et al, 1994) and, therefore, metabolic disorders associated with non-alcoholic fatty liver disease, including insulin resistance and impaired hepatic lipid metabolism (Adams et al, 2005). Because ketosis can be caused by and then perpetuate reduced feed intake and is largely undiagnosed, ketosis in sows could result in uncharacterized herd removal. Therefore, it imperative that sows receive sufficient carbohydrate intake to prevent or reduce ketosis.

1.7 Metabolic tracers and methods

1.7.1 Introduction

Wolfe (1983) reported that the most common technique used for studying human metabolism is to measure the concentration of a substance in the blood at different time points. A change in plasma concentration can be used to determine the rate of appearance or disappearance of the substance and any change in concentration of the measured metabolite would therefore indicate a metabolic event either using or producing the metabolite. The A-V flow technique measures the arterio-venous difference in plasma concentration of a metabolite across a limb or organ. The difference is multiplied by the flow rate to yield a value of net uptake or release. The error of such a measurement is profound, including an estimated 20% error in the measurement of the flow rate alone (Wolfe, 1983). Consequently, Wolfe (1983) states that any conclusions about metabolic events based on plasma concentrations alone "are totally invalid".

In the study of metabolism, interactions with other metabolites, chemical

processes, and any changes in concentration must be known. Attempting to follow the metabolism of a particular substance by directly measuring concentrations tends to be inaccurate or impossible due to the complex of metabolic reactions using and producing the metabolite and inaccessible metabolic pools. The metabolic interactions of a substance can be measured indirectly, however, by using a tracer. Tracers are substances that relate to the metabolism of a specific metabolite, but are easily detected and quantified from accessible metabolic pools.

Tracers are detectable due to isotopic substitutions, either radioactive or stable, at specific locations in the molecule. Depending on the size and characteristics of the tracer being used, a variable number of isotope substitutions can be made within the tracer molecules. Sometimes more than one type of isotope substitution is made per molecule of tracer. Tracers may be the metabolite itself with isotope substitutions or a molecule directly (Wolfe, 1992) or indirectly (Pencharz and Ball, 2003) related to the metabolism of that metabolite. The use of tracers allows compartment visualization and quantification of flow rates of metabolites (Bier, 1997). For example, Arends and Bier (1991) reported that hippurate, α-ketoioscaproate, and α-aminoadipate appear to be useful as direct tracers in stable isotope studies of glycine, leucine, and lysine, respectively, in hepatic protein turnover.

1.7.2 History of isotope tracers in metabolic research

Metabolic studies using tracers began in the 1930s at Columbia University with studies by Schoenheimer and Rittenburg when they used deuterium, a common stable isotope tracer, to study fat metabolism in mice. In the 1950s and

1960s radioactive tracers were often used due to the availability of various detectors of radioactive decay. Radioactive tracers can be quantified with devices such as liquid scintillation counters or gamma detectors. In the 1970s, the use of stable isotopes as metabolic tracers saw a resurgence (Wolfe, 1983). Bos et al (2002) reviewed the application of isotopes in studies of protein and amino acid requirements and concluded that these types of studies are providing a clearer understanding of amino acid requirements.

1.7.3 Principles of isotope tracer studies of protein and amino acid kinetics

The defining principle of an isotopic tracer is that the tracer must accurately indicate the metabolism of the tracee because it is not discriminated from the unlabeled molecule (Wolfe, 1984). Metabolic tracer doses are typically very small and kept minimal such that the administration of the tracer does not perturb the system being observed (Shipley and Clark, 1972).

Kinetics of protein and amino acid metabolism may be studied by compartmental or stochastic analysis. Compartmental analysis relies on the assumption that specific pools, or compartments, can be identified and that movement of the tracer between compartments can be described by exponential equations. Alternatively, stochastic analysis ignores any compartmentalization present within the system (Shipley and Clark, 1972); only transfers between the accessible, sampled pool and the rest of the body are considered (Waterlow et al, 1978).

1.7.4 Common assumptions associated with isotope tracer studies of protein and amino acid kinetics

Isotope tracer studies require that the following assumptions are recognized and that the conditions under which tracer studies are conducted conform to these assumptions (Waterlow et al, 1978):

1) the pool into which the tracer enters and samples are collected must be representative of the precursor pool for protein synthesis,
2) pools are in steady-state and, therefore, do not change in size,
3) material entering a pool mixes uniformly and instantaneously with material already present in the pool, and
4) there is a constant fraction of material which is exchanged per unit time.

When all of the above conditions have been met, either stochastic or compartmental analysis may be used to quantify the kinetics from isotope tracer studies of protein and amino acid metabolism.

Acceptance of an additional assumption is necessary for the carry out of isotopic tracer studies. It is assumed that the metabolic fate of the tracer infused is an end-product which does not allow recycling of the tracer. That is, the source of tracer is assumed to be entirely from the infusion and, therefore, the rate of movement through the system can be quantified, based on the rate of infusion. However, although recycling of the tracer can be measured (Swick, 1958), consideration of tracer recycling is often ignored to simplify analysis (Slevin and Waterlow, 2008).

1.7.5 Stable isotope tracers

The numerous advantages of stable isotopes have been reviewed by

several individuals (Bier, 1997; Koletzko et al, 1998; Rennie, 1999; Wolfe, 1984). Stable isotopes have the obvious advantage that they are non-radioactive, and do not place experimental subjects at risk, allowing for experimentation in infants, even while still *in utero*, adults of child-bearing age, and test subjects with a compromised health status. Repeat measurements can be taken within the same subject, without exceeding maximum dose limits, which must be considered when using radioactive isotopes. Some atoms, namely N and O, have no suitable radioactive isotopes; therefore, stable isotopes are the only choice for use as tracers (Bier, 1997).

1.7.5.1 Mass spectrometers used in metabolism research

Two types of mass spectrometers are commonly used in metabolic research to quantify the abundance of stable isotope tracers. These are the isotope ratio and gas-liquid chromatography mass spectrometers. Both of these techniques can be used in metabolic studies to quantify the rate of appearance or disappearance of the tracer and, therefore, tracee (i.e. the substance being traced). The resurgence of stable isotopes as metabolic tracers was most likely due the increased ease of use and availability of quadrupole mass spectrometers interfaced with gas chromatographs. The usefulness of mass spectrometry was summed up by Bier (1997) as being "rapid, sensitive, specific, and precise". Mass spectrometry provides the molecular mass and structural fragments of the sample molecule, even across many compound classes. Another major advantage of mass spectrometry (MS) is the fact that it can be used to measure anything with mass (i.e. everything) including gases, liquids, and solids (Dass, 2001).

1.7.5.2 Electron ionization

As the sample is injected into the inlet, ions are created by one of many ionization methods. The most common ionization method is electron ionization (EI). EI has an upper mass range of 1000 Daltons and is applicable to most thermally stable and relatively volatile compounds, thus covering a wide range of samples. EI uses energetic electrons to bombard the sample as it is introduced through the inlet into the ionization chamber. The energy of the electrons must be greater than the ionization energy for the molecule in order to cause ionization. Commonly, electrons are produced by heating a thin filament of rhenium wire. The electrons then encounter a weak magnetic field parallel to the electron beam that tends to direct the electrons away from the rhenium wire source in a straight line. This increases the likelihood of ionization of the sample. Ionization occurs when an energetic electron collides with a sample molecule causing an electron to be ejected from the molecule. The result of the electron collision is a positively charged molecule with an unpaired electron, known as a radical cation. This can be represented by the equation: $M + e^- \rightarrow M^{+\bullet} + 2\ e^-$, where M represents the sample molecule, e^- represents an electron and $M^{+\bullet}$ represents the radical cation (Dass, 2001).

The newly formed radical cations are forced out of the heated ionization chamber by a positive repeller voltage. The ions are then further accelerated and focused by a series of negatively charged accelerating plates. Following the ions exiting the ionization chamber, there is a short field-free region. The ions soon enter the mass analyzer where they are separated according to their mass-to-

charge ratio (Dass, 2001).

1.7.5.3 Conventional mass analyzer

In an instrument using a conventional mass analyzer, a magnetic field acts on the charged ions resulting in a curved flight path. In general, the greater the mass-to-charge ratio of a molecule, the larger the radius of curvature of its flight path. Only ions of specific mass-to-charge ratios are able to negotiate the fixed radius path of the mass analyzer and then strike the detector. Therefore, the accelerating voltage or the magnetic field is continuously varied to allow ions of all the different mass-to-charge ratios to strike the detector in sequence.

To improve the differentiation of the various mass-to-charge ratios by the detector, a double-focusing mass spectrometer can be used. In a double-focusing mass spectrometer, the radical cations pass through an electrical field before entering the magnetic field. Together, the electrical and magnetic fields focus the ions according to both energy and direction. Double-focusing may result in fewer ions striking the detector because more ions are filtered out of the ion beam during focusing, however the overall mass-to-charge differentiation at the detector improves. The resolution, R, of a mass spectrometer, is defined by $R = M / \Delta M$ and is a measure of the ability of an instrument to detect the difference, ΔM, between the mass of a particle, M, and the mass of a particle of the next highest mass that can be detected by the instrument (Pavia et al, 2001).

1.7.5.4 Isotope ratio mass spectrometry

Isotope ratio mass spectrometry (IRMS) allows for the measurement of the relative abundance of isotopes very precisely. Wolfe (1983) states that for

metabolic studies an IRMS instrument must be capable of a precision of 10 parts per million for the most important metabolic element ratios. IRMS is capable of the greatest resolution of the lowest tracer-to-tracee ratios of any MS technique. Therefore IRMS is a commonly employed technique when the isotope tracer will be diluted through metabolic events such that no other MS technique can be used. For example, when a labeled amino acid tracer will be incorporated into protein or urea, the isotope tracer will often be too dilute in the sample for any other MS analysis (Wolfe, 1983). The resolution of a sample analyzed by IRMS is possible because the isotope labeled sample is compared to a standard gas under identical conditions (Hachey et al, 1987). In modern instrumentation, a dual-inlet system allows alternating measurements of the sample and the standard gas several times for each analysis (Wolfe, 1992).

Typically each IRMS sample must be 10 – 50 mL in volume. The samples for IRMS must be in pure gaseous form. This means that for the common elements of metabolic studies, hydrogen, nitrogen, carbon, oxygen, and sulfur, gaseous H_2, N_2, CO_2, and SO_2 samples must be prepared before analysis by IRMS. As a result, IRMS has the disadvantage of requiring significant sample preparation. For example, Hachey et al (1987) described the conversion of isotope labeled water samples to hydrogen gas using uranium turnings at 620^{o}C. Sample preparation for IRMS also carries the disadvantage that any information about the location of the isotope label in the metabolite tracer is destroyed (Bier, 1997).

1.7.5.5 Gas-liquid chromatography

Validation of gas-liquid chromatography (GC) as an important analytical

technique by James and Martin (1952) lead to the first commercially available apparatus in 1955. GC allows the separation of volatile compounds based on different chemical properties. As the compounds are forced through a small diameter column by an inert carrier gas, known as the mobile phase, the compounds move at different rates according to interactions with the column, known as the stationary phase. The compounds then reach a detector that records their abundance. GC, as the name implies, requires that the compounds being separated be in the gas phase. To that end, the injection port of a GC is commonly heated to cause vaporization of the injected sample. The powerful separation techniques of GC are often coupled with the powerful identification properties of infrared, NMR, and mass spectrometers (Skoog et al, 1996).

1.7.5.6 Gas-liquid chromatography mass spectrometry

The combination of GC with mass spectrometry (MS) is of particular relevance to metabolic research using stable isotopes to label tracers. Wolfe (1983) points out that mass spectrometers date back to the early 1900s, but biological research using MS was limited by the mass spectrometers available. Specifically, inlet systems and scanning methods limited biological applications. The combination of GC and MS, two already powerful stand-alone analytical techniques, is usually known as GCMS. There is a significant difference in the operating pressures of GC and MS. GC operates at a pressure too high for the sample to be admitted directly into the vacuum required for MS operation. Therefore, one of three main types of separators is found at the interface of the GC output and MS input. A jet, effusion, or indirect membrane separator serve to

reduce the volume and pressure of the carrier gas. As a result, the concentration of the sample increases and pressure of the sample decreases (Wolfe, 1992).

1.7.5.7 Quadrupole mass analyzer

A common modification from conventional magnetic field MS is the quadrupole mass analyzer system. Known by the descriptive name, quadrupole mass analyzer, this system consists of four cylindrical rods. These rods are arranged in a square matrix parallel to the ion beam. The quadrupole system performs mass separation by using an electrodynamic field. Opposite rods are connected as a pair and a direct-current voltage and a radio-frequency component are applied. In one pair of the rods, a positive direct-current potential and radio-frequency component are applied. In the other pair of rods, a negative direct-current potential and a radio-frequency 180^{o} out of phase from the first are applied. Ions are injected at one end of the quadrupole system from the ionization chamber. Provided that the mass-to-charge ratio for the ion is appropriate for the current electrical field, the ion travels between the rods and reaches the detector. Very narrow ranges of mass-to-charge ratios are able to obtain stable trajectories and travel through the field for a particular direct-current potential and a radio-frequency component. Any ions that do not achieve stable trajectories oscillate uncontrollably until they strike one of the rods or are ejected from the field. Therefore, the electrodynamic field can be varied over time by the instrument in order to allow all of the different mass-to-charge ratios to eventually reach the detector (Pavia et al, 2001). A quadrupole mass analyzer mass spectrometer instrument can be set such that only specific ranges or single masses of ions pass

through the mass analyzer and reach the detector, known as selective ion monitoring. Selective ion monitoring offers distinct advantages in metabolic tracer sample analysis because fewer particles reach the detector, reducing noise, and improving resolution (Wolfe, 1983).

1.7.5.8 Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) combines two or more quadrupole mass analyzers in series. This has the advantage of increasing the specificity, sensitivity, and speed with which analyses can be performed. Selected mass ion or multiple reaction monitoring are possible, which increases specificity. Sensitivity is increased by enhanced signal-to-noise ratio because fewer ions strike the detector. Finally, speed is increased because little or no sample derivatization or sample cleanup are necessary before analysis (Rafii et al, 2009). Liquid chromatography can be combined with tandem mass spectrometry for analysis of L-[1-^{13}C]phenylalanine enrichment from plasma or urine (Turner et al, 2006).

1.7.5.9 Fragmentation

Electron ionization is commonly used with IRMS and GCMS. A key feature of EI is that energy above the ionization energy of the sample molecule is transferred to the molecule. The newly formed radical cation may be unstable due to the excess energy. As bonds within an energetic radical cation are broken, energy is released producing more stable products. This process is known as fragmentation. These separate fragments of the sample molecules are then detected after passing through the mass analyzer. Patterns of fragmentation are very indicative of the original structure of the injected sample molecules.

Fragmentation is pivotal for elucidating the exact location of the isotopic substitution or substitutions because fragmentation is so predictable for the vast majority of compounds (Pavia et al, 2001).

1.7.5.10 Mass spectra

A mass spectrum is the plot of abundance of ions versus mass-to-charge ratio from the detector output. The mass-to-charge ratio is often written as m/e or m/z, where e or z refers to the charge on the ion. For the majority of radical cations, the charge will be one. The mass spectrum from modern instruments is converted to a bar graph. The most abundant ion creates the tallest peak, known as the base peak. Using the base peak as a reference, the relative abundance of the other peaks is reported as a percentage. Peaks on the mass spectrum represent fragment ions as a result of fragmentation of the sample in the ionization process, but for one exception. If we ignore the presence of isotopes momentarily, the highest mass-to-charge ratio ion is known as the molecular ion. This peak is a result of loss of one electron from of the original sample molecule. Therefore, the molecular ion, symbolized M^+, represents the molecular weight of the sample molecule. The presence of peaks above M^+ is due to isotopic substitutions. These higher weight peaks typically occur one or two mass units above M^+, so they are commonly known as the M + 1 and M + 2 peaks (Pavia et al, 2001).

1.7.5.11 GCMS data processing

Data from GCMS is rapidly generated. As a result, data must be collected and processed by a computer. Wolfe (1992) points out that there are two specific modes useful during isotope studies for metabolic research. One mode is used for

scanning and storing several complete mass spectra, while the other mode is used for scanning only selected ions. The latter mode, known commonly as SIM, for selected ion monitoring, is used for the measurement of isotope abundances. In this mode, the mass analyzer filters out any ions that are not of interest (Wolfe, 1992).

1.7.5.12 Isotope ratio calculation

The isotope enrichment of a sample can be determined by the ratio of the molecular ion, M^+, peak to the M + q peak, where q is number of mass units of enrichment. The value of q is dependent on the number and type of isotope substitutions that were made to the tracer. The SIM mode is used with a quadrupole mass analyzer to selectively scan for only the M^+ (i.e. tracee) peak and the M + q (i.e. tracer) peak. In order to determine the tracer-to-tracee ratio and take into account the naturally occurring abundance of isotopes, the following approximation is made:

$$\frac{\text{Tracer}}{\text{Tracee}} \approx \left(\frac{M+q}{M^+}\right)_{obs} - \left(\frac{M+q}{M^+}\right)_{nat}$$

In the above equation, adapted from Wolfe (1992), the first term is subscripted "obs" to designate this is the observed data from SIM of the tracer and tracee after isotope infusion. The second term is subscripted "nat" to designate this is the natural abundance of stable isotope as determined by measurement of a background sample before any isotope infusion begins. Measurement of the natural background is necessary because the environment contains a certain level of naturally occurring isotopes (Wolfe, 1992).

1.7.6 Substrate turnover rate equations for metabolic research

Tserng and Kalhan (1983) qualify three equations commonly used in substrate turnover rate studies using stable isotopes. The equations that they found to be accurate enough for metabolic studies are:

Turnover rate = $[(\text{isotope enrichment (\%)})^{-1} - 1] \times (\text{infusion rate})$ and

Turnover rate = $[(100/\text{mole \% excess}) - 1] \times (\text{infusion rate})$.

These equations generate the same answer, but use slightly different units for the isotope data. The above equations require the unique standard curve reported by Tserng and Kalhan (1983). A third equation is more useful when using the conventional GCMS standard curve. The value for the mole ratio of the tracer-to-tracee can be obtained directly from the standard curve regression equation. This equation will give very similar answers to those above, but using slightly different input data. It is:

Turnover rate = (infusion rate) / (mole ratio of tracer-to-tracee).

Below is an example calculation that uses GCMS isotope ratio data from Tserng and Kalhan (1983). Using the isotope enrichment value of 0.69 mole % excess from 240 minutes after the simultaneous constant infusion of [U-$^{13}C_6$]glucose and [6,6-$^{2}H_2$]glucose began, the infusion rate for [6,6-$^{2}H_2$]glucose of 8.68×10^{-2} $\mu mol \bullet kg^{-1} \bullet min^{-1}$, and the first equation from above, the turnover rate for glucose, using [6,6-$^{2}H_2$]glucose as the tracer, can be calculated (Tserng and Kalhan, 1983):

Turnover rate for glucose

from [6,6-$^{2}H_2$]glucose $= [(\text{isotope enrichment (\%)})^{-1} - 1] \times (\text{infusion rate})$

tracer infusion

Turnover rate for glucose

from [6,6-$^{2}H_2$]glucose = [(.69%)$^{-1}$ – 1] x (8.68 x 10^{-2} μmol•kg^{-1}•min^{-1})

tracer infusion

Turnover rate for glucose

from [6,6-$^{2}H_2$]glucose = 12.49 μmol•kg^{-1}•min^{-1}

tracer infusion

1.7.7 Methods in the measurement of protein turnover

Measurement of protein turnover has a long history in the literature (Garlick et al, 1994). For some 50 plus years, protein turnover measurements have been made various subjects and under various physiological conditions. There are two common tracers used in the measurement of protein turnover, namely ^{15}N-glycine and L-[1-^{13}C]leucine. Historically, experiments utilizing the tracer ^{15}N-glycine can be found in the literature earlier than those utilizing labeled leucine (Duggleby and Waterlow, 2005).

1.7.7.1 ^{15}N-glycine

Fern et al (1985) tested nine different ^{15}N-labeled amino acids and single oral or IV dosages of the amino acids to determine which could be used as tracers of protein turnover. Originally, ^{15}N-glycine as a tracer of protein turnover began using urinary urea as the measured end-product. More recently, ammonia was suggested to be a more appropriate end-product due to the small, rapid turnover of the ammonia pool compared to the urea pool (Duggleby and Waterlow, 2005). ^{15}N-glycine was identified as the best tracer because flux calculated from ammonia and urea end-products is the same by oral and IV routes of tracer

administration and flux from ammonia divided by flux from urea shows the smallest deviation from unity (Fern et al, 1985). Thus, the indication is that ^{15}N-glycine behaves as an appropriate tracer by accurately tracing the metabolism of the trace, in this case protein metabolism, when given by either IV or IG routes (Picou & Taylor-Roberts, 1969). The use of ^{15}N-glycine as a tracer qualified the basic assumption of the end-product method that the enrichment of N in the end-product precursor pool is the same as in the precursor pool for protein synthesis (Duggleby and Waterlow, 2005). Bier & Matthews (1982) identified that the distribution of ^{15}N in the amino acids isolated from plasma albumin was similar to that of free amino acids in plasma. This clearly identifies plasma as the precursor pool for albumin synthesis since, due to labeling of the free amino acids in plasma, albumin was labeled similarly. Early protein turnover measurements were performed exclusively with ^{15}N-glycine because of its availability and, relatively, low cost. Support for ^{15}N-glycine as a metabolic tracer of protein metabolism was discussed by Matthews et al (1981). The experiment of Fern et al (1985) allowed Duggleby and Waterlow (2005) to estimate the variability of the method when repeated on the same two subjects; the CV were of the order of 5–6 % which indicates high reproducibility of the measurement protein turnover using ^{15}N-glycine as tracer.

1.7.7.2 Leucine

Leucine has been a popular choice for studies of protein turnover. In fact, when leucine is infused by IV for two or more hours, it is considered the 'gold standard' method for measurement of protein turnover (Duggleby and Waterlow,

2005). Investigations using leucine as the tracer measure the α-ketoisocaproate (α-KIC) enrichment as the precursor of leucine metabolism. However, the appropriateness of leucine as a tracer for protein and amino acid kinetics has been questioned because leucine is known to stimulate the mTOR pathway, which, in turn can stimulate protein synthesis (Anthony et al., 2000; Lynch, 2001). The measured end-product of labeled leucine is expired carbon dioxide which must be corrected for retention within the body carbon pool. The carbon dioxide recovery of sows was reported by Moehn et al (2004b) as 81.0% during frequent feeding, 78.8% during meal-feeding and postprandially, and 58.1% during fasting.

1.7.7.2.1 α-KIC

It is necessary to know the concentration of amino acid directed to protein synthesis, but this cannot be directly measured. Instead, it is possible, using tracers, to determine the oxidation and, by knowing it has an inverse relationship with synthesis, the amino acid used for protein synthesis can be determined. Measurement of protein turnover using α-KIC is proposed to circumvent the assumptions about the pool from which protein is synthesized or oxidized due to the compartmentalization of the trans-amination of leucine to its corresponding alpha-ketoacid (Schwenk and Beaufrere, 1985). The trans-amination occurs in the muscle because of the presence of branched-chain amino acid aminotransferase (EC 2.6.1.42), which is not found in high concentrations in the body, except within the skeletal muscle. Thus, oxidation of the leucine must be initiated in the muscle before the alpha-keto acid is transported to the liver for complete oxidation by branched-chain alpha-ketoacid dehydrogenase complex. However,

trans-amination is not the rate limiting step in leucine catabolism (Harris and Payton, 1985; Schwenk and Beaufrere, 1985) which means that the leucine and α-KIC should equilibrate rapidly. Therefore, it was hypothesized, and subsequently shown, that determining the reciprocal of the infused tracer amino acid (i.e. α-KIC for leucine) in the extracellular plasma pool, will lead to a more accurate description of the intracellular amino acid conversions (Schwenk and Beaufrere, 1985). Flux is calculated from α-KIC enrichment at plateau and the infusion rate of leucine (Matthews et al, 1980).

1.7.7.2.2 Oral versus IV infusion

Biolo and Tessari (1997) measured the splanchnic uptake of leucine. The route by which the tracer is delivered (i.e. oral versus IV) is important when calculating whole-body protein turnover because there are differences in flux calculated from oral intake of the tracer versus IV infusion (Matthews et al, 1993). Biolo and Tessari (1997) determined, during meal ingestion, that one fourth of absorbed leucine is metabolized by the splanchnic tissues. Therefore, oral intake of the tracer results in greater flux being calculated due to the splanchnic utilization of the tracer reducing the appearance of leucine (and therefore α-KIC) into the plasma pool (Matthews et al, 1993). Block (1956) has suggested that appearance of the label in the plasma pool, when given orally, may be delayed due to the significant contribution of free amino acids derived from hydrolysis of digestive enzymes in the gastrointestinal tract.

1.7.7.3 Phenylalanine

Phenylalanine is a good choice for studying effects of the diet on tracer

kinetics because it is oxidized in the liver. Therefore, the metabolism of phenylalanine will be affected by the amino acid profile entering from the portal vein (Ball and Bayley, 1984). For this reason, along with the fact that it has a small, stable pool size which has a, relatively, rapid turnover rate and is irreversibly oxidized to CO_2, measurement of protein turnover can be combined with the indicator amino acid oxidation method for determining amino acid requirements (Ball and Bayley, 1984).

1.7.8 Methods in the determination of amino acid requirements

1.7.8.1 Comparative slaughter

In comparative slaughter experiments, the chemical composition of a group of representative whole carcasses are determined. The carcass energy content is measured by sub-sampling from a homogenous mixture of the ground whole carcass and determining the energy content by complete oxidation in a combustion calorimeter (Close and Stanier, 1984). Comparative slaughter determines the dietary nutrients retained in the body tissues during the experimental period by comparison to the initial slaughter group. The difference between the final and the initial chemical composition of the animal carcasses is taken to represent dietary nutrients retained over the study period. The initial body composition and energy content of the animals is determined by a sample of animals at the beginning of the experimental period (Noblet and Le Dividich, 1982) or by data from previous research (McNutt and Ewan, 1984). Therefore, the comparative slaughter method has the advantage of directly associating carcass composition with energy metabolism (McNutt and Ewan, 1984). However,

disadvantages of the comparative slaughter technique include the competing factors of cost (i.e. labour) and precision. The relative balance between the two must be determined based upon the expected treatment effect, statistical confidence level, and applicability of predicting measurements to determine the number of animals required for the whole of the experiment and representative sub-samples (Kempster et al, 1982). Kempster et al (1982) stated that "live animal assessment in experiments and population studies involving carcase [sic] evaluation is often overlooked" and could "increase the precision of the study considerably". Thus, information collected from live animals, through techniques of *in vivo* body composition measurements and calorimetry, are preferable to comparative slaughter. Calorimetry has the advantage over the serial slaughter technique in that it can be used to measure energy balance over successive short periods of time, even within days, and different components of HE (van Milgen et al., 1997; van Milgen et al, 2000). The calorimetry technique typically gives higher estimates for energy and protein retention than does the comparative-serial slaughter technique (Quiniou et al., 1995; Birkett and de Lange, 2001) because the "material" and endogenous losses are well accounted for.

1.7.8.2 Nitrogen balance

Nitrogen balance experiments have been performed in rats (Hegsted and Neff, 1970; Dreyer, 1975), pigs (Fuller et al, 1987; Scipioni and Martelli, 2001), humans (Rose, 1957; Leverton et al, 1959), and various other species to determine requirements for dietary amino acids. Nitrogen balance experiments have investigated specific physiological conditions, such as the low birthweight infant

(Duffy et al, 1981). In general, N balance experiments are useful as a means to assess nutrient quality and have the sensitivity to detect small changes in dietary amino acid intakes (Low, 1982). The balance concept is simple; what goes into the system must be, in some way, accounted for so that the overall system is balanced. Nitrogen intake from protein (i.e. amino acids) in the diet is accounted for by quantitative collection and determination of losses present in the feces and urine to indirectly determine the N retained by the pig (Gatel and Grosjean, 1992). However, nitrogen balance studies typically overestimate nitrogen retention. Nitrogen intakes are overestimated because measurement errors are difficult to avoid and nitrogen losses (e.g. hair, skin, secretions) are underestimated or ignored (Wallace et al, 1959; Just et al, 1982; van Kempen et al, 2003; Elango et al, 2010). Consequently, nitrogen balance studies result in falsely high estimates and with wide confidence limits (Fuller and Garlick, 1994). The WHO (2007) concluded that the serious technical weaknesses associated with nitrogen balance resulted in estimated requirement values for humans that were too low and concluded that the new gold standard should be indicator amino acid oxidation.

1.7.8.3 Plasma urea nitrogen, plasma free amino acids

It has been suggested that plasma metabolites reflect the adequacy of indispensable amino acid intake. High levels of plasma urea nitrogen indicate an imbalance of amino acid intake or a higher than required intake of protein because significant amino nitrogen has been released during oxidation of the amino acids in excess and must be transported through the body circulation as urea before elimination (Pedersen and Boisen, 2001). Mitchell et al (1968) observed a linear

response of plasma free lysine to graded levels of intake which led them to propose that a period of "metabolic adaptation" to deficient levels of the test amino acid is necessary. This is likely based on the ability of the body to release amino acids from the labile protein pool. Changing dietary intake of the test amino acid in the short-term can be buffered by the breakdown of proteins from the labile protein pool. In the longer term, once the labile protein pool has been exhausted of the amino acid that is deficient in the diet (Young et al, 1968), a broken-line response to graded intake should be expected. In this response, plasma free amino acid levels of the test amino acid increase only after intake of the test amino acid is above the requirement (Mitchell et al, 1964). Therefore, the results from experiments using plasma free amino acid concentration as a criterion for determining amino acid requirements should be interpreted with caution.

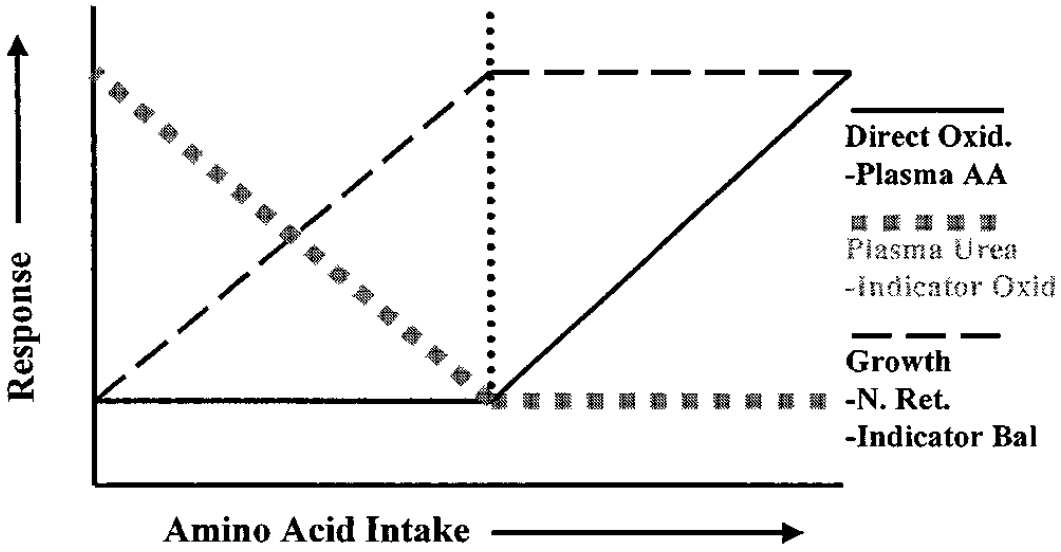
1.7.8.4 Indicator amino acid oxidation

The indicator amino acid oxidation (IAAO) method has been recognized as the gold standard for defining amino acid requirements (WHO, 2007). Increasing levels of the test amino acid are provided, either enterally or parenterally, and the oxidation of the indicator amino acid is measured (Pencharz and Ball, 2003). The IAAO method was originally developed and validated in piglets by comparison to nitrogen balance and growth results (Kim et al, 1983). Theoretically, any indispensible amino acid can be used as the tracer. However, possible indicator amino acids are typically limited to leucine, lysine, and phenylalanine because they are the most common isotope labeled amino acids

(Pencharz and Ball, 2003). Phenylalanine, with additional tyrosine, has been utilized extensively in the IAAO method because of the small, stable pool size and rapid turnover and the fact that it is oxidized in the liver, so its metabolism is directly affected by diet (Ball and Bayley, 1984). The IAAO method is based on two principles of amino acid metabolism:

a) there is no storage of dietary amino acids in the body, except when incorporated into protein. Therefore, dietary amino acids have two, inversely related, fates: oxidation or incorporation into protein. And,
b) oxidation of the indicator amino acid represents the oxidation of the amino acids in excess of the amino acid limiting protein synthesis.

Figure 1.1 – Comparison of the three different metabolic response patterns to graded intake of an indispensible amino acid (Adapted from Pencharz and Ball, 2003)



Advantages of the IAAO method include the fact that adaptation to a new dietary intake level is not required (Moehn et al, 2004a) and that the indicator reflects all entry and degradative pathways of the test amino acid (Bos et al,

2002). From the first advantage, a number of dietary levels can be tested rapidly within the same subject, which reduces the variation of the response (Pencharz and Ball, 2003). The second advantage means that the requirement estimate reflects the true total metabolic requirement for the entirety of metabolism of the test amino acid (Bos et al, 2002).

1.8 Concepts related to protein turnover

1.8.1 Definition

Protein turnover is defined as the ongoing, continuous processes of protein synthesis and breakdown which occurs in the body (Duggleby and Waterlow, 2005). Therefore, proteins of living cells are constantly being broken down and resynthesized from dietary or recycled amino acids and results in the destruction of some of the amino acids (Block, 1956).

1.8.2 Amino acid recycling

The process of protein turnover requires input of amino acids to replace those released from lean tissue during protein breakdown. Most of the necessary indispensable amino acids are derived from the diet and synthesized into protein. However, some of the amino acids from protein breakdown are recycled into new protein products or lean tissue accretion (Waterlow, 1995). The extent of amino acid recycling has been investigated in plants (Davies and Humphrey, 1978) and in animals (Swick, 1958; Klevecz, 1971; Dallman and Manies, 1973). Swick (1958) calculated the probability of reutilization of lysine from degradation of liver proteins to be 50 percent when dietary lysine intake was minimal.

1.8.3 Maintenance versus growth

The balance of the concomitant processes of protein breakdown and synthesis represent whether lean tissue mass of an animal increases, decreases or remains constant. At maintenance, the processes of synthesis and breakdown are balanced, so that no net gain of protein occurs. During periods of growth, the balance of the processes is towards synthesis, but it is important to note that breakdown does not halt completely (Block, 1956). In fact, Waterlow (1995) indicated that protein accretion in the infant is characterized by a high rate of protein breakdown and a higher rate of protein synthesis. And, finally, lean tissue loss occurs when the process of protein breakdown is greater than protein synthesis (Block, 1956).

1.9 Summary

This literature review has attempted to highlight the current knowledge in protein and energy nutrition of non-pregnant, pregnant and lactating modern sows. However, in so doing, gaps and weaknesses in the knowledge have been identified. These gaps include the current estimates of the maintenance energy requirement and dietary lysine requirement, both of which are included in the factorial approach to define nutrient requirements of sows. Furthermore, the lack of knowledge of individual amino acid requirements was discussed. Therefore, the latter part of this literature review was focused on techniques that might be used to improve sow nutrition.

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2.0 RATIONALE, HYPOTHESES AND SPECIFIC OBJECTIVES

2.1 Rationale

Modern sows are a product of genetic selection for improved reproductive capacity and growth rate, especially of lean tissue. This has resulted in sows that are more likely to rebred successfully, even after an intense lactation when significant body stores are mobilized to support the milk production required for the high growth rates of the offspring. Sows are larger and leaner, meaning:

1) they have less body fat which can be mobilized for energy, and
2)greater energy requirements for maintenance to support protein turnover associated with lean tissue mass.

2.2 Null hypotheses

1a) Indirect calorimetry combined with quantitative collection of expired CO_2 will not react independently of each other.

1b) Measurements of protein and energy metabolism will not be different when non-pregnant sows are fed two distinct protein and energy intakes.

1c) Numerical correction factors used to derive 24 h values calculated from periods of differential feeding frequencies or during fasting will not be different.

2a) Protein and energy balances will not be different between days 30, 45, and 105 of gestation when pregnant sows are fed a conventional gestation diet.

2b) Protein and energy balances will not be different between days 7 and 19 of lactation when lactating sows are fed a conventional lactation diet.

2c) Protein and energy balances will not be different between pregnant and lactating sows.

3a) The dietary lysine requirement for modern, non-pregnant sows will not be different than the NRC (1998) estimate of 36 $mg/kg^{0.75}$.

4a) The dietary lysine requirement for modern, pregnant sows will not be different than can be estimated by NRC (1998) during gestation.

4b) The dietary lysine requirement for pregnant sows will not be different comparing early- and late-gestation.

2.3 Specific objectives

Before simultaneous studies of protein and energy metabolism can be undertaken, an appropriate system for the quantification of expired gases must be designed and built. The first objective of the first study was to design, develop, and demonstrate the quantitative operation of an indirect calorimetry system that would allow the simultaneous measurement of heat production and expired CO_2 production for the assessment of amino acid requirements and protein turnover using stable isotopes. However, validation that the system reacts appropriately to changes in a single gas concentration do not necessarily mean that the system will react appropriately to simultaneous changes in gas concentrations. Therefore, the second objective of this study was to determine, simultaneously, parameters of protein and energy metabolism of non-pregnant sows at two energy intake levels

over 24 h to validate that changes in the gas concentrations induced by sow respiration are detectable and quantifiable and reactive to changes in energy and protein intake. It is desirable to conduct future amino acid requirement studies in the fed state over periods of less than 24 h. The third objective of this study was to determine what, if any, numerical correction factors would be required to derive 24 h values of energy and protein metabolism from studies of a shorter duration, including future experiments of amino acid requirements (Chapter 3.0).

Protein and energy metabolism should be studied simultaneously to ascertain if interactions occur. The objectives of this experiment were to simultaneously measure protein and energy balance in gestating and lactating sows to provide data about differential changes in energy and protein metabolism during gestation and lactation and, therefore, identify periods of changing requirements of breeding sows. Secondary outcomes will also be recorded. These include sow body weight change, litter size and growth rate, and milk production (Chapter 4.0).

The objective of this study was to determine the dietary lysine requirement of modern non-pregnant sows using the indicator amino acid oxidation method. Secondary outcomes, including simultaneous measurement of heat production and respiratory quotient, phenylalanine flux, plasma free amino acid concentrations and nutrient digestibility, will also be recorded (Chapter 5.0).

The objective of this study was to determine the lysine requirement of a population of 2nd and 3rd parity sows in early- (day 24 – 45) and late- (day 86 – 110) gestation using the indicator amino acid oxidation method. Secondary

outcomes, including simultaneous measurement of heat production and respiratory quotient, phenylalanine flux, plasma free amino acid concentrations, weight gain, subsequent litter size and nutrient digestibility, will also be recorded (Chapter 6.0).

3.0 INTEGRATED METHODOLOGY FOR SIMULTANEOUS MEASUREMENT OF ENERGY AND PROTEIN METABOLISM IN SOWS INCLUDING VERY LONG TERM CATHETERIZATION

3.1 Introduction

Traditionally, parameters of energy and protein metabolism have been determined separately. Therefore, simultaneous measurements are scarce despite the fact that indirect calorimetry and endproduct measurements of isotope kinetics require similar experimental equipment (Junghans and Chwalibog, 2001). Simultaneous measurements of energy and protein metabolism require that each measurement technique be independent of influences from the other. The first objective of this study was to design, develop, and demonstrate the operation of an indirect calorimetry system that would allow the simultaneous measurement of $^{13}CO_2$ production for assessment of amino acid requirements and protein turnover using stable isotopes. The major benefit of simultaneous measurements is that interactions of energy and protein intake can be ascertained.

Current feeding recommendations (NRC 1998; Aherne et al, 1999) may not be appropriate for optimum productivity of the modern, high producing sow. Modern breeds are a result of constant genetic selection which has resulted in greater growth rates and higher productive and reproductive capacity (Foxcroft et al, 2008) and thus increased daily nutrient requirements. Selection of pigs for greater leanness has increased body protein content, resulting in higher maintenance requirements (Kolstad and Vangen, 1996), due to increased protein turnover (Wenk et al, 1980). Because there are very few current data on sows, the

second objective of this study was to determine, simultaneously, parameters of protein and energy metabolism of non-pregnant sows at two energy intake levels over 24 h to validate that changes in the gas concentrations induced by sow respiration are detectable and quantifiable and reactive to changes in energy and protein intake.

Isotopic studies of amino acid requirement were initially believed to require continuous intravenous infusion for extended periods of time to obtain the steady state necessary for stochastic calculations of amino acid flux and protein turnover (Aub and Waterlow, 1970; Slevin and Waterlow, 2008). However, frequent oral feeding of the isotope has been shown, in humans (Zello et al, 1990; Bross et al, 1998), dogs (Shoveller, personal communication), and growing pigs and sows (Moehn et al, 2004a) to result in an adequate, apparent steady state, within approximately 6 h, in breath and plasma necessary to obtain plateau values in enrichments (Slevin and Waterlow, 2008). Studies of energy metabolism with calorimetry still typically use periods of 24 hr or more (van Milgen et al, 1997). To conduct future studies of energy expenditure rapidly and cost-effectively, in conjunction with isotope studies of amino acid metabolism, periods of measurement shorter than 24 h need to be considered. However, to derive valid 24 h values from such studies, the relationship between a chosen experimental duration and daily values needs to be established (El-Khoury et al, 1994). This relationship was studied by testing a frequent, half-hourly feeding regimen, modified from Moehn et al (2004a,b), for 8 h followed by a bolus meal. Therefore, the third objective of this study was to determine what, if any,

numerical correction factor would be required to derive 24 h values of energy and protein metabolism from studies of a shorter duration.

3.2 Methods and materials

3.2.1 Respiration system

Two independent respiration chambers were constructed; each using a standard farrowing crate with a rear door for the animals to enter and exit the chamber. Access to the animals was through a removable piece of plexiglass on 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 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 in fresh air (250 L/min) through the inlets which was evenly distributed via pipes running the length of the chambers with holes drilled every 30 cm. Total air volume removed from the chambers was recorded by two independent AC630 gas meters (Canadian Meter, Edmonton, AB) and recorded manually every 30 minutes. A sub-sample of the total air volume was drawn by two separate vacuum pumps from the main air flow and fed under positive pressure at a flow of 200 mL/min to the gas analyzers (O_2 – S103; CO_2 – S153; CH_4 – S127, (Qubit Systems, Kingston, ON). The oxygen (O_2), carbon dioxide (CO_2), and methane (CH_4) concentrations of the sample air were recorded in 60 second intervals as the average of 200 samples by C409 data acquisition software (Qubit Systems, Kingston, ON).

3.2.2 Indirect calorimetry system – method of validation

Firstly, the accuracy of the oxygen sensors and recovery of the system were tested by injecting a measured flow of nitrogen (see Appendix A for details) over a known time into the sealed respiration chambers. The total injected nitrogen was calculated and compared to the reduction in measured O_2 by the sensors. Secondly, the CO_2 sensors and the entire system were tested by dissolving a known quantity of $NaHCO_3$ into water. A solution was placed into each of the respiration chambers and concentrated $HCl_{(aq)}$ was delivered from a syringe driven by an injection pump. The chambers were sealed before the syringe pumps were started. As the $HCl_{(aq)}$ was delivered, CO_2 was evolved (see Appendix B for details). The amount of CO_2 measured by the sensors was quantified and compared to the quantity of $NaHCO_3$ added (Samuel, 2008).

3.2.3 Quantitative breath CO_2 collection

Part of the air flow (150 mL/min) was diverted from the total air flow (250 mL/min) for CO_2 trapping to enable analysis of ^{13}C enrichment in breath CO_2. A midget bubbler (Fisher Scientific, Mississauga, ON or VWR International, Edmonton, AB) was suspended in a solution of 1 *N* $NaOH_{(aq)}$ (~11 mL). The solution was sampled and replaced at 30 min intervals. An air flow of 150 mL/min with an anticipated mean CO_2 content of 0.5%, was calculated to achieve a sufficient amount of $^{13}CO_2$ absorbed in the NaOH for isotope analysis.

3.2.4 Long-term, in-dwelling catheterization of sows

Surgery was developed for a surgically implanted in-dwelling catheter and injection port because this catheter had been previously reported to remain patent

and functional for years with low risk of infection and complications (Appelgren et al, 1996; Foley et al, 2002; Swindle et al, 2005). Such a device allows repeated blood sampling over periods of months without the necessity of repeated surgical insertion of a new catheter. Each animal underwent a surgical implantation of a Carmeda Bio-Active Surface (CBAS) (Instech Laboratories, Inc., Plymouth Meeting, PA) coated catheter inserted into the cephalic vein, tunneled under the skin to the side of the neck and terminated with a subcutaneous titanium injection port (Swindle et al, 2005). An incision of approximately 6 cm length was cut medial to the point of the shoulder along an imaginary line at an angle of approximately 45 degrees to the midline. The starting point of the incision is midway between the two tendons elevated by caudal flexion of the leg at the shoulder. The incision is made along the groove that forms between these and the sternocephalicus. The internal cephalic vein was exposed by blunt dissection of the subcutaneous fat and connective tissue. The vessel was dissected free of surrounding fascia. Forceps were passed under the vein and the ends of two 10 cm lengths of 0 silk were grasped. Using another set of forceps to lift the vein, the forceps holding the two 10 cm lengths of 0 silk were drawn under the vein. The loose ties of silk suture string were separated and placed at opposite ends of the vein separated to a distance of 2 cm along the length of the vein. The ends of each suture string were clamped with a pair of forceps such that the suture was looped under the vein to allow it to be elevated at each end. The vein was then elevated using the suture at the proximal end, allowed to fill with blood, and then the distal end was lifted. A small hole was made in the vein in the direction of blood flow.

Approximately 13 cm of the catheter was inserted into the vein. The catheter was tied at the proximal end of the vein around both the catheter and the vessel to prevent bleeding and then at the caudal end using monofilament polypropylene 2-0 or 0. A slightly curved incision (approximately 12 cm diameter) of 10 cm total length was made 10 cm cranial to an imaginary line directly up from the cranial side of the right leg, approximately at the height of and caudal to the base of the ear. A pocket was formed by blunt dissection under the flap of skin created by the incision to allow for the insertion of the subcutaneous port. The catheter was tunneled subcutaneously from the initial incision to the second incision, where it was exteriorized. The port was then sutured into place using monofilament polypropylene. The skin incisions were both closed. The port and catheter was locked with 100 IU/ml heparinized saline through a Huber needle, and then flushed, and re-locked every two weeks. Records were kept of the length of time used and any complications, interventions, and subsequent outcomes.

3.2.5 Animal studies

The experiment described herein was conducted simultaneously with an experiment described previously (Samuel, 2008). Data related to energy metabolism was previously reported by Samuel (2008) and is repeated here for completeness and to assist in interpretation and integration with the data about protein metabolism.

The studies described below were designed to investigate the energy and protein balances of sows fed two distinct feeding levels. Energy and protein balance were measured over 24 h to give daily values. Daily values are required

for the expression of requirements for energy and protein within feed allowances.

3.2.5.1 Animals

All procedures used in this study were approved by the Faculty Animal Policy and Welfare Committee of the University of Alberta. Six Large White/Landrace sows, non-pregnant after their 1st litter (body weight (BW) 174±11 kg) were selected from the herd (Swine Research and Technology Centre, Edmonton, AB) (Samuel, 2008) before being surgically fitted with cephalic vein catheters terminated with a subcutaneous injection port. The sows were left to recover for 1 week post surgery and adapted to the test feed intake for 1 week. Feed intake was determined daily; body weight weekly.

3.2.5.2 Accessing injection ports for blood sampling and isotope infusion

EMLA cream (lidocaine 2.5%/prilocaine 2.5%, AstraZeneca Canada Inc., Mississauga, ON) was applied to reduce sensation of pain over the port at least one hour before access and covered with a bandage. The ports were accessed using aseptic technique, which required two trained persons. The person accessing the port donned sterile gloves using aseptic technique and the assistant used the wrapper as a sterile surface for the sterile materials. The assistant removed the bandage and cleaned the area above and around the port with aseptic soap and water and 70% 2-isopropyl alcohol before surgical iodine solution (Prepodyne, Delaval Inc., Kansas City, MO) was sprayed onto the skin access site and allowed to dry. After drying, the injection port was accessed by the sterile person using a right-angle, non-coring Huber needle with attached extension (Instech Laboratories, Inc., Plymouth Meeting, PA) and a sterile 10 mL syringe (BD –

Canada, Mississauga, ON). The old locking solution (100 IU heparin/mL saline) was aspirated and discarded before at least 15 mL of normal saline was infused using "push-pause-push" on the syringe plunger to create turbulent flow within the catheter. On experimental days, an extension set was attached for infusion of the isotope solution (3.2.5.4) and blood collection (3.2.5.6). Finally, new locking solution (100 IU heparin/mL saline) was infused into the catheter through the injection port. Flushing of the catheters occurred every two weeks.

3.2.5.3 Diets and feeding

The sows were fed the regular dry sow diet used at the SRTC, which was based on wheat and barley with equal contributions of soybean and canola meals (Table 3.1). Diatomaceous earth (Celite®) was included at 10 g/kg of diet as an indigestible marker to allow measurements of nutrient digestibility from smaller samples of feed and feces (McCarthy et al, 1977). The diets were formulated to provide all nutrients needed by a pregnant sow of 175 kg body weight (NRC, 1998) (Consultant Feeds Ltd., Calmar, AB, Canada). The diet was first fed at the intake required to meet the maintenance energy requirement (ME_m) of 458 $kJ/BW^{0.75}$, (ARC, 1981), noted herein as feeding level 1.0 and then twice that amount, noted herein as feeding level 2.0. Sows were fed at each level for one week before measurements of energy and protein metabolism. Grab samples of feces were taken within the last three days on each feeding level.

Table 3.1 – Diet composition of dry sow ration[1]

Ingredient	**g/kg**
Wheat[2]	121
Barley[3]	690
SBM[4]	65
Canola Meal[5]	65
Canola Oil	9
Breeder Premix[6]	40
Celite	10
Calculated	
Ca, %	0.95
Total P, %	0.72
DE, MJ/kg	13.06
CP, %	14.76
Total Lys, %	0.65

[1]: Reproduced from Samuel (2008).

[2]: 12.5% CP

[3]: 11.2% CP

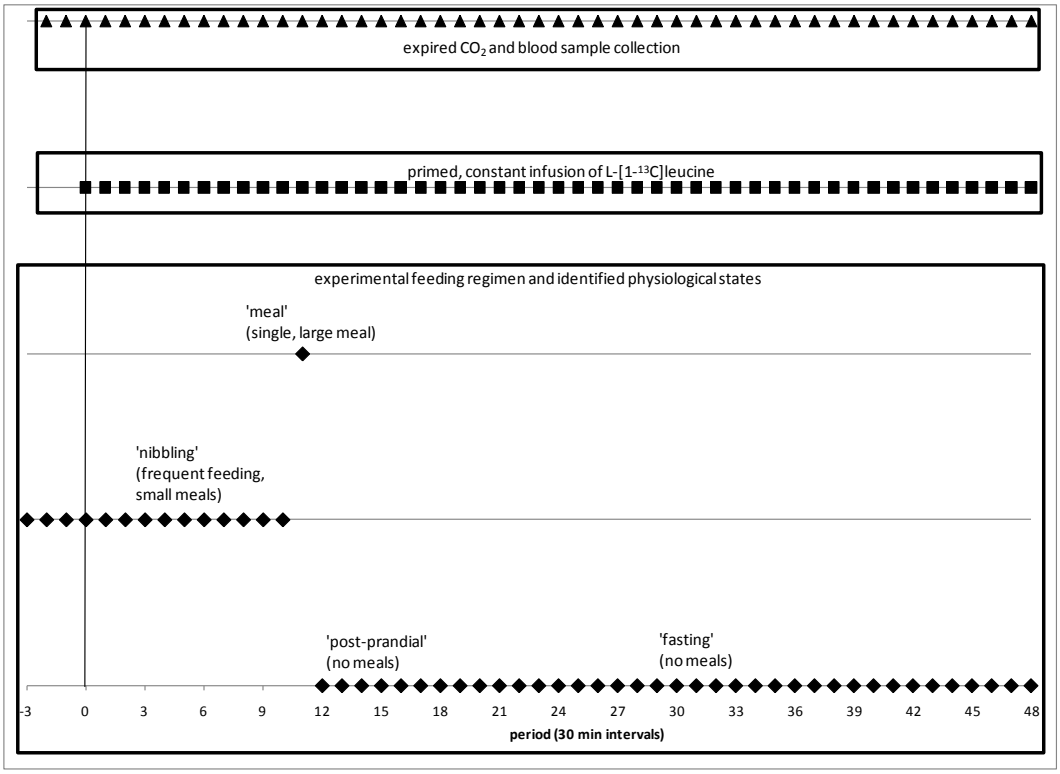
[4]: 48% CP

[5]: 35% CP

[6]: Provided per kilogram of the diet: Ca, 8.6g; P, 3.4g; Na, 1.9g; Mg, 140mg; K, 30mg; Fe, 139 mg; Zn, 119 mg; Mn, 56 mg; Cu, 16 mg; Co, 0.4 mg; I, 0.4 mg; Se, 0.3 mg; vitamin A, 12,000 IU; vitamin D_3, 1200 IU; vitamin E, 62 IU; vitamin K, 2.5mg; biotin, 0.6 mg; folic acid, 2.5mg; niacin, 42 mg; pantothenic acid, 25 mg; pyridoxine, 5mg; riboflavin, 9.5 mg; thiamine, 8.4mg; vitamin B_{12}, 28μg.

Sows were fed twice daily, except for respiration days, when the frequent feeding protocol was tested. On respiration days they were given 16 half-hourly meals equal to 1/32 of their daily feed allowance, followed in the afternoon by the remaining half of the daily allowance. Thus, several physiological states could be identified (Figure 3.1): 'nibbling' during the frequent feeding phase, 'meal fed' during the 1 h needed to consume the afternoon meal, 'postprandial' for 8 h after the afternoon meal, and 'fasting' for the remaining duration of the 24 h study period. This feeding regimen was imposed to test the frequent feeding schedule for future IAAO studies, to test the effects of different feeding strategies on energy and protein metabolism, and to derive relationships between measurements recorded during the identified physiological states and 24 h values.

Figure 3.1 – Sample collection, isotope infusion, and identified physiological states[1] from experimental feeding regimen over 24 h



[1]: frequent feeding = 1/32 of daily feed allowance every 30 minutes; single, large meal = ½ of daily feed allowance after 8 h; post-prandial = starting 2 h after large meal and for 8 h; fasting = 10 h after large meal and for 6 h

3.2.5.4 Indirect calorimetry

Before each study, the S108 oxygen (O_2), S153 carbon dioxide (CO_2), and S126 methane (CH_4) analyzers (Qubit Systems, Kingston, ON) were calibrated to zero with N_2 for the O_2 and CO_2 analyzers and a gas of known [O_2] and the balance N_2 for the CH_4 analyzers. The upper limits of the analyzers were calibrated with a gas of known [O_2] (approximately 21%), [CO_2] (approximately 1.5%), and [CH_4] (approximately 100 ppm) and the balance (approximately 77.5 %) N_2 (Praxair, Edmonton, AB). O_2, CO_2, and CH_4 concentrations were collected by C409 data acquisition system as an average of 200 samples in one-minute intervals during the study (Qubit Systems, Kingston, ON). The gas exchange of the animals was determined by comparison to the ambient air values as recorded immediately before and after the study periods. The analytical values for each of the calibration gases was recorded immediately before and after the study and used to correct for drift of the analyzers, if necessary.

3.2.5.5 Isotope infusion rates

The stable-isotope labeled amino acid tracer L-[1-^{13}C]leucine (Cambridge Isotope, Andover, MA) was used to measure protein turnover (Jahoor, 2003). However, based upon a review of current literature this tracer had not previously been used in sows and therefore the appropriate tracer dose had to be determined. A dose of 0.5 $mg \cdot kg^{-1} \cdot h^{-1}$, chosen based on a review of data from human studies, failed, in a preliminary study (data not shown), to produce $^{13}CO_2$ enrichment sufficiently above the natural background enrichment for measurement of atom percent excess (APE) (Jones et al, 1985). A tracer dose of 1.0 $mg \cdot kg^{-1} \cdot h^{-1}$ was

subsequently found to be adequate, resulting in $^{13}CO_2$ of no less than 0.0003 AP above background and was used thereafter with a priming dose of 1.0 $mg \cdot kg^{-1}$ delivered at the start of the infusion.

For two sows, the isotope was delivered orally with each meal at both feeding levels to simulate the route of administration in future amino acid requirement studies. This also provided a measure of the splanchic metabolism of the tracer required to calculate whole-body protein turnover (WBPT). The remaining three sows received the isotope by IV as a primed, constant infusion using a syringe pump.

3.2.5.6 Quantitative expired CO_2 collection

A sub-sample of the air flow from the respiration chambers flowed through midget bubblers (Fisher Scientific, Mississauga, ON or VWR International, Edmonton, AB) suspended in 1 *N* NaOH solution at a rate of ~150 mL/min. A single midget bubbler in ~11 mL of $NaOH_{(aq)}$ was shown to provide complete and quantitative trapping of CO_2 (3.2.3). The solution was changed every 30 min and transferred equally into two evacuated, glass, 16 x 100 mm, serum collection tubes (Vacutainer, Oakville, ON) and frozen at -20^{o} C until analysis. Expired CO_2 samples were collected at 90, 60, and 30 minutes before the start of the infusion to establish basal production of $^{13}CO_2$ (Jones et al, 1985).

3.2.5.7 Blood collection

Blood samples were collected at 30 min intervals in glass or plastic Vacutainer tubes containing sodium or lithium heparin (BD – Canada,

Mississauga, ON). Blood was centrifuged at 1500 * g, the supernatant was transferred to centrifuge tubes and then stored frozen at -20^{o} C until analysis. Catheters were flushed with 8 mL of 10 IU heparin/mL saline following sample collection before the restart of the isotope infusion. Plasma samples were collected at 90, 60, and 30 minutes before the start of the infusion to establish basal labeled α-KIC enrichment of the plasma (Jones et al, 1985).

3.2.6 Chemical analyses

3.2.6.1 Sample grinding

Feed and freeze dried fecal samples were ground in a commercial coffee grinder before chemical analyses to reduce particle size.

3.2.6.2 Combustion calorimetry

Approximately 1g ground samples were weighed in duplicate into tared metal cups and pressed into pellets. The cup was then placed into the holder in the top of the bomb (LECO Corporation, St. Joseph, MI). A 10 cm length of platinum wire was situated in the slits on the posts of the top of the bomb, just touching the sample in the cup. The top was put onto the bottom of bomb and the ring screwed down to seal the bomb. The bomb was charged with O_2 to 450 psi. The computer was previously readied by clearing old data. The weight of the current sample was entered, 'Enter' was pressed and the computer sequence began by lowering the bomb into the water chamber. After the sample in the bomb had been completely oxidized, the computer sequence ended and the bomb was raised from the water chamber. Excess O_2 was released from the bomb and the bomb was opened. After visually confirming complete oxidation of the sample, distilled H_2O with Methyl

Orange indicator was used to wash the interior and top of the bomb into a beaker. The washing was complete when there was no longer a colour change of the rinse solution. The solution in the beaker was titrated with 0.8 *N* $Na_2CO_{3(aq)}$ to an orange endpoint. The volume of titrant and the length of wire burned (i.e. 10cm – length unburned) was entered to correct for acid formation during combustion, assumed to be nitric acid, and the caloric contribution from the burned wire, respectively. The 'Enter' key was pressed twice and the caloric content (kcal/g) was then printed out by the computer.

3.2.6.3 Acid insoluble ash content

Approximately 2 g samples of ground feed in quadruplicate or 0.5 g samples in duplicate of ground, dried feces were weighed into weighed and labelled 195x12mm glass test tubes. The test tubes were held upright in a 600 mL Pyrex beaker then placed into a 500 ºC oven overnight. The ashed samples were cooled to room temperature before 1.0 ml of 4 *N* $HCl_{(aq)}$ was added to each tube and vortexed. An additional 4 ml of 4 *N* $HCl_{(aq)}$ was then added to each tube. Marbles were placed on top of each of the tubes and heated at 120 ºC overnight. After cooling to room temperature, the sample tubes were centrifuged at 3000 rpm for 10 minutes. The supernatant was removed by vacuum before 5 ml of double distilled H_2O was added to each tube and vortexed. The tubes were then centrifuged at 3000 rpm for 10 minutes before the supernatant was again removed by vacuum. This washing step was repeated for a total of 3 times. After the final washing, the sample tubes were placed in a 90 ºC oven overnight. Once the samples were completely dry, they were placed into a 500 ºC oven for 24 hours.

The ashed samples were removed and cooled to room temperature in a dessicator. Final weights of the tubes were recorded.

3.2.6.4 Nitrogen and carbon contents

Approximately 100 mg of previously ground samples were weighed in duplicate into foil cups. The cups were folded closed around the sample, forming a tear drop-shape. The samples were then loaded into the automatic sample carousel. Nitrogen and carbon contents of the samples were determined by complete combustion. NO_x and CO_2 gas contents were measured by infrared radiation in separate cells of the analyzer (LECO Corporation, St. Joseph, MI). The percent nitrogen and carbon was calculated from the previously entered sample weights by the computer.

3.2.6.5 Crude fat content

Approximately 5 g of sample was weighed in duplicate into tared thimbles in metal holders. A small plug of glass wool was added to the top of each thimble. The metal holders were clipped into place on the Goldfisch fat extraction apparatus, previously switched on and with the condensing water running. Approximately 20 ml of petroleum ether was added to each pre-weighed Goldfisch apparatus beaker before being tightened into place by the metal ring. The heaters were turned on and each heater was raised to within 1 cm of the corresponding beaker. The system was checked for leaks of petroleum ether and, after rectifying any leaks, left to reflux for 4 hours. The heaters were lowered and the beakers allowed to cool. The beakers were removed and the sample holders replaced by glass cups. The heaters were again raised to evaporate the petroleum

ether into the glass cups. When there was at least 3 mm of solution left in the beaker, the heaters were lowered and switched off. The beakers were left overnight in a fume hood to dry off the remaining petroleum ether. The dried beakers were then placed in 110 ºC oven for at least 3 hours before cooling in a dessicator to room temperature. The cooled beakers were then weighed.

3.2.6.6 Neutral detergent fibre content

Between 0.45 – 0.55 g of previously ground sample was weighed and heat-sealed in duplicate into sample bags (ANKOM Technology, Macedon, NY). A maximum of twenty-four bags, including an empty, heat-sealed blank, were arranged in the trays of the apparatus. 1.9 L of NDF solution (30.0 g sodium lauryl sulfate, USP; 18.61g Ethylenediaminetetraacetic disodium salt, dihydrate; 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol per 1 L distilled H_2O) was added on the top the bags held in the apparatus. 20 g of sodium sulfite and 4 ml of heat-stable alpha-amylase were also added. The top of the apparatus was closed and the heat and agitate were turned on. After 75 minutes, heat and agitate were turned off, the solution was drained, and then the lid was opened. After closing the drain valve, 1.9 L of hot water (~80 ºC) and 4 ml of alpha-amylase were added, agitated for 5 minutes and then drained. This rinse process was repeated a total of three times, omitting the alpha-amylase on the final rinse. The bags were removed from the apparatus, excess water was gently pressed out, and then soaked in acetone. After 5 minutes, the excess acetone was gently pressed out. The bags were allowed to dry at room temperature overnight and then at 110 ºC for 4 hours before being

cooled in provided dessicator bags (ANKOM Technology, Macedon, NY). Room temperature samples were weighed.

3.2.6.7 Acid detergent fibre content

Duplicate ground samples of 0.45 – 0.55 g were previously prepared for and run through the NDF procedure (see 3.2.6.6). Twenty-four bags, including one blank bag, were arranged in the trays and placed into the apparatus (ANKOM Technology, Macedon, NY). 1.9 L of ADF solution (20 g cetyl trimethylammonium bromide in 1 L 1.0 *N* H_2SO_4) was added to the apparatus on top of the samples bags. Heat and agitate were turned on. After 60 minutes, heat and agitate were turned off. The ADF solution was drained and the lid was opened. After closing the drain valve, approximately 1.9 L of hot water (~80 ºC) was added. After agitating for 5 minutes, the rinse water was drained. A total of three rinses were performed before the bags were removed. Excess water was gently pressed from the bags before being submerged in acetone. After 5 minutes, the acetone was gently pressed from the bags before overnight drying at room temperature. The next day, bags were dried at 110 ºC for 4 hours before cooling in provided desiccator pouches (ANKOM Technology, Macedon, NY). Room temperature samples were weighed.

3.2.7 Isotope analyses

Expired $^{13}CO_2$ enrichment was measured by continuous flow isotope ratio mass spectrometry (CF-IRMS20/20; PDZ Europa, Cheshire, United Kingdom) (Di Buono et al, 2001). Enrichments of all breath samples collected were expressed as APE $^{13}CO_2$ above a reference standard of compressed CO_2 (Jones et

al, 1985). Plasma α-ketoisocaproate (KIC) enrichment was determined by selective ion-monitoring GCMS analysis of pentaflurobenzyl-esters according to Hachey et al (1991).

3.2.8 Calculation of results

3.2.8.1 Acid insoluble ash content

Acid insoluble ash (AIA) content was calculated as:

$$\frac{\text{Final tube weight} - \text{Initial tube weight}}{\text{Sample weight}} \times 100 = \%\ \text{AIA}$$

3.2.8.2 Digestibility

The digestibility of individual dietary components and energy was determined using diatomaceous earth (Celite®) as an indigestible marker and analysis for acid insoluble ash (AIA) (McCarthy et al, 1977). The following formula using the AIA contents of feed (AIA_{feed}) and feces (AIA_{feces}) was used to calculate the nutrient digestibility of individual nutrients:

$$\text{Nutrient digestibility (\%)} = 1 - \frac{(AIA_{feed} * Nutrient_{feces})}{(AIA_{feces} * Nutrient_{feed})}$$

3.2.8.3 Crude fat content

Crude fat (CF) content was calculated as:

$$\frac{(\text{Final weight of beaker} - \text{Initial weight of beaker}) - \text{Blank beaker weight}}{\text{Sample weight}} \times 100 = \%CF$$

3.2.8.4 Neutral and acid detergent fiber contents

Fiber content (NDF or ADF) was calculated as:

$$\frac{\text{Final bag weight} - (\text{Initial bag weight} \times \text{Blank bag correction})}{\text{Sample weight}} \times 100 = \%\ \text{fiber}$$

Where,

Blank bag correction = (Final Blank bag weight/Initial Blank bag weight)

3.2.8.5 Volumes of gases

Litres of gases consumed and produced were calculated using the difference between recorded gas exchange values during the experiment and the room air values recorded immediately before and after the experimental period. The values recorded for each minute were corrected for drift of the analyzers, if statistically significant. The difference between the experimental and room air percent gas compositions were multiplied by the air flow out of the respiration chambers for each 30 minute period. Therefore, litres of gas consumed or produced were calculated for each 30 minute period, as follows:

V_{O2} (L/30 min) = total air flow (L)/30 min * (% O_2 room air - % O_2 test period)

V_{CO2} (L/30 min) = total air flow (L)/30 min * (% CO_2 test period - % CO_2 room air)

V_{CH4} (L/30 min) = total air flow (L)/30 min * (% CH_4 test period - % CH_4 room air)

3.2.8.6 Heat production

The formula by Brouwer (1965) was used to calculate heat production (HP) from indirect calorimetry. The formula was abbreviated by omitting the urinary nitrogen term. According to Weir (1949), 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. Therefore, the formula used to calculate HP from gas exchange was:

$$HP = (16.195 \times V_{O2}) + (5.02 \times V_{CO2}) - (2.19 \times V_{CH4})$$

where V_{O2}, V_{CO2}, and V_{CH4} represent volumes (L) of O_2 consumed and CO_2 and CH_4 produced, respectively. Daily HP was calculated as the summation of HP for each 30 minute of the 24 h period.

3.2.8.7 Respiratory quotient

The respiratory quotient (RQ) was calculated as:

RQ = V_{CO2} produced (L/30 minute period) / V_{O2} consumed (L/ 30 minute period)

3.2.8.8 Assumptions relevant to subsequent calculations

The energy content of protein and lipid were assumed to be 23.8 kJ/g and 39.0 kJ/g, respectively (ARC, 1981). The efficiency for utilization of ME from dietary sources was 70% and from body tissue was 80%, as reported by Verstegen et al (1971). The energy cost of protein accretion is 44.4 kJ/g of protein and the energy cost of fat accretion is 52.3 kJ/g of fat (NRC, 1998). Water contents of protein and fat were assumed to be 75% (Mohrmann et al, 2006) and 10% (Frisch, 1987), respectively.

3.2.8.9 Energy balance

Energy balance was calculated as:

energy retention (ER) = ME intake – measured HP

3.2.8.10 Isotope kinetics

For the IV isotope application, flux (Q) was calculated using the standard stochastic equations assuming steady state conditions where intake (I) plus breakdown (B) are equal to synthesis (S) and oxidation (O), according to Jahoor (2003):

Q = I + B = S + O

Flux, for IV and constant oral isotope (i.e. nibbling) applications, was calculated according to Matthews et al (1980):

Q = isotope dose * [(99/plasma α-KIC enrichment) - 1]

For single-dose oral isotope application (i.e. bolus meal), flux was calculated as

the cumulative excretion of the label, according to Waterlow et al (1978):

$$Q = (\text{rate of } CO_2 \text{ production}) \times (\text{dose}/e_x)$$

Where e_x represents the quantity of endproduct containing the isotope label. Intake of leucine was calculated as the total leucine intake; dietary intake was multiplied by the apparent digestibility of leucine, estimated using values given by NRC (1998), and added to the isotope dose given during a 24 h period.

Total leucine oxidation was determined according to Jahoor (2003):

$$O = \frac{CO_2 \text{ recovery} \times {}^{13}CO_2 \text{ enrichment in breath}}{\alpha\text{-KIC enrichment in plasma}}$$

The appropriate carbon dioxide recovery was included in the calculation, according to Moehn et al (2004b): 81.0% for the nibbling phase, 78.8% for meal-feeding and postprandially, and 58.1% for fasting.

Leucine for protein synthesis was calculated as:

$$S = Q - O$$

Leucine appearance from body protein breakdown was calculated as:

$$B = Q - I$$

For the oral isotope application, parameters of protein turnover were calculated analogously for the frequent feeding phase of the experiment. Data for the remaining time of the experiment were omitted from the analysis because sows did not consume their meals as planned. Because orally infused labeled leucine is utilized by the gastro-intestinal tract (Matthews et al, 1993), flux rates differ between oral and intravenous application (Biolo and Tessari, 1997). To equalize differences in leucine flux caused by the route of tracer application, the flux determined with intravenous isotope application was adjusted using the ratio

of oral:intravenous α-KIC (see Appendix C for details).

3.2.8.11 Protein and lipid balance

Protein balance was calculated by converting S into units of protein by dividing by the concentration of leucine, which was taken as 7 g/100 g protein (Pettigrew and Yang, 1997):

Protein retention was calculated as:

Protein retention = leucine retention / (7 g/100g protein)

Lipid balance was calculated assuming ER in excess of (or below) that required for the deposition of protein retention was stored (or lost) entirely as fat, therefore:

Lipid retention was calculated as:

$$\text{Lipid retention} = \frac{\text{ER} - (\text{protein retention} * 0.0444 \text{ J/g})}{0.0523 \text{ J/g}}$$

3.2.8.12 Calculation of 24 h HP from identified physiological states

Daily HP was calculated from identified physiological states by multiplication of the mean HP calculated per minute within the physiological state by 1440 minutes to extrapolate to 24 h.

3.2.9 Statistical evaluation

Data are presented as means ± SEM, unless otherwise stated. Values were considered significant at $P < 0.05$. Statistical analysis was performed using mixed procedure in SAS (SAS Inst. Inc., Cary, NC). The classification variable was feeding level and individual animals were treated as random variables. Model statements were tested using the Kenward-Roger degrees of freedom method. Least square means were compared using the 'pdiff' option. Significance was

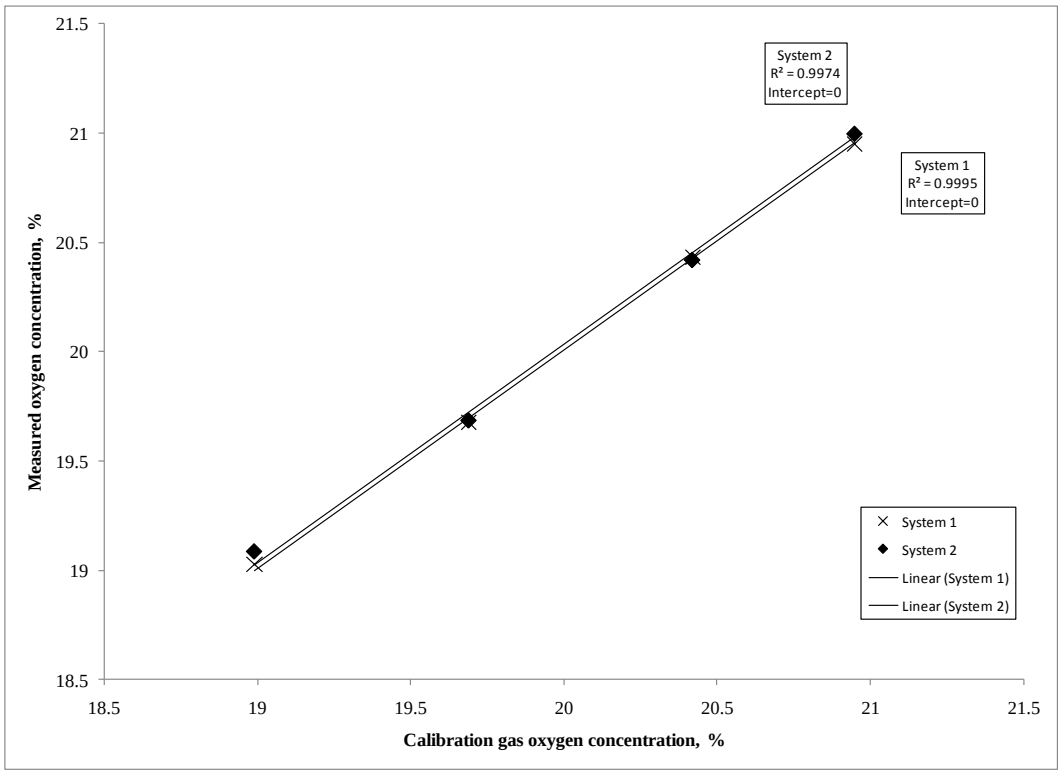
taken at $P < 0.05$, while $P < 0.1$ was regarded as a tendency.

3.3 Results

3.3.1 Respiration system development and validation

Validation of the respiration system was previously discussed (Samuel, 2008) and is reproduced here for completeness. The efficiency of the nitrogen injection test was 102.6 ± 0.6% for the O_2 sensors over repeated (n=6) measurements. The linearity of the response of the O_2 analyzers was tested using five gases of known O_2 concentrations (0, 18.99, 19.69, 20.42, 20.94). The analyzers responded linearly ($R^2 = 0.995$, CV = 0.26%, $P<0.001$) to a series of gases with O_2 contents between 0 and 21% (Figure 3.2).

Figure 3.2 – Response of the system 1 and 2 S103 O_2 sensors to gases of known O_2 concentration



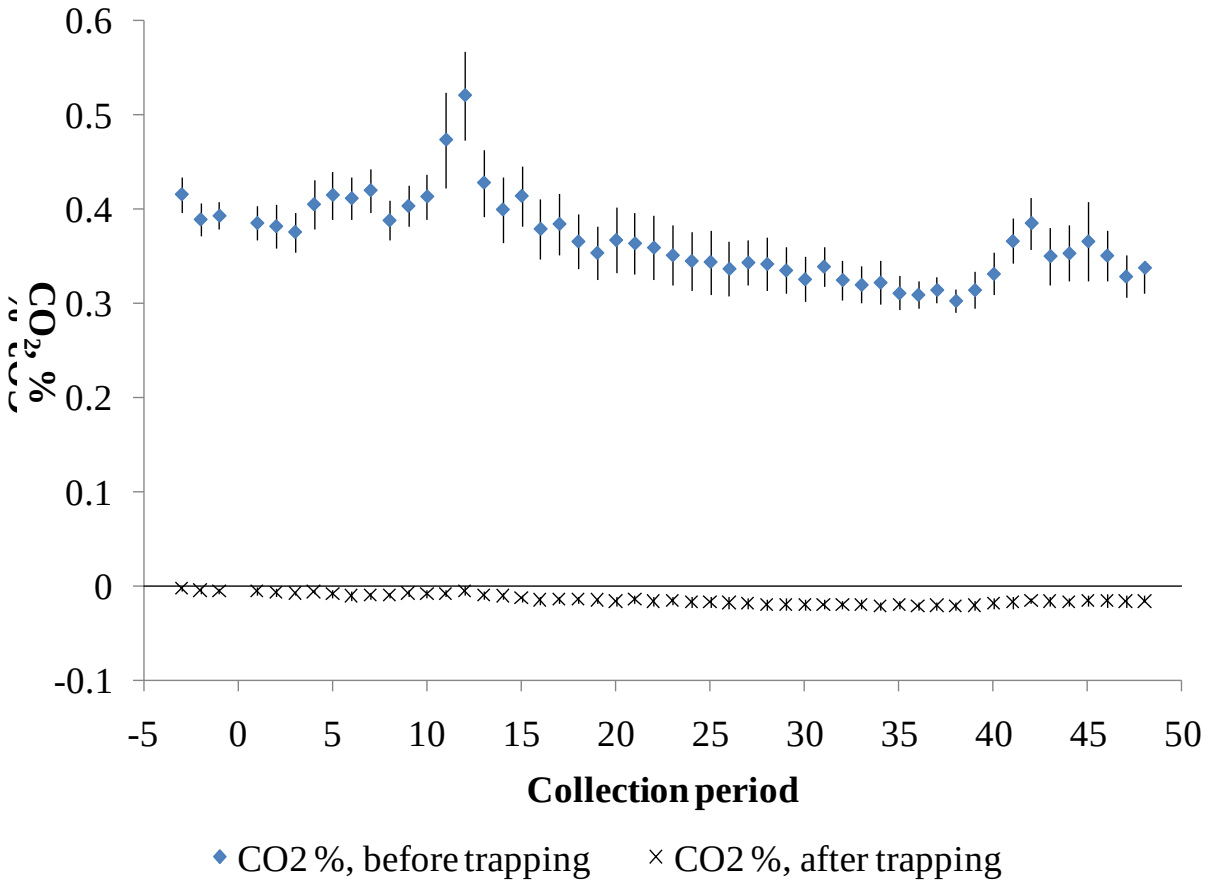
The recovery of CO_2 was 107.3 ± 2.1% over repeated (n=15) measures for both chambers, with no difference between chambers (P>0.50). The CO_2 analyzers response to changing CO_2 content of the air was a non-linear response curve. The curve was repeatedly (n=5) determined using seven gases of known CO_2 concentration (0, 0.04, 0.08, 0.4, 0.8, 1.2, and 1.5%). The measured CO_2 concentrations were corrected to the known concentration according to the non-linear response curve:

$$CO_2 = -0.18653x^5 + 1.563x^4 – 4.9975x^3 + 7.8458x^2 – 5.5865x + 1.4242$$

3.3.2 Quantitative breath collection

Quantitative collection (100%) was verified by measurement of the CO_2 content of the sample air, by independent CO_2 analyzers (Model LB-2, Beckman Instruments Ltd, Schiller Park, IL), following CO_2 collection (Figure 3.3). Collected CO_2 was transferred to evacuated 10 mL Vacutainer tubes (BD – Canada, Mississauga, ON) and stored at -20 °C until analysis.

Figure 3.3 – CO_2 concentration (mean ± SE, n = 10) of chamber air before and after trapping in 1 *N* NaOH through a single midget bubbler over 24 h



3.3.3 Long-term catheterization of sows

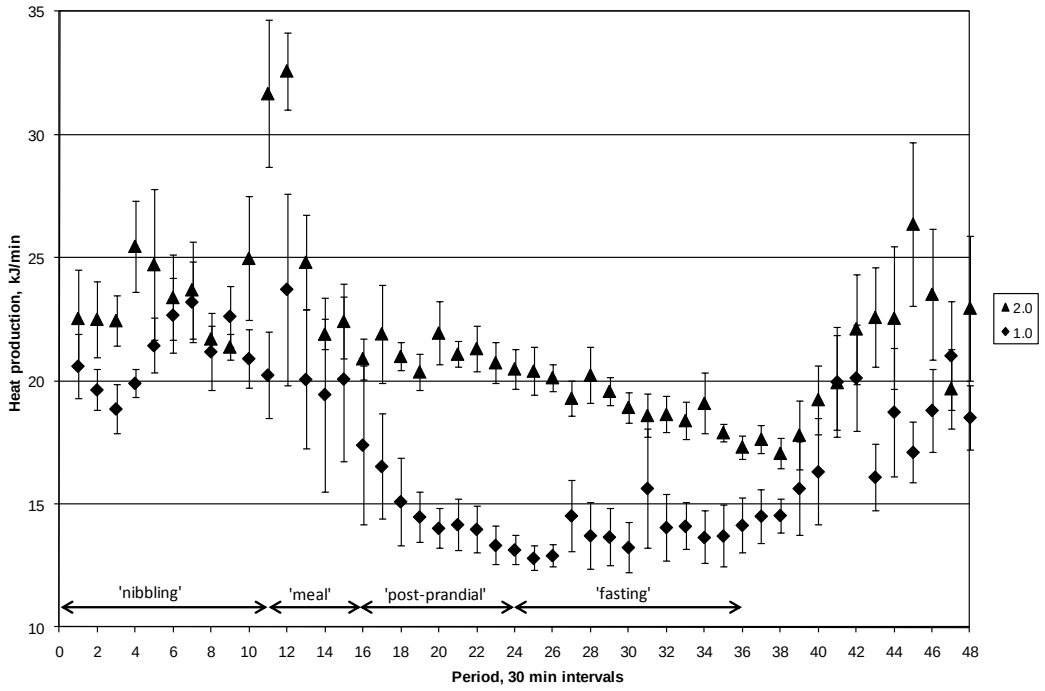
Seven sows were catheterized in development of the long-term catheterization protocol. The catheter of the first sow was deemed not functional post-surgery after multiple attempts to aspirate or inject saline were unsuccessful. She was euthanized within one week of surgery in order to ascertain the reason for the lack of flow. It was concluded that the catheter was occluded by the positioning of the injection port due to the presence of excess catheter. One sow suffered post-surgical complications (i.e. an infection of the lower incision) which did not respond to the oral antibiotic Cephalexin. The infection spread to the upper incision, burst, and the port became exteriorized before she was euthanized one month after surgery. The port of one sow became exteriorized through loss of body condition or injury after four months (no infection), the catheter came out, and the sow, subsequently, recovered. Of the remaining four catheters, two were patent at the time the animals were euthanized (seven months later), and two catheters could be infused, but no blood could be withdrawn for the final three weeks preceding euthanasia.

3.3.4 Energy metabolism

Parameters of energy metabolism were previously discussed (Samuel, 2008). Briefly, there were no significant interactions between feeding level and identified physiological states ($P > 0.1$); therefore, means for identified physiological states were calculated across feeding levels (Table 3.3). Data from the orally dosed sows were omitted after the nibbling phase because they did not consume their remaining feed allowance according to the planned experimental

schedule. Sows on both feeding levels had significantly greater heat production when nibbling or meal eating compared to the post-prandial and fasting states (Figure 3.4).

Figure 3.4 – Mean heat production of sows fed feeding level 1.0 versus feeding level 2.0 according to collection 'period' over 24 h (reproduced from Samuel, 2008)



3.3.5 Protein metabolism

Leucine flux was greater for oral than IV isotope application, so that for some sows given IV isotope, negative body protein breakdown was calculated due to the lower flux. Application of IV isotope bypasses the splachnic bed and results in greater appearance of the tracer in the sample pool than compared to oral isotope application. As a result, leucine appearance from body tissue breakdown, calculated as B = Q – I, resulted in negative values because I was greater than Q. Therefore, a conversion factor was calculated by dividing the mean α-KIC

enrichment of IV isotope by that from oral isotope application (3.95 vs. 15.5, SEM=2.4) (Appendix C). This factor – 3.9 – was applied to the flux values obtained with IV isotope application in the current and subsequent study (Chapter 4.0).

Measured enrichments of the three background samples collected before initiation of the isotope delivery were consistent and comparable within and across feeding levels and animals (n=30, 1.0964±0.0004 AP, CV=0.21%). Leucine flux was similar (P=0.23) for both energy intakes (Table 3.2). Dietary leucine intake was twice as much for feeding level 2.0, in accordance with the experimental design. Leucine released from body protein breakdown, oxidation, and leucine used for protein synthesis were not different (P>0.16) between feeding levels. Sows on both feeding levels had positive protein retention (113.0 vs. 277.5 g/d), but retention was greater for feeding level 2.0 (P=0.02). Calculated lipid retention (-84.0 vs. 168.3 g/d), however, was negative for sows fed 1.0 and significantly (P<0.01) lower that the markedly positive lipid retention when fed 2.0 (Table 3.2).

Leucine flux, leucine derived from body protein breakdown, and leucine used for protein were not different among identified physiological states (Table 3.3). Intake of leucine was greater during nibbling and meal-fed compared to fasting states and post-prandial states. Protein and lipid retention were not different during identified physiological states.

Protein metabolism parameters were not different when expressed as percent of leucine flux between feeding levels. There was a trend for increased

oxidation, as a percent of flux, for sows fed feeding level 2.0 (Table 3.4). Protein metabolism parameters were not different when expressed as percent of leucine flux between identified physiological states (Table 3.5).

Table 3.2 – Parameters of energy and protein metabolism (g/d) of sows fed feeding level 1.0 or 2.0

	Feeding level			
	1.0	2.0	SEM	P-value
n	5	5		
Body weight[2], kg	174.1	186.6	3.0	**<0.001**
Feed intake[2], kg/d	1.84	3.69	0.31	**<0.001**
Metabolizable energy intake[2], MJ/d	23.9	44.9	3.8	**0.001**
Heat production[2], MJ/d	24.0	32.3	1.5	**0.037**
Maintenance energy expenditure[3], MJ/d	24.3	25.6	0.3	**0.001**
Energy retention[2], MJ/d	-0.1	12.6	3.1	**0.004**
Isotope dose, g/d	4.2	4.8	2.9	0.27
KIC enrichment, MPE	9.4	12.4	2.4	0.35
Leucine flux, g/d	217.9	163.4	34.2	0.23
Leucine intake[1], g/d	14.2	28.4	2.4	**<0.001**
Leucine from body protein breakdown, g/d	203.4	134.4	35.7	0.17
Leucine oxidation, g/d	11.0	18.2	4.6	0.16
Leucine for protein synthesis, g/d	214.1	156.1	33.0	0.20
Protein retention, g/d	113.0	277.5	43.5	**0.02**
Lipid retention, g/d	-84.0	168.3	57.4	0.004

[1]Intake of total leucine plus 24 h dose of L-[1-^{13}]leucine

[2]Reproduced from Samuel (2008)

[3]Calculated using 506 kJ/$BW^{0.75}$ (Samuel, 2008)

Table 3.3 – Effect of identified physiological state of sows on parameters[1] of energy and protein metabolism (g/d) of sows

	Nibbling	Meal-fed	Postprandial	Fasting	SEM	P-value
Heat production[2], MJ/d	33.1ab	29.8b	25.2c	26.6b	0.8	**0.03**
Respiratory quotient[2]	1.04b	1.14ab	1.20a	1.06b	0.02	**0.04**
KIC enrichment	10.7	10.9	10.8	11.1	1.2	0.99
Leucine flux, g/d	189.8	197.4	187.8	187.6	14.9	0.98
Leucine intake, g/d	21.3a	21.3a	3.9b	3.9b	1.1	**<0.01**
Leucine from body protein breakdown, g/d	168.1	175.6	183.9	183.7	15.5	0.94
Leucine oxidation, g/d	13.1	12.1	18.4	14.7	2.1	0.32
Leucine for protein synthesis, g/d	185.2	191.2	180.6	183.4	14.4	0.99
Protein retention, g/d	206.3	199.5	186.4	189.1	20.3	0.98
Lipid retention, g/d	37.1	40.3	46.2	45.0	27.0	1.00

[1]All values extrapolated to 24 h

[2]Calculated with data from Samuel (2008)

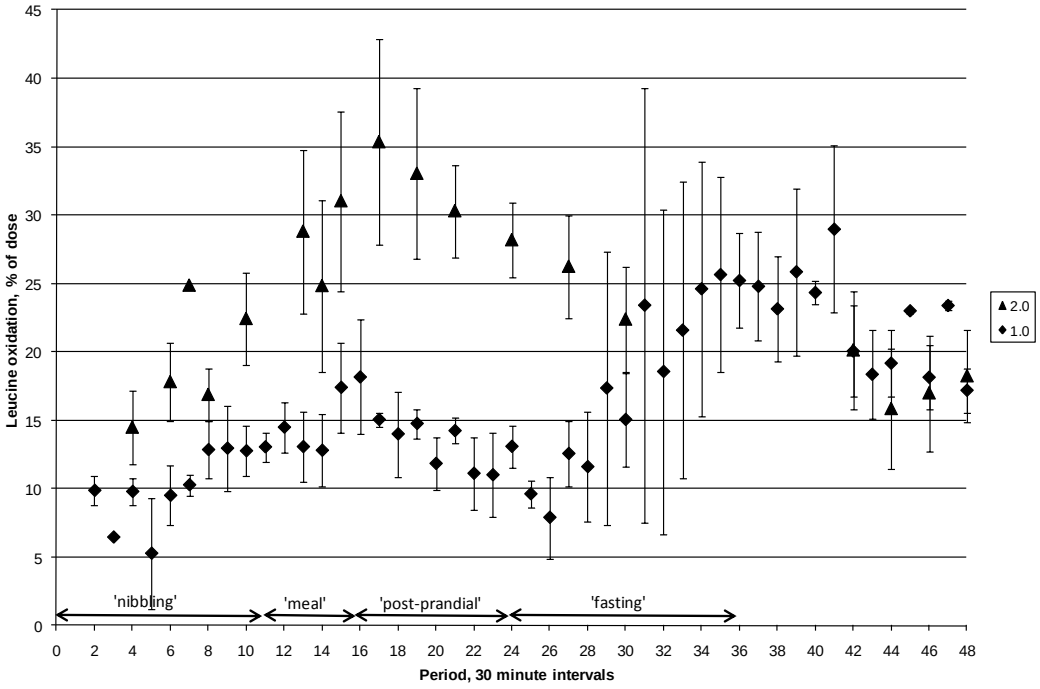
Table 3.4 – Partitioning of leucine (% of leucine flux) by feeding level from non-pregnant sows fed feeding level 1.0 and 2.0

	Feeding level			
	1.0	2.0	SEM	P-value
Leucine from dietary intake	2.6	7.4	1.5	0.15
Leucine from body protein breakdown	97.4	92.6	1.5	0.15
Leucine oxidation	2.6	4.2	0.5	0.08
Leucine for protein synthesis	97.4	95.8	0.7	0.11
Leucine retention	4.7	16.4	3.9	0.17

Table 3.5 – Partitioning of leucine (% of flux) during identified physiological states from non-pregnant sows fed feeding level 1.0 and 2.0

	Nibbling	Meal-fed	Postprandial	Fasting	SEM	P-value
Leucine from dietary intake	13.4a	14.2a	2.4b	2.5b	2.0	<0.01
Leucine from body protein breakdown	86.6a	85.8a	97.6b	97.5b	2.0	<0.01
Leucine oxidation	2.7	2.7	3.7	3.9	0.3	0.26
Leucine for protein synthesis	98.0	97.3	96.3	97.1	0.5	0.66
Leucine retention	10.6	11.5	10.5	11.6	2.0	0.99

Figure 3.5 – Mean leucine oxidation by sows fed feeding level 1.0 versus feeding level 2.0 according to collection ‘period’ over 24 h



3.3.6 Relationships between measurements

There were no significant interactions of energy and protein metabolism (i.e. leucine flux, appearance from body protein breakdown, oxidation, leucine for protein synthesis), except that protein gain was increased by greater energy balance from sows fed feeding level 2.0.

3.3.7 Comparison to 24 h values

Heat production, extrapolated to 24 h, during the nibbling phase exceeded the daily heat production by a mean factor of 1.17 ($P<0.001$) with no difference between feeding levels ($P>0.65$) (Table 3.6). The RQ was not different within the identified physiological states compared to the 24 h values, except when sows fed feeding level 1.0 were nibbling and when sows fed feeding level 2.0 were fasting. Leucine oxidation during the identified physiological states was not related to the daily mean, therefore correction factors could not be prepared (Table 3.7).

Table 3.6 – Comparison of HP measured during identified physiological states extrapolated to 24 h to daily measured HP

Physiological state	Fasting	Nibbling	Post-prandial	Meal	SEM
HP extrapolated from state[1]	26.6	33.1	25.2	29.8	0.338
Recorded daily HP[1]	28.1	28.1	28.1	28.1	0.141
P-value for the relation between extrapolated and recorded HP	<0.001	<0.001	<0.001	0.48	-
Correction factor	0.95±0.02	1.17±0.02	0.90±0.01	-	-

[1]Reproduced from Samuel (2008)

Table 3.7 – Comparison of RQ and leucine oxidation measured during identified physiological states to 24 h measured values

Feeding level	Physiological state	Fasting	Nibbling	Post-prandial	Meal	SEM	P-value
1.0	RQ recorded from state	1.02	0.96	1.15	1.08	0.01	<0.001
	Mean RQ over 24 h	1.05	1.05	1.05	1.05	0.01	-
	Difference between mean and recorded RQ	0.67	<0.001	0.13	0.61	-	-
	Extrapolated leucine oxidation from state	21.7	12.3	14.4	12.8	0.44	<0.001
	Recorded daily leucine oxidation	15.9	15.9	15.9	15.9	0.44	-
	Difference between extrapolated and recorded daily leucine oxidation	0.24	0.41	0.22	0.70	-	-
2.0	RQ recorded from state	1.09	1.12	1.25	1.21	0.01	<0.001
	Mean RQ over 24 h	1.16	1.16	1.16	1.16	0.01	-
	Difference between mean and recorded RQ	0.04	0.43	0.35	0.58	-	-
	Extrapolated leucine oxidation from state	17.8	20.3	29.7	24.8	0.85	<0.001
	Recorded daily leucine oxidation	23.6	23.6	23.6	23.6	0.85	-
	Difference between extrapolated and recorded daily leucine oxidation	0.21	0.23	0.93	0.36	-	-

Table 3.8 – Comparison of O_2, CO_2, and CH_4 measured during identified physiological states to 24 h measured values

Feeding level	1.0	Fasting	Nibbling	Post-prandial	Meal
P-value for the relation between extrapolated and recorded O2		<0.001	0.03	0.02	0.12
P-value for the relation between extrapolated and recorded CO2		<0.001	0.16	0.81	0.009
P-value for the relation between extrapolated and recorded CH4		0.71	0.36	0.37	0.43
Feeding level	2.0	Fasting	Nibbling	Post-prandial	Meal
P-value for the relation between extrapolated and recorded O2		<0.001	0.31	0.29	0.18
P-value for the relation between extrapolated and recorded CO2		<0.001	0.13	0.08	0.19
P-value for the relation between extrapolated and recorded CH4		<0.001	0.08	0.48	0.74

3.4 Discussion

3.4.1 Respiration system development

The respiration system was developed exclusively by S. Moehn from two sets of individual S103 O_2, S153 CO_2, and S126 CH_4 analyzers coordinated with customized C409 data acquisition software (Qubit Systems, Kingston, ON) and two custom manufactured, independent respiration chambers. Each of the two chambers was built around a standard farrowing crate, making them safe and acceptable by the sows, and can be expanded to accommodate the sow plus litter for lactation studies. The chambers are air tight, except for the air inlets which draw in fresh air from the room due to a slight negative pressure induced by vacuum pumps withdrawing air from the chambers.

3.4.2 Indirect calorimetry system validation

The development of an indirect, open-circuit respiration system for sows was successful. The accuracy of the oxygen sensors (102.6 ± 0.6%) and carbon dioxide sensors (107.3 ± 2.1%) were not different than 100%, indicating that the sensors responded efficiently and correctly to changing gas concentrations induced within the respiration chambers in the range of interest. It is imperative that the sensors respond correctly, rapidly, and independently to small changes in gas concentrations. The gas concentrations were generally different by less than 0.5% from room air concentrations. The O_2 analyzers responded linearly and the measured CO_2 concentrations were corrected according to the non-linear response curve in *post hoc* analysis of the respiration data above and below the range of

interest. Therefore, based on the responsiveness and accuracy of the analyzers to changes in gas concentration within the range of interest (Samuel, 2008), the indirect calorimetry system has been validated.

3.4.3 Quantitative breath CO_2 collection

To correctly quantify the $^{13}CO_2$ content of expired breath, collection of breath CO_2 must be entirely complete to avoid the possibility of preferential absorption of the unlabeled versus labeled CO_2. Mass differences of the reactants can cause detectable isotope effects where reactions involving the heavier, labeled atoms generally proceed more slowly than for the unlabeled substance. However, because $^{13}CO_2$ is only one mass unit heavier than CO_2, any isotope effects would be less than for $^{14}CO_2$ and would be difficult to detect. By ensuring that the trapping of expired CO_2 is complete, any possible isotope effects are avoided.

3.4.4 Long-term, in-dwelling catheterization of sows

The development of the final protocols regarding surgical placement and maintenance of long-term, in-dwelling catheters in sows had a number of steps. The catheter of the very first sow was not functional immediately following surgery because the catheter was kinked. This occurred due to placement of the catheter attachment on the injection port at a right-angle to the direction of the tunneled catheter. This was done in an effort to provide extra catheter length below the incision which would allow future growth of the sows without straining the catheter. Subsequently, excess catheter was limited to 2 - 3 cm within the lower incision and was carefully sutured into place before closing the incision. Also, the catheter attachment to the port was always positioned in the direction of

the tunneled catheter.

Exteriorized catheters typically require daily flushing of heparin to maintain patency and increase the occurrence of loss in patency (Foley et al, 2002) and infection (Appelgren et al, 1996; Foley et al, 2002). In-dwelling catheters coated with CBAS and terminated with injection ports can be flushed days apart and patency can be maintained (Foley et al, 2002). Initially, flushing of the catheters was performed days apart and, gradually, a day or two was added to the interval based on the fact that the more frequent blood is aspirated through the catheter and injection port, the more likely the occurrence of thrombosis or infection. Finally, a two-week interval between flushing was deemed most appropriate based on the increasing number of catheters which could not, initially, be aspirated when the interval was increased beyond two weeks.

Catheters which could not be aspirated after accessing the port required an injection of saline before aspiration was possible or, failing that, were subsequently aspirated during the next scheduled flushing period. All catheters were flushed with normal saline and locked with 100 IU heparin/mL saline following access of the injection port. The push-pause-push injection technique was used which created turbulent flow within the port and catheter to ensure complete flushing. The patency of catheters from the initial set of sows reported herein was limited to seven months. However, a subsequent set of three sows (Chapter 4.0) were catheterized and the catheters were maintained patent for 18 months before the sows were euthanized at the end of a series of studies.

3.4.5 Animal studies

3.4.5.1 Energy metabolism

Daily feed intake was greater for sows fed feeding level 2.0, in accordance with the experimental design. Sows fed feeding level 1.0 were in negative energy balance, as confirmed by the observed negative weight gain (-198 ± 96 g/d). Sows fed feeding level 2.0 were in positive energy balance (12.6 MJ/d), and gained 1290 g/d. Sows fed feeding level 2.0 consumed more energy and had greater HP than sows fed feeding level 1.0 (Samuel, 2008).

The 24 h HP of sows was affected by the different feeding frequencies in a similar manner for both of the feeding levels. When sows were consuming small meals every 30 minutes, HP was greater ($P<0.01$) than when sows were fasting or following the large meal (postprandial). The HP of the eating sows, whether consuming the frequent, small meals or consuming the single large meal was not different (Samuel, 2008).

3.4.5.2 Protein metabolism

A correction factor relating IV to oral flux was calculated based on the mean plasma enrichment of α-KIC (Appendix C). Biolo and Tessari (1997) observed that one-quarter of the leucine was taken up by the splanchnic metabolism and does not appear in plasma. Further, they recorded a lower efficiency of α-KIC production from splanchnic tissues compared to the whole-body. Application of the conversion factor corrected the flux from IV infusions of the isotope for splanchnic metabolism, which is bypassed, for calculation of WBPT. This factor increased the flux, thus increasing leucine appearance from

body protein breakdown and leucine used for protein synthesis because they were calculated as Q – I and Q – O, respectively. The correction factor changed the partitioning of protein metabolism parameters as a percent of flux, but does not change the pattern of the response observed. Without application of the correction factor, flux was so low that negative breakdown was calculated for some sows. Therefore, a correction factor must be applied to calculate meaningful results from IV application of isotope in this experiment.

Leucine flux was not different between intakes (P=0.23). Protein synthesis represented 97.4% and 95.8% of flux for sows fed feeding level 1.0 and 2.0, respectively. Leucine appearance from body protein breakdown was numerically greater in sows fed feeding level 1.0 (97.4 vs. 92.6 % of flux) compared to sows fed feeding level 2.0. Because energy intake was inadequate for feeding level 1.0, body protein was likely being catabolized for energy and this resulted in lower protein gain (113.0 vs. 277.5 g/d, P=0.02). This is in agreement with Reeds et al (1980) who reported a high correlation of total energy and protein intake on total protein synthesis. Sows fed the higher energy and protein intake utilized dietary protein for protein synthesis with a numerical greater efficiency (7.4 vs. 2.6 % of flux) than sows fed feeding level 1.0. Thus, sows fed adequate energy and protein showed somewhat reduced body protein breakdown and incorporated dietary amino acids more efficiently into body protein. Sows fed the lower energy intake had numerically increased body protein breakdown and amino acids were oxidized for energy, demonstrating the significant impact that dietary energy intake has on amino acid requirements of sows. In agreement with Reeds et al

(1980), it appears that requirements associated with maintenance proportionally increase as feed intake is reduced.

Attempts to calculate cumulative excretion of the isotope for the orally dosed sows were unsuccessful because the sows did not consume their large meals according to the planned experimental schedule.

During long isotope infusions (i.e. greater than 6 h) recycling of the tracer must be considerd (Aub and Waterlow, 1970). Recycling of the tracer occurs when the tracer has been incorporated into protein and is then later released as proteins are broken down during protein turnover. It is not possible to correct for the increase in dose due to tracer recycling, but it may have had an impact in this experiment because the tracer was infused over 24 h. Tracer recycling increases the plateau enrichment and, therefore, reduces the flux estimate. There were no differences in flux detected between the identified physiological states in this experiment, however, the results are difficult to interpret due to the possibility of recycling of the tracer as the time of the experiment progressed.

The IV tracer infusion and blood sampling utilized the same catheter. However, because enrichment was determined in α-KIC, which is derived from metabolism of the infused leucine, the plasma α-KIC could be collected from the same catheter as the infusion of the tracer.

3.4.5.3 Protein and energy interactions

Protein gain was greater when energy and protein intake were higher for sows fed feeding level 2.0. Interaction of energy intake, heat production, or energy balance on additional measures of protein metabolism began to approach

significance (P-values 0.12 – 0.17) due to high variability in results and low numbers of animals.

Energy and protein metabolism reacted oppositely during the nibbling feeding frequency. The measured HP during this period exceeded the daily mean HP. This greater HP is due, in part, to the energy expenditure associated with standing and consuming the meal and, the other part, due to digestion of the consumed meal (van Milgen et al, 1997). Increasing feeding frequency improves the efficiency of protein metabolism because the delivery of amino acids is more gradual (Leveille et al, 1965; Allee et al, 1972) whereas LeBlanc and Diamond (1986) proposed that increasing feeding frequency without adaptation would be energetically inefficient as a result of the significant HIF response to the meal, regardless of size or actual consumption. Therefore, the frequency of meal delivery to sows should be considered in the diet formulation (Samuel, 2008).

3.4.5.4 General results from animal studies

The mean digestibility of nutrients and energy in this experiment were previously discussed (Samuel, 2008). Briefly, the values are similar to those reported by Noblet and Henry (1993) and the calculated values based on data by NRC (1998).

The daily weight gain of sows predicted by Samuel (2008) was much greater than the calculated gain from energy and tracer balance. This is due to different assumptions for each calculation. The predicted gain calculated by Samuel (2008) above assumes 100% availability of the additional energy above that for protein accretion for body lipid deposition, which is not physiologically

true. In fact, the energy balance for feeding level 2.0 was 12.6 MJ/d, so body lipid accretion would be reduced to 267.7 g/d. The Samuel (2008) calculation also calculates lower protein retention, based on the dietary lysine being the limiting amino acid, than was measured from the tracer balance study which indicates an incorrect assumption in the calculation or an incorrect result from tracer balance was calculated. Therefore, the underlying assumptions in calculation of body tissue deposition from energy balance studies require further characterization.

3.5 Conclusions

The design and validation of an integrated methodology for simultaneous measurements of energy and protein metabolism in sows has been described. Custom built respiration chambers were combined with individual gas analyzers to create an indirect calorimetry system. Responses of the analyzers were previously validated (Samuel, 2008). The O_2 analyzers responded linearly and the CO_2 analyzers responded in a predictable non-linear manner to changing gas concentrations. The analyzers responded accurately, as shown by recoveries of 100% of the predicted volume of O_2 reduction or CO_2 release. Quantitative collection of CO_2 was achieved using a single midget bubbler suspended in NaOH. Protocols for the long-term catheterization procedure were established which subsequently reduced complications associated with long-term catheterization of sows. Results from sows fed approximately the ME_m and twice that amount indicate that 24 h values of energy and protein metabolism can be accurately extrapolated from measurement periods of shorter duration and during frequent feeding, as will be utilized in future indicator amino acid experiments.

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4.0 PROTEIN TURNOVER AND ENERGY METABOLISM DURING GESTATION AND LACTATION

4.1 Introduction

Sows are the basis of pork production by virtue of their ability to reproduce. Sows represent the productive unit of pork production systems as they are required to produce and rear new piglets into the systems (Elsley, 1971). Maximal reproductive capacity and growth rate are, theoretically, determined by genetics but, practically, limited by nutrition. Therefore, the importance of providing adequate and correct nutrition to sows on the overall efficiency of pork production should not be discounted.

Pregnant sows must be energy restricted to avoid excessive maternal weight gain. Commonly, sows are fed a fixed diet throughout pregnancy. Pregnant sows allowed *ad libitum* access to feed consume more energy than required for maintenance and growth of the products of conception (NRC, 1998; Noblet and Close, 1980). A moderate amount of maternal gain during pregnancy is desirable in all parities as an indicator of sufficient nutrient intake and to provide necessary nutrients for lactation, through maternal tissue mobilization, when nutrient intake is insufficient (Beyer et al, 2007). Maternal gain should be expected for adolescent (i.e. parity ≤ 4) sows because they have not achieved adult body mass (Close and Mullan, 1996; Cooper et al, 2001). However, too much maternal gain during gestation is costly; unnecessary feed costs are incurred (Young and Aherne, 2005) and often results in poor lactation performance (Dourmad, 1991; Pettigrew et al, 1993: Coffey et al, 1994; Young et al, 2004)

through reduced milk production (Clowes et al, 2003) or agalactia (Persson et al, 1989).

Energy and protein metabolism of the sow changes during gestation (Kim et al, 2009). Thus, it seems illogical to follow the recommendations of NRC (1998) who suggest that constant feeding values are appropriate for the entirety of gestation. Instead, diets should be adjusted according to the changing requirements in order to optimize sow nutrition. However, data on energy metabolism in gravid sows are scarce and, somewhat, conflicting. Ramonet et al (2000) was able to show positive protein and lipid gain throughout gestation, whereas Close et al (1985) observed that gilts lost body fat in late gestation at constant feed intake. Available recommendations may not be reflective of current high producing animals, thus limiting the potential for growth, production, and efficient nutrient utilization (Ball et al, 2008).

It is well recognized that under current feeding recommendations, lactation requires such an enormous quantity of nutrients that sows and, especially, first parity animals, cannot consume enough feed to avoid catabolism of body tissue (Aherne and Foxcroft, 2000; Cameron et al, 2002). Commonly, a step-up feeding program is used in early-lactation, resulting in nutrient restriction, before the sows are allowed access to feed *ad libitum* (Neill and Williams, 2010). If nutrient availability is restricted severely enough within a single lactation, sows may suffer significant lean-tissue losses which may affect milk production or impact rebreeding (Clowes et al, 2003; Vinsky, 2006; Pluske et al, 2009). More importantly, nutrient insufficiency over multiple lactations limits the longevity of

the sows in the pork production system and increases culling rates (Mahan, 1977; Mahan, 1981; Aherne and Kirkwood, 1985; Dourmad et al, 1994a,b; Gaughan et al, 1995; Young and Aherne, 2005; Anil et al, 2006; Tvrdoň and Marková, 2007).

Protein and energy metabolism interact and should be studied simultaneously (Chapter 3.0). However, there are not even any data available about protein turnover in gestation and lactation in sows. Therefore, interactions of energy and protein metabolism in gestating and lactating sows must be investigated.

The objectives of this experiment were to simultaneously measure protein and energy balance in gestating and lactating sows to provide data about differential changes in energy and protein metabolism during gestation and lactation and, therefore, identify periods of changing requirements of breeding sows. Secondary outcomes will also be recorded. These include sow body weight change, litter size and growth rate, and milk production.

4.2 Methods and materials

The experiment described herein was conducted simultaneously with an experiment described previously (Samuel, 2008). A portion of the data related to energy metabolism was previously reported by Samuel (2008) and is repeated here for completeness and to assist in interpretation and integration with the data about protein metabolism.

4.2.1 Animals

Sows (n=5, 192±2 kg) previously selected from the University of Alberta's Swine Research and Technology Centre (SRTC) and surgically fitted

with cephalic vein catheters terminated with a subcutaneous injection port (Chapter 3.0) were bred and confirmed pregnant. A second group of sows (n=3,190.0±2.9 kg), previously confirmed pregnant, were surgically fitted with cephalic vein catheters on approximately d 80 of gestation. This second group of sows was added to supplement the number of animals studied in late-gestation and beyond due to issues with catheter patency and illness (Chapter 3.0). All procedures used in this study were approved by the Faculty Animal Policy and Welfare Committee of the University of Alberta.

Piglets received intramuscular injections of iron dextran (1 mL, Ferroforte®, MTC Pharmaceuticals, Cambridge, ON), Penicillin G (1 mL, Vetoquinol NA Inc., Chemin Georges, Lavaltrie, QC), and piglets' teeth and tails were clipped within two days of birth. Piglets were cross fostered, with 48 hours after birth, to achieve a minimum litter size of 8 piglets; all fostering events were recorded. Male piglets were castrated on day 12 following parturition. All of the above procedures were performed according to standard operating procedures of the SRTC.

4.2.2 Diets and feeding

Specific, specialized diets were not prepared for this experiment. Instead, diets, as provided to the SRTC, were used, with two exceptions:

1) an indigestible marker, Celite®, was added and mixed into individual batches of feed at 1% and
2) A) additional leucine, equivalent to the isotope dose, was added and mixed into individual batches of feed during gestation or

B) (for the first group of five sows) additional leucine, equivalent to the isotope dose, was added and mixed into 1 kg bags of feed and fed immediately after the leftover feed from the previous day was collected in the morning or

C) (for the second group of three sows) additional leucine was added to the complete feed at 0.6 g/kg to provide leucine intake equivalent to the isotope dose.

Gestation and lactation diets were formulated to provide 13 MJ DE/kg and 0.65% lysine total lysine and 14 MJ DE/kg and 1.02% total lysine, respectively (Table 4.1) (Consultant Feeds Ltd., Calmar, AB). Diets were composed of barley, wheat, and canola and soybean meals. Sows were housed individually and fed one-half of their daily feed allowance twice daily during gestation. Gestation feed allowances were determined individually according to measured P2 backfat thickness and body weight post-breeding to achieve sufficient maternal tissue gain to support lactation (Aherne and Foxcroft, 2000). Individual nipple drinkers provided free access to water at all times. Sows consumed all feed provided up to parturition, so there were no feed refusals to collect.

Gestation diet was provided, according to individual feed allowances, until sows were moved into individual farrowing crates in temperature-controlled rooms on approximately day 109 of gestation, immediately following the end of open circuit calorimetry. Sows were then offered and consumed 3.0 kg/d of lactation diet until parturition once daily, in accordance with standard industry practice. Following farrowing, additional feed was offered in 0.5 kg/d increments

until sows were consuming 5 kg/d. Additional feed was then offered in 1 kg increments provided that feed refusal of the previous day was less than 0.5 kg to, effectively, achieve *ad libitum* feed intake. Sows were offered feed three times daily (0700, 1200, 1530 h) during lactation and, when necessary, were encouraged to stand up and consume their feed allowance. Supplemental piglet creep feed was not provided during lactation, which is a common industry practice, to avoid confounding effects on piglet growth. Piglets had free access to water via a nipple drinker in the farrowing crate.

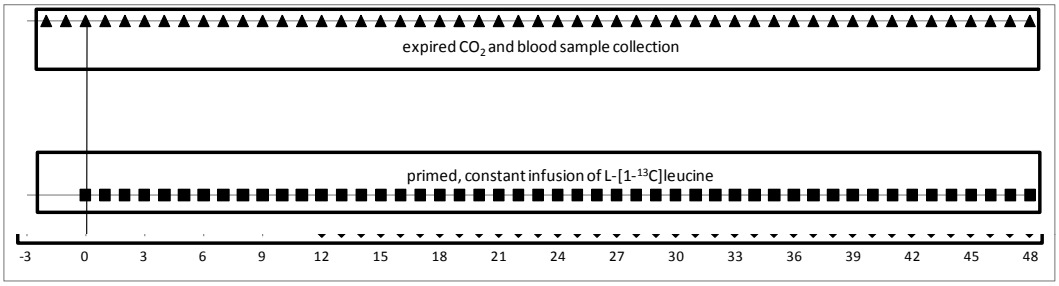
4.2.3 Isotope dosage, delivery, and feeding

The tracer dose of 1.0 $mg \cdot kg^{-1} \cdot h^{-1}$ for the stable-isotope labeled amino acid tracer L-[1-^{13}C]leucine (Cambridge Isotope Labs, Andover, MA) was previously determined to produce $^{13}CO_2$ enrichment sufficiently above the natural background enrichment for the measurement of atom percent excess (APE) (Chapter 3.0). The tracer was delivered as a primed-constant IV infusion over 24 h to measure protein turnover (Jahoor, 2003).

Sows were fed a small meal, equivalent to 1/13th of their daily ration, upon entering the chamber in the morning. Sows were then fed three small meals, equivalent to 1/26th of their daily ration, to establish basal production of $^{13}CO_2$ (Jones et al, 1985). Sows were then fed large meals twice daily during gestation; once after the three background measurements and once 6 h later. Sows were fed *ad libitum* during lactation by providing feed in 1 kg increments once the majority of feed had been consumed, as determined by visual inspection. Actual feed intake was determined by subtracting uneaten feed collected at the end of the

experiment from the total weight of the feed offered.

Figure 4.1 – Sample collection and isotope infusion in pregnant and lactating sows over 24 h



4.2.4 Indirect calorimetry

Sows were individually housed in respiration chambers for 24 h measurements of heat production by open-circuit indirect calorimetry on days 30, 45, and 105 of gestation and with their litters on days 7 and 19 of lactation. The 24 h gas exchange was recorded for O_2, CO_2, and CH_4 in 1 min intervals. The earliest day in gestation that sows could be confirmed pregnant was 28 days after breeding, therefore, day 30 was chosen to represent early-gestation. Fetal secondary muscle fiber development (Cerisuelo et al, 2009) and dichotomy of fetal size (Kim et al, 2009) has been reported to occur around day 45 of gestation, so this was chosen to represent mid-gestation. Sows were to be relocated to the farrowing rooms on day 109 of gestation; in order to accommodate, day 105 was chosen to represent late-gestation.

Before each study, the S108 oxygen (O_2), S153 carbon dioxide (CO_2), and S126 methane (CH_4) analyzers (Qubit Systems, Kingston, ON) were calibrated to zero with N_2 for the O_2 and CO_2 analyzers and a gas of known [O_2] and the balance N_2 for the CH_4 analyzers. The upper limits of the analyzers were

calibrated with a gas of known [O_2] (approximately 21%), [CO_2] (approximately 1.5%), and [CH_4] (approximately 100 ppm) and the balance (approximately 77.5 %) N_2 (Praxair, Edmonton, Alberta). O_2, CO_2, and CH_4 concentrations were collected by C409 data acquisition system as an average of 200 samples in one-minute intervals during the study (Qubit Systems, Kingston, ON). The gas exchange of the animals was determined by comparison to the ambient air values as recorded immediately before and after the study periods. The analytical values for each of the calibration gases was recorded immediately before and after the study and used to correct for drift of the analyzers, if necessary.

Two independent open-circuit indirect calorimetry chambers were previously described (3.2.1), except that the volume of the chambers was increased during lactation studies to allow for the litter to be housed with the sows. Due to the greater CO_2 production of lactating sows with piglets, the airflow through the chamber was increased to maintain CO_2 below 1%.

4.2.5 Sample Collection

4.2.5.1 Quantitative expired CO_2 collection

A sub-sample of the air flow from the respiration chambers flowed through midget bubblers (Fisher Scientific, Mississauga, Canada or VWR International, Edmonton, Canada) suspended in 1 *N* NaOH solution at a rate of ~150 mL/min. A single midget bubbler in ~11 mL of $NaOH_{(aq)}$ was shown to provide complete and quantitative trapping of CO_2 (Chapter 3.0). The solution was changed every 30 min. Charged solution was transferred equally into two evacuated, glass, 16 x 100 mm, serum collection tubes (Vacutainer, Oakville, Ontario) and frozen at -20 ˚C

until analysis.

4.2.5.2 Blood collection

The IV infusion of isotope was halted and detached from the infusion solution for approximately three minutes to allow blood to be withdrawn from the catheter. The isotope solution plus at least two ml of undiluted blood were collected from the catheter before blood samples of ~5 mL sample size were collected every 30 min through the catheter extension attached to the subcutaneous injection port. The isotope solution and diluted blood were reinjected into the catheter and flushed with 10 IU/ml heparin in saline before the infusion was reestablished (Chapter 3.0). Samples were transferred into glass or plastic 13 x 100 tubes containing lithium heparin (Vacutainer, Oakville, Ontario) before centrifugation at 1500 x g for 15 min. Plasma was harvested and stored frozen at -20 °C until analysis. Grab samples of feces (because they contained an indigestible marker, see 4.2.6.3) (at least 200 g) were taken on the day of respiration before the change of diet, stored frozen at -20 °C, and then freeze dried before further analysis. Small random samples of the mixed diets were collected frequently following mixing during the preparation of individual meals. The small samples were pooled as representing a particular diet before and were stored at room temperature before further analysis.

4.2.6 Chemical analyses

4.2.6.1 Sample grinding

Feed and freeze dried fecal samples were ground in a commercial coffee grinder before chemical analyses to reduce particle size. The results are

summarized in Table 4.2.

4.2.6.2 Combustion calorimetry

(see 3.2.6.2)

4.2.6.3 Acid insoluble ash content

(see 3.2.6.3)

4.2.6.4 Nitrogen and carbon contents

(see 3.2.6.4)

4.2.7 Amino acid concentrations by HPLC

Amino acid concentrations in feed and milk were measured by reverse-phase HPLC (Waters Corp. Mississauga, Canada) after 24 h hydrolysis in 6 N HCl (Llames and Fontaine, 1994) using phenylisothiocyanate derivatives with norleucine added as internal standard, as previously described (Bidlingmeyer et al, 1984; Murch et al, 1996). Methionine, cysteine, and tryptophan concentrations were not determined.

4.2.8 Isotope analyses

(see 3.2.7)

4.2.9 Calculation of results

4.2.9.1 Acid insoluble ash content

(see 3.2.8.1)

4.2.9.2 Digestibility

(see 3.2.8.2)

4.2.9.3 Volumes of gases

(see 3.2.8.5)

4.2.9.4 Heat production

(see 3.2.8.6) Additionally, for lactating sows, total heat production was apportioned into ‘sow only’ heat production by subtraction of ‘piglet’ heat production, which was calculated according to the ratio of the metabolic live weight of the sow to the sum of the piglets (Jakobsen et al, 2005).

4.2.9.5 Respiratory quotient

(see 3.2.8.7)

4.2.9.6 Assumptions

(see 3.2.8.8)

4.2.9.7 Isotope kinetics

(see 3.2.8.10) However, carbon dioxide recovery was calculated according to Moehn et al (2004) at 73.3% for gestation and 61% during lactation (Motil et al, 1989).

4.2.9.8 Protein and lipid balance

(see 3.2.8.11)

4.2.10 Measurements

4.2.10.1 Sow body weight and composition

Sow body weight (±0.1 kg, Detecto, Webb City, MO) and real-time ultrasound measurements of backfat and loin thickness and loin area were recorded on the days immediately preceding or following each of the three measurements of energy expenditure and isotope dilution during gestation and the two measurements of energy expenditure and isotope dilution during lactation.

4.2.10.2 Milk production

The weigh-suckle-weigh (WSW) technique was used to measure milk production. Measurements of milk production were conducted on days 6, 7, or 8 (6.7±0.4) and days 16, 19, 18, 19, or 20 (19.7±1.3) of early- and late-lactation, respectively, between 0800 or 0900 and 1600 h. Seven repeated measurements were performed on each day.

At 0800 or 0900, piglets were moved away from the sow to a nearby, empty pen. Approximately 20 minutes later, sows received an intramuscular injection of 20 IU of oxytocin (Bimeda®-MTC Animal Health Inc., Cambridge, Ontario) into the neck muscle to stimulate milk expression. Samples of milk were manually expressed from random mammary glands to obtain samples of 10 to 15 g of milk. When sufficient milk sample could not obtained at the beginning of the day, sows were given an intramuscular injection of 20 IU of oxytocin in the neck 20 minutes following the seventh suckling period. Milk samples were frozen at -20 ˚C until further analysis.

After the morning milk sample collection, the piglets were kept away from the sow for an additional 20 minutes before WSW measurements began. Litter weight was recorded to ±0.2 g (Sartorius EB15, Sartorius AB, Gottingen, Germany) before the litter was placed in the pen with the sow. The duration of suckling was recorded as the time from weighing to the time when the piglets stopped suckling and were returned to the empty pen, away from the sow. Urination and defecation events by piglets during the timed period (i.e. time with sow or during weighing) were recorded. Weight was corrected according to

Klaver et al (1981) for urination events:

urine voided (g) = $2.9*W^{0.75} + 18.7$, and

by 10 grams for each defecation event (Sinclair et al, 1999). Piglet metabolic and evaporative losses during suckling were corrected using a formula adapted from Noblet and Etienne (1986):

milk intake/suckling (g) = $(W_f - W_i) + 0.210(t)*W^{0.75}$

where W_f and W_i are the final and initial litter weight in grams, respectively, t is the duration of suckling in minutes, and $W^{0.75}$ is the mean metabolic body weight of the piglets, multiplied by the number of piglets in the litter. According to Noblet and Etienne (1986, 1989) and Speer and Cox (1984) the first two WSW events of each day should be treated as an adaptation period, so were not included in the determination of milk production. If, following a WSW event, the litter weight was similar or lower than the initial litter weight, then the measurement was regarded as a non-nutritive suckling event (Spinka et al, 1997) and, therefore, not included in the calculation of final milk yield.

4.2.11 Statistical analysis

Data are presented as mean ± SEM, unless otherwise stated. Statistical analysis was performed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC.) The classification variable was either day of gestation or lactation. Data were analyzed within and between gestation and lactation. Individual animals, nested in respiration chamber, were treated as random variables. Model statements were tested using the Kenward-Rogers degrees of freedom method. Least-square means were compared using the ‘pdiff’ option. Significance was

accepted at $P<0.05$; a tendancy at $P<0.1$.

4.3 Results

Daily weight gain of sows (190 g/d) was lowest ($P<0.01$, SEM=50) from breeding to day 30 of gestation (Samuel, 2008) compared to day 30 to day 45 (389 g/d) or day 45 to day 105 (558 g/d) (Table 4.3). Sow body weight increased ($P<0.01$, SEM=3) from 183.8 kg at breeding to 193.9 kg on day 30 up to 199.3 kg on day 45 and finally to 214.3 kg on day 105 of gestation (Table 4.3). Ultrasonic measurements of body composition were not different within gestation or lactation, but lean percent was greater during lactation than on day 45 of gestation (Table 4.3). Measured backfat thickness increased ($P<0.05$) from breeding (18.8 mm) to late gestation (20.8 mm) (Samuel, 2008) and tended to decrease from early (19.1 mm) to late lactation (17.5 mm). Backfat thickness was lower in lactation than on days 45 and 105 of gestation. Sows lost weight at over 2.6 kg/d during lactation up to day 7 of lactation before weight loss decreased to 600 g/d up to day 19 of lactation. Loin area was not different ($P=0.84$) from breeding to late gestation or from early to late lactation (Table 4.3).

Sows were fed 2.4±0.1 kg of gestation diet daily resulting in 6.39, 6.22, and 5.84 $kJ \cdot kg^{-1} \cdot h^{-1}$of energy intake for the periods between measurement days (Table 4.4) (Samuel, 2008). Feed intake was greater on day 7 of lactation and further increased by day 19 of lactation (Table 4.3).

Total milk production (7.14 vs. 9.90 kg/d) (Table 4.5) increased ($P<0.05$) and milk production per piglet (682 g/d vs. 935 g/d) tended to increase from day 7 to day 19 of lactation. Litter size was standardized and remained constant during

lactation (Table 4.5). Piglet daily gain increased from 173 g/d, from birth to day 7, to 251 g/d, from day 7 to day 19, of lactation ($P<0.01$). Milk required per kg of piglet gain remained constant.

Parameters of energy metabolism were previously discussed by Samuel (2008). Briefly, energy intake was restricted during gestation by feed intake and energy retention was numerically negative on day 105 of gestation. The RQ for sows at day 45 was greater than 1, indicating lipogenesis (Table 4.5) (Samuel, 2008). Sow plus litter heat production (63.4 vs. 87.9 MJ/d) (Table 4.6), calculated (Jakobsen et al, 2005) sow only heat production (49.6 vs. 69.0 MJ/d) and energy intake from early to late lactation (60.9 vs. 79.7 MJ/d) increased ($P<0.05$) from day 7 to day 19 of lactation. The RQ of sows on day 19 of lactation was lower compared to day 7 of lactation. Energy retention was negative throughout lactation and increasingly negative on day 19 of lactation. The difference between energy intake and output (14 MJ) was entirely accounted for by increased milk energy output on day 19 of lactation (Table 4.6).

Leucine oxidation and appearance from body protein breakdown were not different ($P>0.10$) during gestation. Leucine flux was not different either within or between gestation and lactation. Leucine for protein synthesis was similar in gestation and greater than in lactation ($P<0.01$), but did not change from day 7 to day 19 of lactation. Leucine oxidation was greater in lactation than during gestation, but was not different within gestation or lactation. Leucine intake increased as feed intake increased during lactation (Tables 4.4 & 4.6) and tended to be lower on day 105 of gestation when expressed by unit body weight (Table

4.4 and Figures 4.2 and 4.3). Protein retention was not different during gestation, but tended to be greater on day 19 of lactation when expressed per unit body weight (Table 4.4 and Figures 4.2 and 4.3). Calculated lipid retention was consistently negative during gestation and lactation, except for day 45 when it tended to be greater and numerically positive within gestation (Table 4.6). Calculated daily gain was similar during gestation and lactation, but was lower during lactation (Table 4.6).

Heat production was greater than 100% of ME intake on day 105 of gestation and greater than on days 30 or 45 of gestation (calculated with data from Samuel, 2008). Sow only heat production was not different between day 7 and 19 of lactation and was less than 100% of ME intake. Maintenance energy expenditure was highest on day 105 of gestation, but was lower and decreased during lactation from 50% to 35% of ME intake. Leucine used for protein synthesis (as percent of flux) was greater and leucine oxidation and intake (as percent of flux) lower during gestation than lactation, but were not different within gestation or lactation. Leucine retention (as percent of flux) was greatest on day 19 of lactation, but not different within gestation or lactation. Energy exported in milk was 60 – 65% of ME intake and not different during lactation (Table 4.7).

4.4 Discussion

The experiment described herein has provided first-time measurements of protein turnover in gestating and lactating sows, which was recorded simultaneously with energy metabolism. Sow body weight gain during gestation,

litter size, and body weight loss during lactation were typical for modern, high producing sows.

The energy intake of sows during gestation must be restricted to avoid excessive weight gain which can compromise milk production (Clowes et al, 2003) and subsequent rebreeding success due to body protein loss (Vinsky, 2006; Pluske et al, 2009). Wallace et al. (2003) also reported that maternal over-nutrition leads to reduced placental growth, reduced vascularization, and decreased efficiency of nutrient transport. It has also been shown that maternal over-nutrition in the period after breeding reduces embryo survival, and, therefore, litter size. Pregnant sows allowed *ad libitum* access to feed consume more energy than required for maintenance and growth of the products of conception (NRC, 1998). Therefore, sows in this experiment were fed a constant allowance of 29.9±0.3 MJ ME/d to achieve nominal weight gain during gestation (NRC, 1998). Daily weight gain of the sows was lowest from breeding to day 30 of gestation and numerically greater from day 45 to day 105 (Samuel, 2008). However, significant weight gain begins to occur beyond this time as the mammary glands begin to grow and develop (Ji et al, 2005). Overall, sow body weight increased from breeding to parturition and was consistent with the prediction of NRC (1998).

As a result of constant feed intake, sows on day 105 of gestation had energy expenditure that exceeded energy intake. Maintenance energy expenditure was highest on day 105 of gestation. Noblet et al (1997) reported that the energy concentration in the products of conception increases during gestation. It should

be noted that, in contrast to the current experiment, Ramonet et al. (2000) showed that sows maintained a positive energy balance throughout gestation. However, the sows used by Ramonet et al. (2000) were older than those used in the current experiments. Results from previous experiments in our lab indicate that second parity sows lost backfat between mid- and late-gestation, but not third parity sows, indicating older sows are less energy restricted in late-gestation (McMillan, 2003). It has been reported that sows will have achieved adult body weight by their forth parity (Close and Mullan, 1996); Cooper et al, 2001). High energy intake in lactation cannot compensate for energy intake below recommendations (NRC, 1998) during gestation, with respect to milk production and rebreeding success (Coffey et al, 1994; Beyer et al, 2007), therefore, it is imperative to feed sows sufficient energy intake during gestation. However, Weldon et al (1991) cautioned that the extent of body tissue mobilization varies widely by individual, as shown by the variability of energy balance in late-gestation.

Sow only heat production during lactation was not greater than energy intake, but coupled with the additional energy required for milk production, energy intake was not sufficient and resulted in negative energy retention. Thus, sows were in negative energy balance by day 105 of gestation which continued through the duration of lactation.

Sows, like most mammalian species, progressively develop reversible insulin resistance as gestation and lactation continues. Insulin resistance is an adaptive mechanism to partition nutrients to the gravid uterus and mammary glands. Without the effects of insulin on the maternal tissues, body tissue reserves

of lipid are mobilized, protein breakdown is increased, and plasma glucose concentration increases. As a result, glucose and amino acids are available to be transferred to the growing fetuses. However, lipids cannot be transferred by the porcine placenta and, therefore, mobilized body lipids are utilized for energy by the maternal tissues and may result in ketosis, due to increased levels of ketones in the blood. Backfat thickness increased from breeding up to day 105 of gestation, but was numerically greatest on day 45, indicating the development of insulin resistance between day 45 and day 105. In the extreme, Anderson (1975) and Hard and Anderson (1979) reported that starved sows made available protein and energy from maternal stores for embryonic and placental development when progesterone and estrogen levels were adequate, clearly demonstrating that sows are capable of maintaining litter size during malnutrition (Baumen and Currie, 1980; Pond et al, 1991).

Backfat thickness of sows was numerically greater, lean percent was numerically lower, RQ was greater than one, and calculated lipid retention was positive on day 45 of gestation, indicating lipogenesis (Samuel, 2008). Butte and King (2005) observed that humans deposit body fat in mid-gestation to prepare for the greater energy demands associated with fetal growth in late-gestation and milk production during lactation. Similarly, Ramonet et al. (2000) observed numerically greater energy retention and RQ on day 57 of gestation which, subsequently, had decreased by day 97 of gestation. It is not possible to suggest that energy intake reduction would be feasible during mid-gestation under current feeding recommendations. Improper nutrition in mid-gestation of cattle has long-

term effect on offspring muscle fiber content, but when nutrient restriction reduces muscle size in late-gestation, the effect is recoverable through compensatory post-natal growth (Du and Zhu, 2009).

Samuel (2008) calculated that the weight gain of sows in late-gestation could almost entirely be accounted for by fetal growth, assuming it was similar to the fetal gain of approximately 50 g/d per piglet that was reported by McPherson et al. (2004). The calculated lipid loss of 154 g/d from the current experiment was similar to value of 140 g/d reported by Close et al. (1985). Therefore, this population of sows would have required an additional 9 MJ ME/d to achieve zero lipid retention in late-gestation. This value is similar to the recommendation by GfE (2008) to provide an additional 8 MJ ME/d for first and second parity sows. However, it is important to note that pregnant, growing mothers use extra nutrients for maternal growth, not fetal development (Reynolds and Redmer, 1995). Miller et al (2000) observed that sows fed greater energy and protein for the final two weeks of gestation gained almost 4 kg and 0.2 mm of backfat and they did not observe any differences in piglet performance. Alternatively, Beyer et al. (1994) caution that energy intake above requirements negatively impacts the development of secreting tissue in the mammary glands. Therefore, optimal feeding of sows requires careful consideration of energy intake.

Sows were in severe negative energy balance during lactation, which was similar for day 7 and day 19 of lactation. The extent of energy deficit is important because Theil et al. (2004) reported that milk production was limited by the energy supply. Milk production increased from day 7 to day 19 of lactation and

tended to increase per piglet, consistent with reports by McMillan (2003), Jakobsen et al. (2005), and Renaudeau and Noblet (2001). Excess dietary energy consumed on day 19 of lactation was almost entirely exported in milk. In contrast, Verstegen et al. (1985) reported a smaller and Jakobsen et al. (2005) reported a greater energy deficit in late-gestation than the current experiment. The energy deficit reported in the current experiment was slightly greater than previous reports (Verstegen et al, 1985; Ramonet et al, 2000; Theil et al, 2004) even though energy intake and milk production were similar. Milk energy and protein content from the current experiment was similar to Renaudeau and Noblet (2001). The quantity of energy exported in milk was fairly high (60 – 65% of ME intake) and consistent during lactation. This is in contrast to Theil et al. (2004) who indicated that the efficiency for utilization of dietary ME for milk production increased as lactation progressed. Litter size was standardized following parturition and remained constant. Piglet daily gain increased during lactation and was similar to reports by Verstegen et al. (1985) and Renaudeau and Noblet (2001). The quantity of milk required per kg of piglet gain was constant, which agrees with 3.7 kg milk/kg gain of Noblet and Etienne (1989). Energy intake is the determining factor for milk production, so increasing the energy intake of lactating sows could increase piglet growth and, by reducing sow body tissue mobilization, sow longevity.

Leucine flux was not different either within or between gestation and lactation and leucine oxidation, appearance from breakdown of or incorporation into body protein were not different during gestation. Leucine for protein

synthesis was greater during lactation than during gestation, due to the production of milk protein, but was not different between days of lactation. Protein retention was numerically greater during late- (P=0.10) than early-lactation and greater than during gestation (P=0.03), demonstrating the effect of increasing energy intake on protein retention. Ultrasonic measurements indicated sufficient protein intake since there were no differences in loin area during pregnancy or lactation and protein retention was consistently positive. Leucine oxidation was greater in lactation than during gestation, suggesting an inefficiency in protein nutrition of lactating sows. This may be due to insufficient energy intake, resulting in amino acids catabolism or it may be due to a limiting amino acid, resulting in high oxidation of the other amino acids. Given that our tracer was one of the BCAA which can be readily oxidized to provide energy to the muscle and the severity of the measured energy deficit, it was most likely that leucine was being oxidized at a high rate to supplement energy intake. Protein retention, calculated from leucine retention, tended to be greater on day 19 of lactation, when expressed per unit body weight. The increase in leucine intake was likely the cause of increased leucine and protein retention. Also as leucine intake increased during lactation, there was a numerical increase in the utilization of leucine from the diet (as percent of flux) and a corresponding decrease in the appearance of leucine from body protein breakdown. Leucine from body protein breakdown contributed fairly extensively (80 %) and consistently to leucine for protein synthesis, which was the majority of leucine flux during gestation, suggesting a consistent remodeling of body protein. Leucine for protein synthesis was consistently greater than

leucine from body tissue breakdown, resulting in net accumulation of body protein during gestation.

Quantitative estimates of protein synthesis and protein retention from isotope kinetic studies rely on assumption of the recovery of CO_2. The weighted average of CO_2 recovery in non-pregnant sows was used (73.7%) during gestation. In the absence of data for lactating sows, the mean recovery (61%) from Motil et al (1989) was applied.

In the absence of protein kinetics data available for sows, comparisons to human data will be discussed. It should be noted that pregnant or lactating women have much smaller total nutrient requirements compared to litter bearing sows. Maternal tissue growth occurs in gestating sows, but much less so, if at all, in humans (Butte and King, 2005) and porcine fetal growth is 10-fold greater in sows (McPherson et al, 2004) compared to humans (Butte and King, 2005) in late-gestation. Also, milk production was much greater from sows in this experiment than from lactating women.

Leucine flux (211 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) was similar to Kalhan et al (1998) who reported values ranging from 135 – 170 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ in pregnancy and Sunehag et al (2003) who reported 195 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ from lactating women. Kalhan et al (1998) reported a numerical increase in flux in mid-pregnancy, which was similarly observed in this experiment. Also similar to Kalhan et al (1998), leucine oxidation was unchanged during gestation. In contrast to Motil et al (1989), leucine oxidation in this experiment was greater during lactation than during gestation. Leucine for protein synthesis during lactation was similar to humans

(Motil et al, 1989; Sunehag et al, 2003). Leucine intake, as a percent of flux, was similar between sows (54.9%) and humans (59%) (Sunehag et al, 2003). Leucine appearance from protein breakdown was also similar (Sunehag et al, 2003). Therefore, remarkable similarities exist between protein kinetics of sows and humans.

Practically, sows are fed a single, fixed diet throughout pregnancy. However, growing evidence suggests that significant changes in fetal and maternal metabolism occur during gestation (Kim et al, 2009). Dietary energy intakes for late-gestation and lactation were insufficient. Feeding a diet greater in energy to sows in late-gestation is required to ensure sufficient energy intake and avoid a catabolic state before lactation. Also, it may be appropriate to reconsider the amino acid content of the diet during late-gestation. Lactating sows required 30% more energy than was consumed in order to improve protein metabolism and to maximize milk production and longevity.

Table 4.1 – Ingredient composition (g/kg) of diets fed to sows during gestation and lactation

Ingredient	Gestation diet[1]	Lactation diet
Wheat[2]	120	500
Barley[3]	690	200
Soybean meal[4]	65	225
Canola meal[5]	65	0
Canola oil	10	25
Celite	10	10
Mineral-vitamin premix[6]	40	40
Calculated		
Ca, %	0.95	0.98
Total P, %	0.72	0.71
DE, MJ/kg	13.06	14.06
CP, %	14.76	19.78
Total Lys, %	0.65	1.02

[1]: reproduced from Samuel (2008)

[2]: 12.5% CP

[3]: 11.2% CP

[4]: 48% CP

[5]: 35% CP

[6]: Provided per kilogram of the diet: Ca, 8.6g; P, 3.4g; Na, 1.9g; Mg, 140mg; K, 30mg; Fe, 139 mg; Zn, 119 mg; Mn, 56 mg; Cu, 16 mg; Co, 0.4 mg; I, 0.4 mg; Se, 0.3 mg; vitamin A, 12,000 IU; vitamin D_3, 1200 IU; vitamin E, 62 IU; vitamin K, 2.5mg; biotin, 0.6 mg; folic acid, 2.5mg; niacin, 42 mg; pantothenic acid, 25 mg; pyridoxine, 5mg; riboflavin, 9.5 mg; thiamine, 8.4mg; vitamin B_{12}, 28μg.

Table 4.2 – Analyzed nutrient and amino acid contents (g/kg) of diets fed to sows during gestation and lactation

	Gestation	Lactation
Digestible energy (MJ/kg)	14.7	15.2
Crude protein	162.5	210.9
Crude lipids	27.2	42.4
Neutral detergent fibre	159.5	134.3
Acid detergent fibre	73.8	71.6
Crude ash	8.7	15.2
Arginine	3.4	12.4
Histidine	5.6	5.1
Isoleucine	11.2	8.0
Leucine	6.6	15.0
Lysine	2.7	9.3
Phenylalanine	8.0	3.1
Threonine	5.6	10.7
Valine	7.3	10.7

Table 4.3 – Feed intake, body weight, daily gain, and measurements of body composition (i.e. lean percent and backfat thickness) of sows during gestation and lactation

	Gestation[1]					Lactation				Comparison between gestation and lactation	
	Day 30	Day 45	Day 105			Day 7	Day 19				
Number of sows	5	5	7	SEM	P-value	7	7	SEM	P-value	SEM	P-value
Feed intake, kg	2.38x	2.38x	2.40x	0.03	1.0	4.69y	6.13z	0.35	<0.01	0.32	<0.001
Body weight, kg	193.9a,x	199.3b,x	214.3c,z	3.0	<0.01	215.9z	206.8y	4.0	<0.01	2.5	<0.001
Daily gain, g/d	190a,xz	389b,x	558b,x	50	<0.01	-2651y	-602z	383	<0.01	251	<0.001
Lean percent	39.1xy	37.8x	38.9xz	0.4	0.21	39.9yz	40.6y	0.6	0.12	0.4	0.024
Backfat, mm	20.7x	23.8y	20.8yz	1.1	0.19	19.1xz	17.5x	1.3	0.09	0.9	0.007
Loin, mm	53.5	54.0	54.2	1.1	0.99	53.2	51.2	1.0	0.28	0.7	0.831
Loin area, cm^2	38.9	39.2	39.3	0.7	0.98	38.6	37.2	0.7	0.28	0.5	0.836

[1]: reproduced from Samuel (2008)

[a,b,c]: values which share a common letter are not different within gestation or lactation

[x,y,z]: values which share a common letter are not different between gestation and lactation

Table 4.4 – Energy (MJ/d) and protein metabolism (g/d) of sows during gestation and lactation

	Gestation					Lactation				Comparison between gestation and lactation	
	Day 30	Day 45	Day 105			Day 7	Day 19				
Number of sows	5	5	7	SEM	P-value	7	7	SEM	P-value	SEM	P-value
Metabolizable energy intake[1], MJ/d	29.8a,x	29.8a,x	30.0a,x	0.3	1.0	60.9y	79.7z	4.6	0.030	4.2	<0.001
Heat production[1], MJ/d	25.5a,x	23.9a,x	32.9b,x	1.4	0.036	63.4y	87.9z	5.8	0.012	5.1	<0.001
Sow only heat production[2], MJ/d	-	-	-	-	-	49.6	69.0	5.3	0.021	-	-
Maintenance energy expenditure[3], MJ/d	26.3a,v	26.9a,vxy	28.4b,xz	0.3	<0.001	28.5z	27.6y	0.4	0.003	0.3	<0.001
Energy retention[4], MJ/d	4.2ab,x	5.9b,x	-2.9a,x	1.4	0.031	-24.4y	-38.8z	4.7	0.111	3.7	<0.001
Energy exported in milk[5], MJ/d	-	-	-	-	-	35.7	49.5	3.5	<0.05	-	-
Respiratory quotient	1.09xy	1.17x	0.99yz	0.04	0.081	1.01yz	0.95z	0.02	<0.05	0.02	<0.05
Leucine flux, g/d	140.6	153.8	145.6	5.8	0.573	123.2	129.3	13.0	0.789	6.7	0.510
Leucine intake, g/d	25.3a,x	25.5ab,x	26.4b,x	0.3	0.016	65.1y	86.7z	5.5	0.024	5.6	<0.001
Leucine from body protein breakdown, g/d	115.3x	128.2x	119.3x	5.7	0.607	58.1y	42.6y	11.5	0.569	9.4	<0.001
Leucine oxidation, g/d	9.6x	10.4x	11.7x	0.5	0.200	20.9y	23.8y	2.3	0.562	1.5	<0.001
Leucine used for synthesis, g/d	131.0x	143.4x	134.1x	6.1	0.680	74.9y	72.0y	13.2	0.935	9.3	0.008

Leucine exported in milk, g/d	-	-	-	-	-	24.6	32.8	2.3	0.74	-	-
Leucine retention, g/d	15.7x	15.2x	14.9x	0.6	0.860	16.8x	24.4y	3.8	0.101	1.9	0.029
Protein retention[5], g/d	206.4x	199.5x	195.4x	7.4	0.860	221.0x	387.4y	50.6	0.101	24.4	0.029
Lipid retention[6], g/d	-30.7x	26.9x	-153.8x	34.6	0.092	-962y	-1289z	129	0.148	126	<0.001
Calculated daily gain[7], g/d	730x	768x	552xz	51	0.238	-251y	1yz	200	0.581	123	0.011

[1]: Gestation data reproduced from Samuel (2008)

[2]: Calculated according to Jakobsen et al, 2005

[3]: Calculated as 506 kJ/$BW^{0.75}$ (Samuel, 2008)

[4]: Calculated as: Metabolizable energy intake (MJ/d) – Heat production (gestation) or Sow only heat production (lactation) (MJ/d)

[5]: Calculated as: Mean energy content of milk (MJ/kg) * mean milk production (kg/d)

[6]: Calculated as: leucine retention (g/d) / 0.076 (the leucine content of body protein)

[7]: Calculated as:

lipid retention (g/d) = [Energy retention (MJ/d) – (energy content of protein (23.8 kJ/g) * protein retention (g/d)]*(1/energy content of lipid (39.0 kJ/g) (ARC, 1981)

[8]: Calculated as:

daily gain = [(protein retention (g/d) * (1/mean water content of protein) + (lipid gain)*(1/mean water content of lipid)

[a,b,c]: values which share a common letter are not different within gestation or lactation

[v,w,x,y,z]: values which share a common letter are not different between gestation and lactation

Table 4.5 – Lactation production performance measurements of sows (i.e. number of piglets, milk production, and piglet weight gain)

	Early-lactation	Late-lactation	SEM	P-value
Number of piglets suckling	10.6	10.6	0.3	1.00
Milk production, kg/d	7.14	9.90	0.71	0.045
Milk production, g/piglet	682	935	67	0.054
Piglet daily gain, g/d	173	251	14	0.006
Milk consumed per piglet gain, g/g	4.02	3.76	0.27	0.657

Table 4.6 – Energy ($kJ \cdot kg^{-1} \cdot h^{-1}$) and protein metabolism ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) of sows during gestation and lactation

	Gestation					Lactation				Comparison between gestation and lactation	
	Day 30	Day 45	Day 105			Day 7	Day 19				
Number of sows	5	5	7	SEM	P-value	7	7	SEM	P-value	SEM	P-value
Metabolizable energy intake[1], $kJ \cdot kg^{-1} \cdot h^{-1}$	6.39a,x	6.22a,x	5.84b,x	0.09	<0.001	11.69y	16.09z	0.93	0.011	0.82	<0.001
Heat production[1], $kJ \cdot kg^{-1} \cdot h^{-1}$	5.49x	4.99x	6.43x	0.26	0.157	12.26y	17.86z	1.28	0.010	1.03	<0.001
Sow only heat production[2], $kJ \cdot kg^{-1} \cdot h^{-1}$	-	-	-	-	-	9.6	14.0	1.1	0.016	-	-
Maintenance energy expenditure[3], $kJ \cdot kg^{-1} \cdot h^{-1}$	5.66a,x	5.62a,xz	5.52b,xy	0.31	<0.001	5.52y	5.58z	0.03	0.005	0.02	<0.001
Energy retention[4], $kJ \cdot kg^{-1} \cdot h^{-1}$	0.9ab,x	1.2b,x	-0.6a,x	0.3	0.023	-4.8y	-8.0z	1.0	0.096	0.8	<0.001
Energy exported in milk[5], $kJ \cdot kg^{-1} \cdot h^{-1}$	-	-	-	-	-	6.9	10.1	0.8	0.071	-	-
Leucine flux, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	227.5	243.0	200.1	10.0	0.307	185.6	199.3	16.8	0.707	9.5	0.353
Leucine intake, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	41.5x	40.8x	39.4x	0.4	0.091	95.2y	133.9z	16.6	0.005	8.3	<0.001
Leucine from body protein breakdown, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	186.5x	202.8x	163.8x	9.6	0.376	87.1y	63.2y	3.2	0.544	14.4	<0.001

Leucine oxidation, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	15.7x	16.5x	17.4x	0.7	0.608	30.4y	36.6y	8.3	0.353	2.2	<0.001
Leucine used for protein synthesis,$\mu mol \cdot kg^{-1} \cdot h^{-1}$	212.0x	226.7x	184.2x	10.4	0.347	112.3y	108.8y	18.7	0.932	14.0	0.006
Leucine exported in milk, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	-	-	-	-	-	36.2	50.6	3.9	0.097	-	-
Leucine retained, $\mu mol \cdot kg^{-1} \cdot h^{-1}$	25.8x	24.3x	22.1x	1.0	0.318	24.3x	44.9y	5.7	0.066	2.7	0.019
Leucine intake, $mg \cdot kg^{-1} \cdot h^{-1}$	5.5x	5.4x	5.2x	0.1	0.091	12.6y	17.7z	1.1	0.005	1.1	<0.001
Leucine retained, $mg \cdot kg^{-1} \cdot h^{-1}$	3.4x	3.2x	2.9x	0.1	0.318	3.2x	5.9y	0.8	0.066	0.4	0.019
Retained protein[5], $mg \cdot kg^{-1} \cdot h^{-1}$	44.9x	42.3x	38.4x	1.7	0.318	42.2x	78.1y	10.0	0.066	4.7	0.019
Retained fat[6], $mg \cdot kg^{-1} \cdot h^{-1}$	-6.7x	5.8x	-31.0x	7.1	0.115	-190.4y	-268.1z	29.0	0.115	26.3	<0.001

[1]: Gestation data reproduced from Samuel (2008)

[2]: Calculated according to Jakobsen et al, 2005

[3]: Calculated as 506 $kJ/BW^{0.75}$ (Samuel, 2008)

[4]: Calculated as: Metabolizable energy intake (MJ/d) – Heat production (gestation) or Sow only heat production (lactation) (MJ/d)

[5]: Calculated as: Mean energy content of milk (MJ/kg) * mean milk production (kg/d)

[6]: Calculated as: leucine retention (g/d) / 0.076 (the leucine content of body protein)

[7]: Calculated as:

lipid retention (g/d) = [Energy retention (MJ/d) – (energy content of protein (23.8 kJ/g) * protein retention (g/d)]*(1/energy content of lipid (39.0 kJ/g) (ARC, 1981)

[8]: Calculated as:

daily gain = [(protein retention (g/d) * (1/mean water content of protein) + (lipid gain)*(1/mean water content of lipid)]

[a,b,c]: values which share a common letter are not different within gestation or lactation

[w,x,y,z]: values which share a common letter are not different between gestation and lactation

Table 4.7 - Energy (% of metabolizable energy intake) and protein metabolism (% of leucine flux) of sows during gestation and lactation

	Gestation					Lactation				Comparison between gestation and lactation	
	Day 30	Day 45	Day 105			Day 7	Day 19				
Number of sows	5	5	7	SEM	P-value	7	7	SEM	P-value	SEM	P-value
Heat production[1]	85.9ab	80.3a	109.8b	4.6	0.031	-	-	3.8	0.489	-	-
Sow only heat production[2]	-	-	-	-	-	82.8	86.0	4.9	0.528	-	-
Energy retention[3]	14.1ab,xy	19.7a,y	-9.8b,x	4.6	0.031	-43.2z	-49.7z	6.4	0.525	6.4	<0.001
Maintenance energy expenditure[4]	88.7a,x	90.5ab,x	94.7b,w	1.1	<0.001	50.1y	35.6z	4.8	0.043	4.8	<0.001
Energy exported in milk[5]	-	-	-	-	-	60.4	63.7	4.8	0.745	-	-
Leucine intake	18.4x	16.9x	18.3x	0.6	0.681	54.9y	74.4z	5.8	0.254	5.8	<0.001
Leucine from body protein breakdown	81.6x	83.1x	81.7x	0.6	0.681	45.1y	25.6z	5.8	0.254	5.8	<0.001
Leucine oxidation	7.1x	7.1x	8.0x	0.5	0.728	17.6y	19.4y	1.4	0.609	1.4	<0.001
Leucine used for protein synthesis	92.9x	92.9x	92.0x	0.5	0.728	57.9y	49.1b	4.8	0.492	4.8	<0.001
Leucine exported in milk	-	-	-	-	-	24.6	31.4	5.0	0.523	-	-
Leucine retention	11.3x	9.9x	10.2x	0.3	0.179	12.7x	23.5y	1.4	0.140	1.4	0.005

[1]: Gestation data reproduced from Samuel (2008)

[2]: Calculated according to Jakobsen et al, 2005

[3]: Calculated as: Metabolizable energy intake (MJ/d) – Heat production (gestation) or Sow only heat production (lactation) (MJ/d)

[4]: Calculated as 506 kJ/$BW^{0.75}$ (Samuel, 2008)

[5]: Calculated as: Mean energy content of milk (kJ/kg) * mean milk production (kg/d)

[a,b,c]: values which share a common letter are not different

Figure 4.2 – Protein, lipid, and energy balance of pregnant sows during gestation

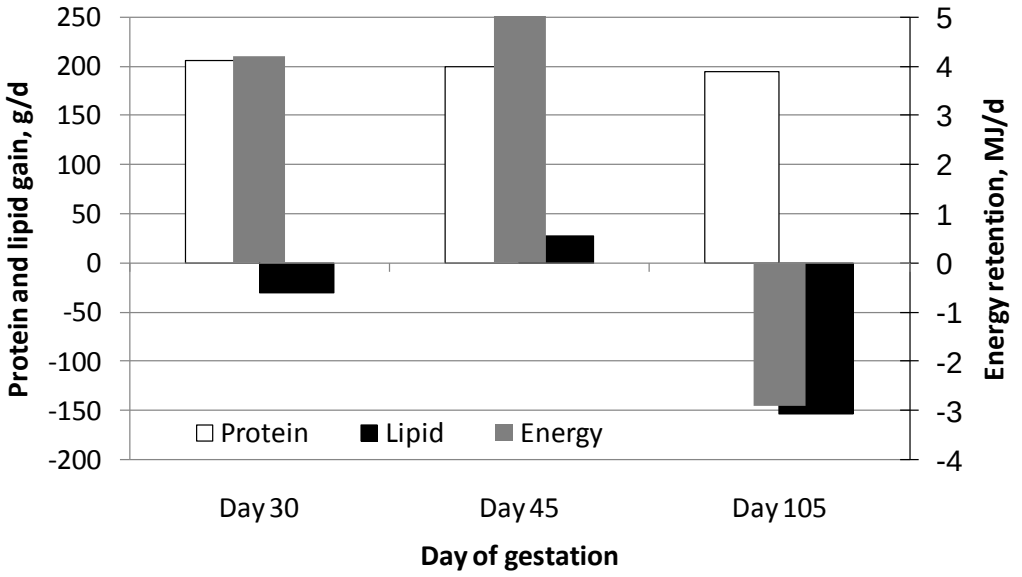
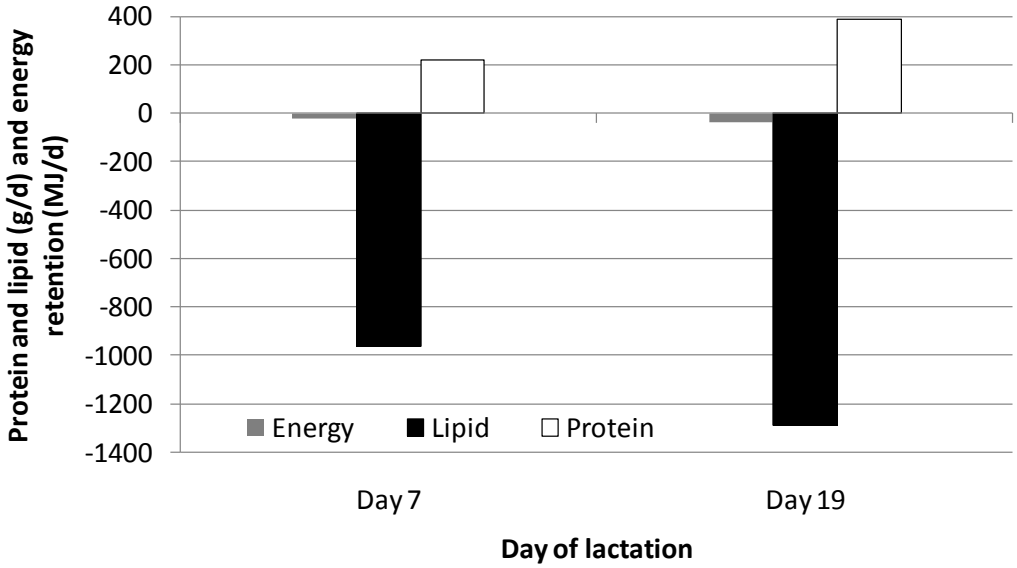


Figure 4.3 – Protein, lipid, and energy balance of lactating sows



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5.0 LYSINE REQUIREMENT FOR MAINTENANCE IN NON-PREGNANT SOWS

5.1 Introduction

Dietary lysine is the limiting amino acid in typical rations for high producing, modern sows. Estimates of the lysine requirement for maintenance in sows were summarized by the National Research Council (NRC 1998) to be 36 mg/$BW^{0.75}$. However, the estimate appears to be based mainly on data from Wang and Fuller (1989) who studied growing pigs. Interestingly, Rippel et al. (1965b) identified that estimates of nutrient requirements for sows according to the NRC (1959) were similarly extrapolated from data obtained in the growing-finishing pig. Previous estimates of the lysine requirement for reproduction (Rippel et al 1965a; NRC 1973; Woerman and Speer 1976) were determined during late pregnancy and, therefore, do not represent the requirements for maintenance. Pettigrew (1993) stated that he took the value reported by Fuller et al (1989) and divided by a, seemingly, arbitrary apparent digestibility coefficient to arrive at a dietary lysine maintenance requirement of 49 mg/$BW^{0.75}$. Therefore, previous estimates of the dietary maintenance requirement for lysine in sows are highly questionable.

The requirement for lysine in sows, like other indispensable amino acids, has often been determined by nitrogen balance. However, nitrogen balance studies tend to overestimate nitrogen retention because nitrogen intakes are overestimated and nitrogen losses (e.g. hair, skin, secretions) are underestimated, ignored, and measurement errors are difficult to avoid (Wallace, 1959; Just et al, 1982; van

Kempen et al, 2003; Elango et al, 2010). Therefore, nitrogen balance studies usually produce falsely high estimates and often have wide confidence limits (Fuller and Garlick, 1994). The WHO (2007) concluded that the serious technical weaknesses associated with nitrogen balance resulted in estimated requirement values for humans that were too low and concluded that the new gold standard should be indicator amino acid oxidation.

Productivity of modern sows has dramatically increased due to genetic advances produced by selective breeding (Dourmad and Étienne, 2002; Ball et al, 2008). Simultaneously, consumer pressure to improve the nutritional value of pork products has resulted in sows with lower lipid and greater muscle carcass contents. Modern sows have a 2.1% greater lean tissue percentage than their predecessors (Tess et al. 1984a,b). Wenk et al. (1980) suggested that leaner animals have increased maintenance energy needs due to higher rates of lean tissue (protein) turnover, and because protein turnover requires a dietary supply of amino acids, this suggests that amino acid requirements have increased. Despite the fact that sow reproductive output has been substantially improved, along with concomitant changes in body composition and protein turnover, little research has been conducted recently on amino acid requirements of sows. Therefore, it is necessary to determine the lysine maintenance requirement of modern sows.

The objective of this study was to determine the dietary lysine requirement of modern, non-pregnant, sows using the indicator amino acid oxidation method. Secondary outcomes, including simultaneous measurement of heat production and respiratory quotient, phenylalanine flux, plasma free amino acid concentrations,

and nutrient digestibility were recorded.

5.2 Materials and methods

5.2.1 Animals

Non-pregnant Large White/Landrace sows [n=4, parity=2] were selected from the University of Alberta breeding herd (Swine Research and Technology Centre, Edmonton, AB, Canada) before being surgically fitted with cephalic vein catheters terminated with a subcutaneous injection port (Chapter 3.0). All procedures used in this study were approved by the Faculty Animal Policy and Welfare Committee of the University of Alberta.

5.2.2 Diets & Feeding

A semi-synthetic diet, based on corn, amino acids, and premix, containing 14.0 MJ ME/kg was formulated and mixed to provide 55% of the lysine requirement of 36 mg/kg $BW^{0.75}$ suggested by NRC (1998) (Table 5.1). Additional lysine was added to the base diet to deliver six graded levels of lysine intake. Each sow received six experimental diets, in random order. Three intake levels were below the presumed requirement (19.8, 25.2, 30.6 mg/$kg^{0.75}$) and three intake levels were above the requirement (41.4, 46.8, 52.2 mg/$kg^{0.75}$), representing 55, 70, 85, 115, 130, and 145% of the requirement, according to NRC (1998), respectively. Sows consumed 1.1 kg of feed, twice daily, throughout the experimental period, except on study days (see 5.2.3) to achieve energy intake of 506 kJ/$BW^{0.75}$ (Samuel, 2008). Sows were fed a diet containing 1.09 g/kg lysine (equivalent to 41.4 mg/$kg^{0.75}$ or 115% of NRC (1998) requirement) for an

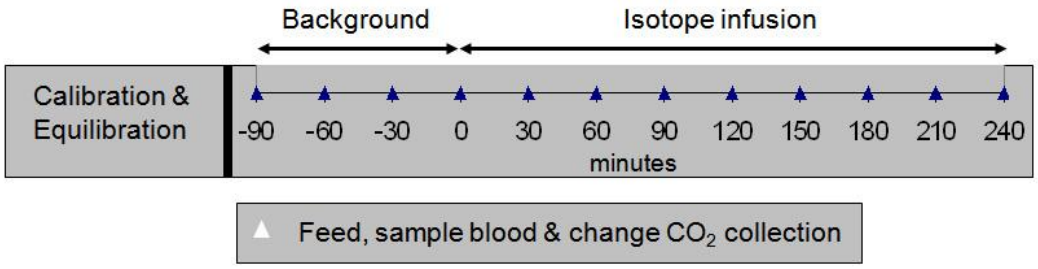
adaptation period of one week before oxidation studies began. All diets were formulated to be isoenergetic and isonitrogenous. A generous tyrosine intake of 4.9 g/d (approximately three times the requirement for a 225 kg sow at maintenance) was provided by the experimental diets in order to minimize the use of phenylalanine as a source of tyrosine, thereby channeling tyrosine formed from phenylalanine towards oxidation (Zello et al, 1990). Phenylalanine was provided by the diets at two times the daily maintenance requirement for a 225 kg sow and included the isotope dose. Unlabeled phenylalanine, equivalent to the isotope dose, was mixed into individual batches and fed during adaptation periods between study days. All other amino acids were included at 150% of requirement. Diets were formulated on the basis of true ileal digestible (TID) amino acid content of the feedstuffs. An indigestible marker was included at 10 g/kg of diet and analyzed as acid insoluble ash (AIA) (McCarthy et al, 1977). Sows were adapted to each lysine intake level for 3 meals. Adequacy of the adaptation period was previously verified (Moehn et al, 2004a).

5.2.3 Isotope Delivery and Feeding

On study days, sows were fed 169.2 g of diet after they were secured into a respiration chamber. The hourly feeding regimens of Moehn et al (2004a,b) were modified so that sows received 11 meals equivalent to $1/26^{th}$ of their daily ration every 30 minutes during the 5.5 hour study period. The first three meals did not contain labeled phenylalanine and were fed to estimate individual $^{13}CO_2$ abundance background profiles (Jones et al, 1985). The oral dose of 2.0 $mg \cdot kg^{-1} \cdot h^{-1}$ L-[1-^{13}C]phenylalanine (Cambridge Isotope Laboratories Ltd.,

Andover, MA) was mixed into the experimental feed allowance as dry, crystalline powder before being divided into eight individual meals. A priming dose of 1.75 times the constant dose of L-[1-^{13}C]phenylalanine was added to the first dosed meal (Moehn et al, 2004a). The constant oral infusion dose of 1.0 mg·kg^{-1}·h^{-1} L-[1-^{13}C]phenylalanine was determined, in a preliminary experiment (data not shown), to produce $^{13}CO_2$ sufficiently above the natural background ^{13}C enrichment in breath CO_2 for subjects in the fed state from a diet with similar ^{13}C content for accurate measurement by isotope ratio mass spectrometry (IRMS) (Jones et al, 1985; Di Buono et al, 2001).

Figure 5.1 – Experimental feeding and blood and CO_2 sampling protocol



5.2.4 Indirect Calorimetry

Before each study, the S108 oxygen (O_2), S153 carbon dioxide (CO_2), and S126 methane (CH_4) analyzers (Qubit Systems, Kingston, ON) were calibrated to zero with N_2 for the O_2 and CO_2 analyzers and a gas of known [O_2] and the balance N_2 for the CH_4 analyzers. The upper limits of the analyzers were calibrated with a gas of known [O_2] (approximately 21%), [CO_2] (approximately 1.5%), and [CH_4] (approximately 100 ppm) and the balance (approximately 77.5 %) N_2 (Praxair, Edmonton, AB). O_2, CO_2, and CH_4 concentrations were collected by C409 data acquisition system as an average of 200 samples in one-minute

intervals during the study (Qubit Systems, Kingston, ON). The gas exchange of the animals was determined by comparison to the ambient air values as recorded immediately before and after the study periods. The analytical values for each of the calibration gases was recorded immediately before and after the study and used to correct for drift of the analyzers, if necessary.

Two independent open-circuit indirect calorimetry chambers were each fitted with a 10cm diameter capped PVC tube which allowed feed to be dropped into the feeder and a nipple drinker for *ad libitum* fresh water intake. Access to the animals was through a clear plexiglass window on the top of each chamber. The calorimetry chambers were designed with two air inlets each consisting of 2.5 cm diameter PVC pipe the length of the chamber with holes drilled approximately every 30cm and capped at the opposite end. Ambient air was drawn into the chamber through these inlet pipes by vacuum displacement as air was withdrawn from the chamber. Air flow out of the chambers of 250 L/min was required to maintain [CO_2] below 1.0%. The animals were directed into the chambers through a rear door that was closed to isolate air flow to the inlet and outlet pipes. The volume of air from the outlet of each box was measured by separate gas meters (Model AC630, Canadian Meter Company, Cambridge, ON) and manually recorded every 30 minutes. A sub-sample (250 ml/min) from each chamber was dried over a column of drierite before being split and two-thirds of the air flow directed to the analyzers and the remaining air flow to the CO_2 collection.

5.2.5 Sample Collection

5.2.5.1 Quantitative expired CO_2 collection

A sub-sample of the air flow from the respiration chambers flowed through midget bubblers (Fisher Scientific, Mississauga, ON or VWR International, Edmonton, AB) suspended in 1 *N* NaOH solution at a rate of ~80 mL/min. A single midget bubbler in ~11 mL of $NaOH_{(aq)}$ was shown to provide complete and quantitative trapping of CO_2 (Chapter 3.0). The solution was changed every 30 min. Charged solution was transferred equally into two evacuated, glass, 16 x 100 mm, serum collection tubes (Vacutainer, Oakville, Ontario) and frozen at -20 ˚C until analysis.

5.2.5.2 Blood collection

Blood samples of 5 mL sample size were collected every 30 min through the subcutaneous injection port (Chapter 3.0) and transferred into glass or plastic 13 x 100 tubes containing lithium heparin (Vacutainer, Oakville, Ontario) before centrifugation at ~2000 x g for 15 min. Plasma was harvested and stored frozen at -20 °C until analysis. Grab samples of feces (at least 200 g) were taken on the day of respiration before the change of diet, stored frozen at -20 °C, and then freeze dried before further analysis. Small random samples of the mixed diets were collected frequently following mixing during the preparation of individual meals. The small samples were pooled as representing a particular diet before and were stored at room temperature before further analysis.

5.2.6 Calculations

5.2.6.1 Volumes of gases

Litres of gases consumed and produced were calculated using the difference between recorded gas exchange values during the experiment and the room air values recorded immediately before and after the experimental period. The values recorded for each minute were corrected for drift of the analyzers, if statistically significant. The difference between the experimental and room air percent gas composition was multiplied by the air flow out of the respiration chambers for each 30 minute period. Therefore, litres of gas consumed or produced were calculated for each 30 minute period, as follows:

V_{O2} (L/30 min) = total air flow (L)/30 min * (% O_2 room air - % O_2 test period)

V_{CO2} (L/30 min) = total air flow (L)/30 min * (% CO_2 test period - % CO_2 room air)

V_{CH4} (L/30 min) = total air flow (L)/30 min * (% CH_4 test period - % CH_4 room air)

5.2.6.2 Heat production

Heat production was calculated according to Brouwer (1965) from the drift-corrected data, if necessary, collected during open-circuit indirect calorimetry. The formula was abbreviated by omitting the urinary nitrogen term because planned attempts to collect urine were unsatisfactory; thus, urinary nitrogen results are not included. According to Weir (1949), 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. Therefore, the formula used to calculate HP from gas exchange was:

$$HP = (16.175 \times V_{O2}) + (5.02 \times V_{CO2}) - (2.17 \times V_{CH4})$$

where V_{O2}, V_{CO2}, and V_{CH4} represent volumes (L) of O_2 consumed and CO_2 and CH_4 produced, respectively. Daily HP was calculated as the summation of HP for each 30 minute of the 4 h isotope delivery period, divided by the previously determined factor (1.17) to extrapolate to a 24 h value (Chapter 3.0).

5.2.7 Isotope analysis

Expired $^{13}CO_2$ enrichment was measured by a continuous flow IRMS (CF-IRMS20/20; PDZ Europa, Cheshire, United Kingdom). Enrichments of all breath samples collected were expressed as atom percent excess (APE) $^{13}CO_2$ above a reference standard of compressed CO_2 gas (Jones et al, 1985; Di Buono et al, 2001).

The enrichment of L-[1-^{13}C]phenylalanine in plasma was analyzed using a triple quadrupole mass analyzer (API4000, Applied Biosystems/MDS SCIEX, Concord, Canada) coupled to an Aligent 1100 HPLC system (Aligent, Mississauga, Canada)(LC-MSMS), as previously described by Turner et al (2006).

5.2.8 Data analysis

Attainment of isotopic steady state in the metabolic pool was shown by plateaus in $^{13}CO_2$ enrichment in breath and plasma [^{13}C]phenylalanine. Plateaus were achieved by 120 min from the start of isotope infusion and were maintained to the end of the study at 240 min (Figure 5.2). Breath and plasma isotope enrichment values were determined as atoms percent (AP). The last two of the three background samples at baseline and at least three samples taken during plateaus were used for further analysis. Plasma [^{13}C]phenylalanine and $^{13}CO_2$

values did not attain plateaus for a single sow/diet combination; this single treatment was excluded from further analysis. The coefficients of variation for plasma [^{13}C]phenylalanine enrichment determinations (mean ± SD) for the remaining 23 studies were 7.9 ± 6.3 and 12.2 ± 5.6 % for baseline and plateau enrichments, respectively. The coefficients of variation for breath $^{13}CO_2$ enrichment were 0.065 ± 0.045 and 0.091 ± 0.047 % for baseline and plateau enrichments, respectively.

Plasma phenylalanine flux was determined at isotopic steady state from the equation of Matthews et al. (1980):

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

where E_i was the enrichment of L-[1-^{13}C]phenylalanine infused (MPE), E_p was the enrichment of plasma phenylalanine above baseline at isotopic plateau (MPE), and I was the rate of L-[1-^{13}C]phenylalanine infused ($mg \cdot kg^{-1} \cdot h^{-1}$). The percent of the phenylalanine dose oxidized (Ox) was calculated as follows:

$$Ox = [(V_{CO2}*APE/100)/(22.4*(288.15/273.15)*1000*165.19*(99/100)]/dose*100$$

where V_{CO2} is the volume of CO_2 expired during a 30 min period, APE was determined by IRMS analysis (see 6.2.9), 22.4 mol/L*(288.15 K/273.15 K) is the correction for the molar volume of an ideal gas at the non-STP temperature of 288.15 K (equivalent to 15 ˚C[1]), 165.19 g/mol is the molecular weight of phenylalanine, and 99% is the enrichment of the L-[1-^{13}C]phenylalanine, as reported (Sigma-Aldrich Canada Ltd., Oakville, ON).

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[1]The air volume measured by the AC 630 gas meters is temperature corrected to 15 ˚C.

5.2.9 Plasma free lysine concentration

Previously frozen plasma was thawed at room temperature before plasma amino acid concentrations were measured by reverse-phase HPLC using phenylisothiocyanate derivatives as previously described (Bidlingmeyer et al, 1984; Murch et al, 1996).

5.2.10 Statistical analysis

Data are presented as means ± SEM. Values were considered significant at $P < 0.05$. Statistical analysis was performed using mixed procedure and non-linear regression analysis (Robbins et al, 2006) in SAS version 9.1 (SAS Inst. Inc., Cary, NC). The classification variable was lysine intake and individual animals were treated as random variables. Model statements were tested using the Kenward-Roger degrees of freedom method. Least square means were compared using the 'pdiff' option.

5.3 Results

Mean sow BW increased numerically during the 12 day experimental period (220.9±1.6 vs. 224.7±2.1 kg), but the difference (3.8 kg) was not significant (P=0.20). All animals remained healthy throughout the experimental period and consumed all meals shortly after feeding. This included all of the study meals that were provided to the animals while in the respiration chambers. The AIA content of the base diet was 1.04±0.03%. The AIA content of the feces collected (results pooled because there were no differences) was 13.3±0.5%. There were no differences in the crude protein (CP) (P=0.48) or carbon (C) (P=0.43) contents of the base diets. Apparent total tract digestibility of nitrogen (73.7±1.0%) and C

(93.7±0.2%) were not different for any of the six graded levels of lysine intake. Concentrations of plasma free lysine increased as dietary lysine intake increased up to 0.81 g/kg of diet and then did not increase any further. However, plasma free lysine concentration was lower than the plateau value ($P<0.05$) when sows were fed 1.24 g/kg of diet (Figure 5.5).

Phenylalanine flux was not affected by dietary lysine intake ($P=0.39$). Oxidation of the indicator amino acid was lower ($P<0.05$) when lysine intake was greater than 1.09 g/kg of diet (Table 5.2) and was supported by results from non-linear breakpoint analysis which determined the requirement as 49±11 mg/kg$^{0.75}$ ($R^2=0.65$). In fact, oxidation of the indicator amino acid decreased linearly and quadratically from the lowest level of dietary lysine intake to 1.24 g of lysine/kg of diet. The RQ and CO_2 production decreased linearly from the lowest level of dietary lysine intake to 1.24 g of lysine/kg of diet (Table 5.2). Average APE values, independent of CO_2 production, showed a similar pattern to the oxidation values (Figure 5.4). Heat production per 30 minute period and the mean RQ were lowest ($P<0.001$ and $P<0.05$, respectively) and there was a trend ($P<0.10$) for lower CO_2 production when sows were fed 1.24 g of lysine per kg of diet (Table 5.2). These results were evaluated by non-linear breakpoint analysis. The breakpoint of 46.8±0.02 mg/kg$^{0.75}$ was calculated from heat production per 30 min period ($R^2=0.67$), respiratory quotient ($R^2=0.63$), and CO_2 production ($R^2=0.40$).

5.4 Discussion

These results provide evidence that improved efficiencies of energy and protein metabolism occurred simultaneously when sows were fed a diet with sufficient lysine. Oxidation of the indicator amino acid, heat production per 30 min period, and respiratory quotient were lowest when sows were fed 1.24 g of lysine per kg of diet, equivalent to 46.8 mg/$BW^{0.75}$. Compared to the previously reported value of 36 mg/$BW^{0.75}$ from NRC (1998), an additional 0.5 g/d of lysine for a 200 kg sow would be required to cover the daily lysine requirement for maintenance.

Non-linear breakpoint analysis determined the lysine maintenance requirement to be 49±11 mg/$kg^{0.75}$ for these sows (R^2=0.65). It may be argued that breakpoint analysis cannot define the requirement from these results because there are not three points above the requirement with which to define a plateau. However, the simultaneous improvement in energy metabolism represents a second, independent measure of the lysine maintenance requirement. Energy and protein metabolism are intertwined; insufficient energy intake limits protein synthesis and excess amino acids must be oxidized for energy. The RQ is defined as the CO_2 produced divided by the O_2 consumed. The RQ was lower for sows fed 46.8 mg/$kg^{0.75}$. There are only two scenarios by which this can occur; either CO_2 production decreased or O_2 consumption increased. The latter clearly did not occur because the HP was also lowest at this intake (Table 5.2). The HP of these sows was measured by indirect calorimetry and calculated according to the Brouwer formula where 75% of the calculated HP is due to O_2 consumption.

Therefore, small changes in O_2 consumption have a large impact on the HP. If changing O_2 consumption was the reason for the decrease in RQ, we would expect a concomitant rise in HP when in fact a decrease in HP was observed. Insufficiencies in specific amino acids leading to an amino acid imbalance can decrease the net energy value of a feed by increasing the heat increment of feeding associated with that feed. This is because amino acids consumed as part of the diet have two metabolic fates; either they are utilized for protein synthesis or oxidized for energy. As protein synthesis is increased, overall HP should decrease to reflect a reduction in amino acid oxidation and an improvement in overall energy metabolism.

The second possible scenario to explain the observed reduction in RQ when sows were fed 1.24 g of lysine per kg of diet is a reduction in CO_2 production. This situation follows what was actually observed (Figure 5.3). The reduction of CO_2 production equates to greater C retention by the sows at this lysine intake. Carbon that has been oxidized, as opposed to retained, by the body is expelled as CO_2 and can be measured as an increase in CO_2 production indicative of HP (Christensen et al, 1988). Sows fed 1.24 g of lysine per kg of diet were metabolically more efficient at C retention than when fed below or above the newly determined lysine maintenance requirement. This represents an improved efficiency of nutrient metabolism because C retention indicates storage of dietary nutrients as body tissue or utilization of the C skeletons for other metabolic processes, such as the production of enzymes and hormones.

Phenylalanine oxidation decreased linearly from the lowest and second

lowest dietary lysine intakes and quadratically from the lowest dietary lysine intake to 1.24 g of lysine/kg of diet. Temporarily removing the lowest level of dietary lysine intake from analysis was performed because phenylalanine oxidation was numerically lower than the next highest level of dietary lysine intake. Reduced indicator amino acid oxidation at the lowest intake of the test amino acid has been previously observed by Kim et al (1983) and Moehn et al (2004c). Kim et al (1983) proposed that reduced oxidation of the indicator amino acid at low dietary concentrations of the test amino indicates that lysine was conserved. Alternatively, reduced oxidation of the indicator amino acid may indicate that the enzyme systems responsible for oxidation of excess amino acids has become overwhelmed and, therefore, oxidation of the indicator amino acid is limited. For these reasons, removing the lowest intake of lysine is the more correct analysis.

Plasma free lysine concentration of sows fed the diet containing 1.24 g of lysine per kg of diet were lower than the plasma lysine concentrations in sows fed dietary lysine intakes of 0.81, 1.09, and 1.38 g/kg. Mitchell et al (1968) observed that the plasma free concentration of the amino acid of interest does not increase with graded addition of the amino acid below the dietary requirement. In fact, the plasma free amino acid concentration started to increase only after the requirement value, determined by nitrogen balance, had been exceeded resulting in a broken-line response with the inflection point at the requirement. Without sufficient “metabolic adaptation” time to the dietary lysine intake, the broken-line response in plasma free lysine was not observed. Instead, Mitchell et al (1968)

observed a linear increase in plasma free lysine concentration with increasing dietary intake of lysine. Pigs with a starting weight of 10 kg were adapted to a diet containing adequate levels of all the indispensable amino acids. Pigs were then fed a diet providing a new, graded intake of lysine as their evening meal before plasma was harvested the following morning. The linear response of plasma free lysine to graded levels of intake lead Mitchell et al (1968) to propose that a period of "metabolic adaptation" to deficient levels of the test amino acid is necessary. The ability of the body to release amino acids from the labile protein pool may be the basis of "metabolic adjustment"; changing dietary intake of the test amino acid in the short-term can be buffered by the breakdown of proteins from the labile protein pool. In the longer term, once the labile protein pool has been exhausted of the amino acid that is deficient in the diet, the broken-line response to graded intake should be expected. The growing pigs used in the experiments reported by Mitchell et al (1968) would have a comparatively small labile protein pool compared to sows (Young et al, 1968). In the present experiment, sows were fed diets with different lysine contents for only three meals before the oxidation experiments (equivalent to 1.5 days), thereby limiting the time for adaptation of plasma free lysine concentrations to the new diet. Therefore, the lower plasma free lysine at the newly determined requirement suggests an increase in lysine utilization, which is in agreement with improved C retention observed in this experiment.

Breakpoint analysis determined the lysine maintenance requirement for these modern sows to be 49 mg/kg$^{0.75}$, which appears greater than model

calculations of the GfE (2008) which suggest 38 mg/kg$^{0.75}$. The lysine requirement value determined in this experiment was previously suggested by Pettigrew (1993) based on the data from Fuller et al (1989). Pettigrew (1993) divided the reported maintenance requirement values by an arbitrary apparent digestibility coefficient (0.80) in order to determine the dietary amino acid requirements for maintenance. The reasoning for this manipulation was that the diets of Fuller et al (1989) contained mostly crystalline amino acids and were assumed to be 100% digestible. In a similar manner, the dietary intake of lysine was mostly from L-lysine·HCl in this experiment and the uncertainty of dietary requirements has been removed by formulation of the base diets based on TID of the amino acids (NRC, 1998). Therefore, it is essential that nutrient requirement values and the bioavailability of nutrients from different feedstuffs be expressed on a consistent basis.

5.5 Conclusion

Improvement in the efficiency of protein and energy metabolism evidenced by improved C retention, lower indicator amino acid oxidation, lower heat production, and lower respiratory quotient were observed when non-pregnant sows were fed 1.24 g of lysine per kg of diet, equivalent to 46.8 mg/kg$^{0.75}$. Therefore, the lysine maintenance requirement for modern, high productivity sows appears to be not less than 46.8 mg of TID lysine/kg$^{0.75}$. However, future work is required to further define the dietary lysine requirement for sows.

Table 5.1 – Formulation of the base diet for non-pregnant sows fed six graded levels of lysine

Ingredient ID	Amount in diet (g/kg)
Corn	255
Starch	292
Sugar	292
Solkafloc	74
Canola oil	10
Ile	0.24
Lys[1]	0
Meth	0.89
Phe	0.06
Tyr	1.66
Thr	1.12
Trp	0.17
Asn	10
Premix[2]	38
Choline chloride	1.9
Dicalcium phosphate	4.9
Sodium chloride	1.4
Potassium bicarbonate	5.75
Vitamin B6	0.00038
Thiamine	0.00031
Indigestible marker (AIA)	10

[1]Addtion of crystalline lysine·HCl to the base diet provided six graded levels of lysine intake.

[2]Provided per kilogram of the diet: Ca, 8.6g; P, 3.4g; Na, 1.9g; Mg, 140mg; K, 30mg; Fe, 139 mg; Zn, 119 mg; Mn, 56 mg; Cu, 16 mg; Co, 0.4 mg; I, 0.4 mg; Se, 0.3 mg; vitamin A, 12,000 IU; vitamin D_3, 1200 IU; vitamin E, 62 IU; vitamin K, 2.5mg; biotin, 0.6 mg; folic acid, 2.5mg; niacin, 42 mg; pantothenic acid, 25 mg; pyridoxine, 5mg; riboflavin, 9.5 mg; thiamine, 8.4mg; vitamin B_{12}, 28μg.

Table 5.2 – Mean oxidation of the indicator amino acid (percent of dose), mean heat production per 30 min period (kJ), mean respiratory quotient, mean litres of CO_2 production per 30 min (L), and mean plasma free lysine concentration (µmol/L) from sows fed six graded levels of lysine

								P-value				
Lys content of diet (g/kg)	0.52	0.67	0.81	1.09	1.24	1.38	SEM	ANOVA	Linear		Quadratic	
Phe oxidation (percent of dose)	9.0[a]	11.6[a]	9.3[a]	8.6[a]	2.8[b]	4.2[b]	0.98	<0.05	0.03[1]	0.01[2]	0.02[1]	0.36[2]
Heat production (kJ/30 min)	26.3[a]	26.0[a]	26.2[a]	25.7[a]	23.4[b]	25.4[a]	0.29	<0.001	0.09[1]	0.07[2]	0.12[1]	0.46[2]
Respiratory quotient	1.10[a]	1.05[a]	1.03[a]	1.09[a]	0.84[b]	1.05[a]	0.01	<0.01	0.02[1]	0.08[2]	0.19[1]	0.15[2]
CO_2 production (L/30 min)	40[a]	38[a]	38[a]	39[a]	29[b]	37[a]	0.60	<0.05	0.02[1]	0.04[2]	0.11[1]	0.21[2]
Plasma free lysine (µmol/L)	87[a]	137[a]	183[b]	186[b]	96[a]	186[b]	10.4	<0.01	0.59[1]	0.24[2]	<0.01[1]	<0.01[2]

[1]: Lysine levels 0.52 to 1.24 g/kg, inclusive (n=19)

[2]: Lysine levels 0.67 to 1.24 g/kg, inclusive (n=15)

[a,b,c]: values which share a common letter are not different

Figure 5.2 – Effect of diet (time<0) and L-[1-^{13}C]phenylalanine intake (time>0) on breath $^{13}CO_2$ enrichment in a typical subject. The last four data points were used to calculate the plateau

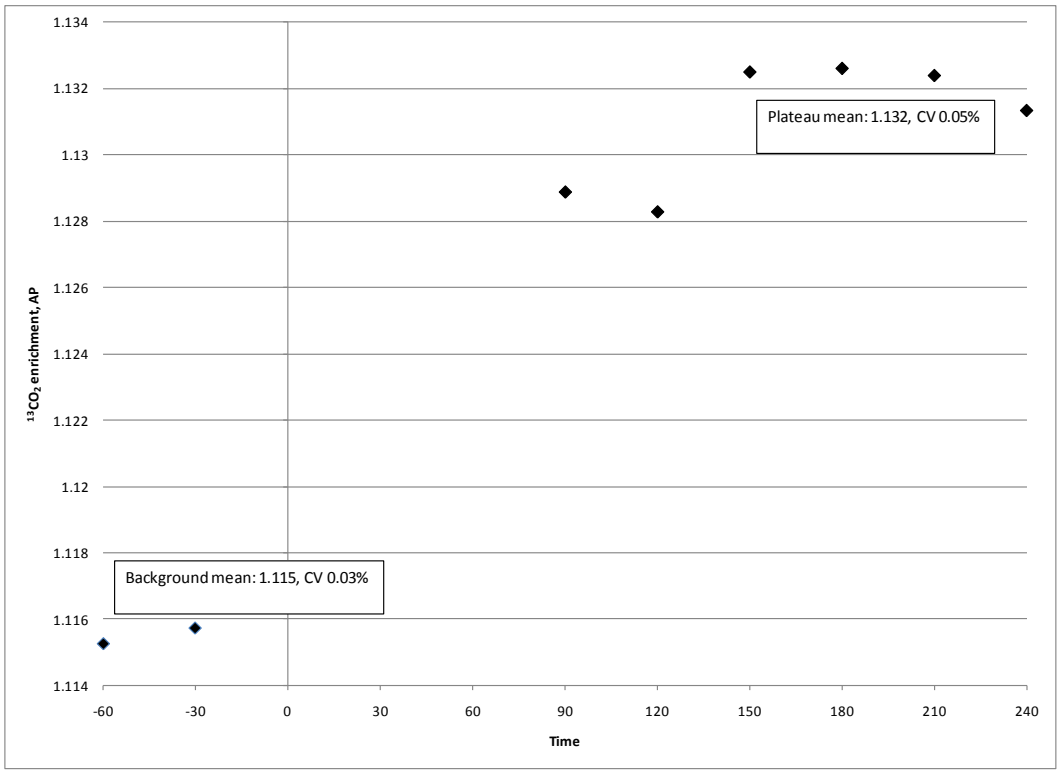


Figure 5.3 – Mean CO_2 production, O_2 consumption, and respiratory quotient from sows fed six graded levels of lysine

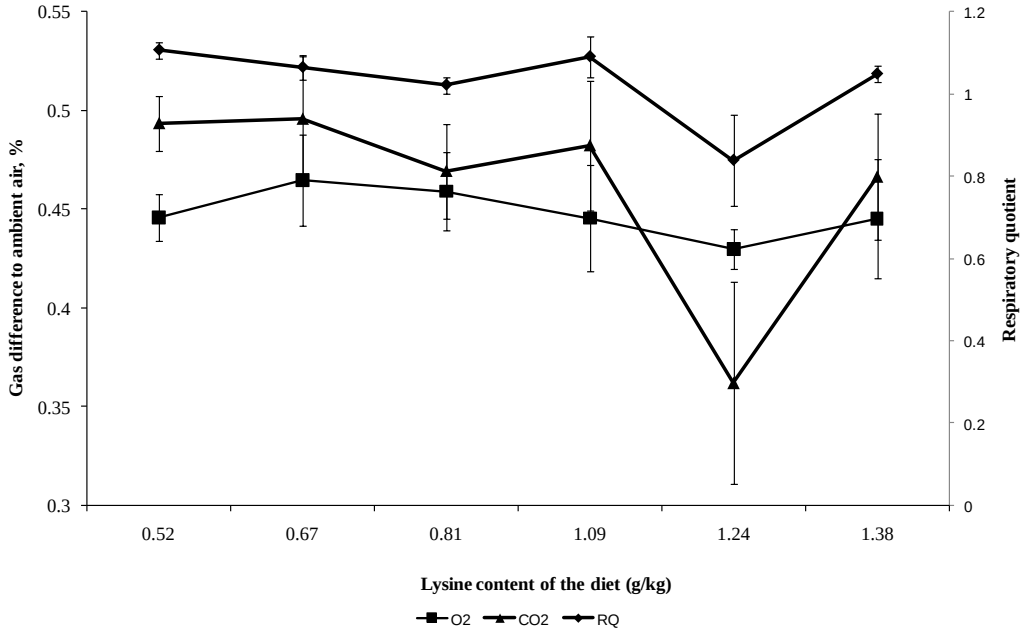


Figure 5.4 – Mean ^{13}C enrichments (APE)x1000 of expired CO_2 from sows fed six graded levels of lysine

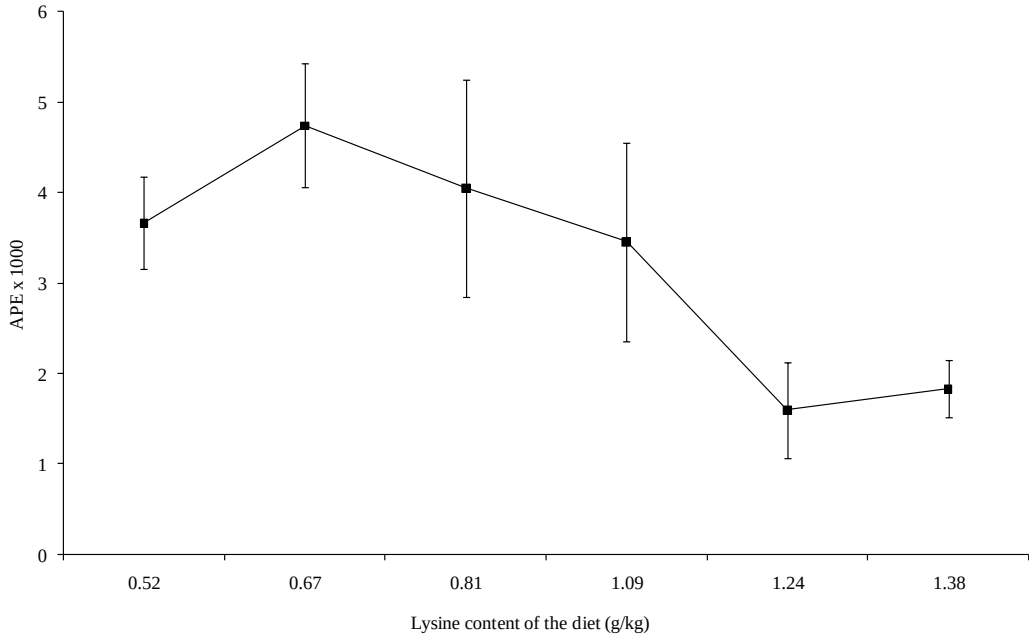
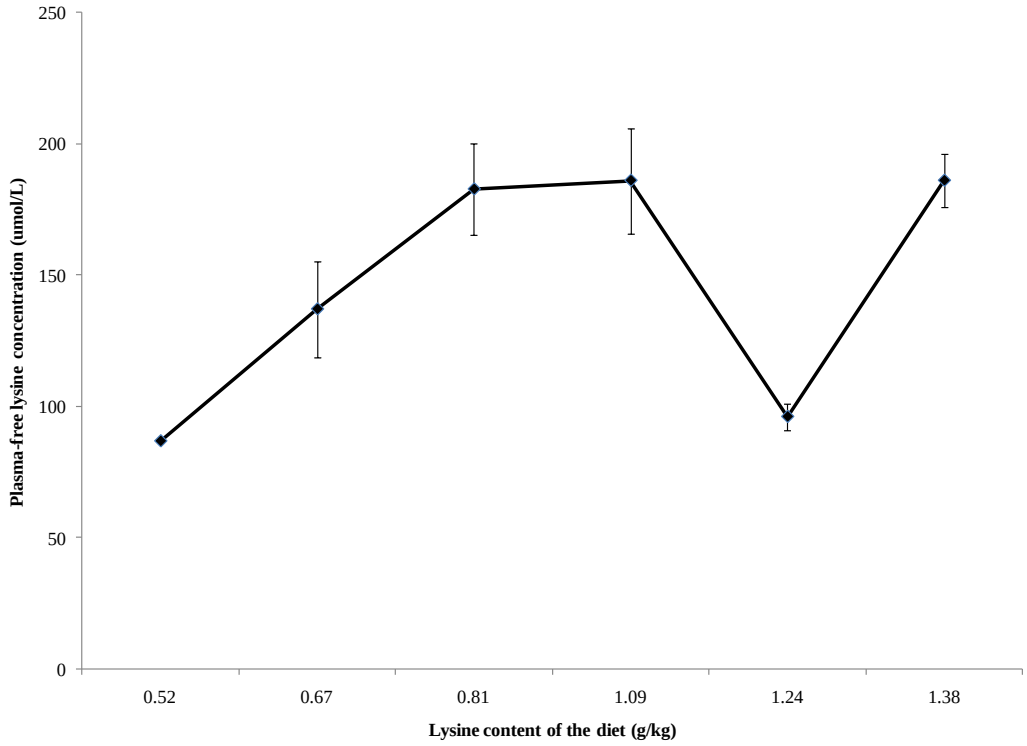


Figure 5.5 – Plasma-free lysine concentration from sows fed six graded levels of lysine



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6.0 LYSINE REQUIREMENT DURING GESTATION IN SOWS

6.1 Introduction

Productivity of modern sows has dramatically increased due to genetic advances produced by selective breeding (Dourmad and Étienne, 2002; Ball et al, 2008). Simultaneously, consumer pressure to improve the nutritional value of pork products has resulted in sows with lower lipid and greater muscle carcass contents. Modern sows have a greater lean tissue percentage than their predecessors (Tess et al. 1984a,b) resulting in increased nutrient requirements associated with maintenance (Chapter 3.0). Wenk et al. (1980) suggested that leaner animals have increased maintenance energy needs due to higher rates of lean tissue (protein) turnover.

The ongoing processes of protein synthesis and breakdown that, together, are protein turnover require a dietary supply of amino acids (Sprinson and Rittenberg, 1949). Higher rates of protein turnover require greater dietary inputs of amino acids to replace those lost during the process (Reeds and James, 1983). Therefore, the dietary lysine requirement has increased for maintenance (Chapter 5.0) and, we hypothesize, reproduction. Previous estimates of the lysine requirement for reproduction (Rippel et al, 1965; NRC, 1973; Woerman and Speer 1976) were determined during late pregnancy. However, significant alterations to the growth rates of specific maternal and fetal tissues occur during gestation, suggesting that the late-gestation requirement would be much higher than in early-gestation (Kim et al, 2009). As examples, the weight of the placenta increases linearly as pregnancy advances (Anderson, 1975; Ji et al, 2005), fetal

weight increases linearly after day 70 of gestation (McPherson et al, 2004), and development of the mammary gland occurs close to parturition (Kim et al, 1999). The development of each of these tissues influences the total daily nutrient requirements for energy and protein for the gravid sow and clearly changes requirements during gestation. Practically, however, most sows are fed a single, fixed diet throughout pregnancy. In contrast, based on the identified alterations in maternal and fetal metabolism and growth of individual tissues, a fixed diet during the entire pregnancy seems illogical. Fittingly, Dourmad et al, (1994b, 1999a,b), Srichana (2006), GfE (2008), and Kim et al (2009) report that the dietary lysine requirement for pregnant sows is greater in late- than early-gestation.

Simultaneous measurement of energy and protein metabolism provides valuable information about interactions of protein and energy metabolism. However, energy and protein metabolism have often been measured separately, forcing authors to conjecture about the effects of one upon the other. The techniques of indicator amino acid oxidation (IAAO) and open-circuit calorimetry can be used simultaneously (Chapter 3.0) and therefore provide better information about the relationships among protein and energy metabolism (Chapter 5.0). For example, in Chapter 5.0, it was identified that energy and protein metabolism was most efficient at the newly determined dietary lysine requirement for maintenance.

Pregnant sows must be energy restricted to avoid excessive maternal weight gain during gestation because significant body lipid accumulation

negatively impacts lactation performance by reducing feed intake (NRC, 1998). Nutrient restriction during gestation has little or no effect on subsequent litter size or piglet birth weight (Elsley, 1971; Duée and Sève, 1978; Pettigrew, 1993), except in extreme restriction (Anderson, 1975; Hard and Anderson, 1979; Pond et al, 1991). Sows' feed intakes are restricted, thus, it is necessary to ensure that adequate nutrients are available within individually prescribed feed allowances. Lysine is the first limiting amino acid in typical diets fed to swine and, as such, the daily requirement for lysine must be known to ensure adequate fetal and maternal growth throughout gestation. In addition, the lysine requirement must be accurately known to calculate ideal amino acid ratios, which are most often expressed in relation to lysine.

The objectives of this study were to determine the lysine requirement of a population of 2nd and 3rd parity sows in early- (day 24 – 45) and late- (day 86 – 110) gestation using the indicator amino acid oxidation method. Secondary outcomes were also assessed to determine the applicability of these results to the larger population. Simultaneous measurement of heat production, respiratory quotient, and energy digestibility were recorded to ensure that energy intake is not limiting. Phenylalanine kinetics were measured to determine if dietary lysine intake will affect protein turnover. Nutrient digestibility and plasma free amino acid concentrations were assessed as a measure of adequacy of dietary nutrient and lysine intake, respectively. Body weight gain and subsequent litter size have been reported to serve as a measure of the productivity of these sows.

6.2 Materials and methods

6.2.1 Animals

Large White/Landrace Hypor Hybrid (Hypor Inc) sows, pregnant after their first (n=4) or second (n=3) litters (185.7±9.6 kg BW) were selected from the University of Alberta breeding herd (Swine Research and Technology Centre, Edmonton, AB, Canada) before being surgically fitted with cephalic vein catheters terminated with a subcutaneous injection port (Chapter 3.0). All procedures used in this study were approved by the Faculty Animal Policy and Welfare Committee of the University of Alberta.

6.2.2 Diets and feeding

Three semi-synthetic diets (14.0 MJ ME/kg) based on corn were formulated and mixed to produce test diets (Table 6.1); base diet (60% of NRC (1998) dietary lysine requirement) and summit diets for early- and late-gestation (150% and 185% of NRC (1998) dietary lysine requirement, respectively). All other dietary indispensible amino acids were provided at the level of the summit diet above their estimated requirement (NRC, 1998), in the base and summit diets. Vitamin and mineral premixes provided vitamins and minerals at least 125% of the NRC (1998) requirement estimates. An indigestible marker, Celite®, was added at a rate of 1% of the diets. Each sow received 6 different test diets, in random order, from 60 to 150% of the requirement suggested by NRC (1998) in early- (Table 6.2) and from 60 to 185% of the requirement suggested by NRC (1998) in late-gestation (Table 6.3). Diets were allocated individually as graded

percentages of the mean dietary lysine requirement estimate (NRC, 1998) so that no two sows were assigned the same diet formulation. Dietary lysine requirements were estimated using the software provided with *The Nutrient Requirements of Swine* (NRC, 1998) using sows' individual breeding weights and an expected litter size of 12. Sows were ordered from lowest to highest predicted dietary lysine requirement. Diet formulations were assigned to individual animals, in that order, so that each animal received three dietary intakes below and three dietary intakes above the mean dietary lysine requirement estimate (NRC, 1998). Forty-eight[2] graded percentages were assigned from 60 to 83%, by single percentage increments, and from 110 to 150%, by 1.75% increments, of the mean dietary lysine requirement estimate (NRC, 1998) in early-gestation. Similarly, forty-two graded percentages were assigned from 60 to 120%, by 3% increments, and from 145 to 185%, by 2% increments, of the mean dietary lysine requirement estimate (NRC, 1998) in late-gestation. Individual diet formulations were prepared by combining and mixing the appropriate quantities of the basal and summit diets. A quantity of the diet slightly greater than half (at least 3%) of the daily feed allowance was reserved for the study day diet before L-phenylalanine, equivalent to the isotope dose, was mixed into individual batches and fed for adaptation periods between study days. Due to differences in feed intakes, the order of the graded percentages of the mean dietary lysine requirement estimate (NRC, 1998) did not mirror the individual lysine intakes. Sows were housed individually and

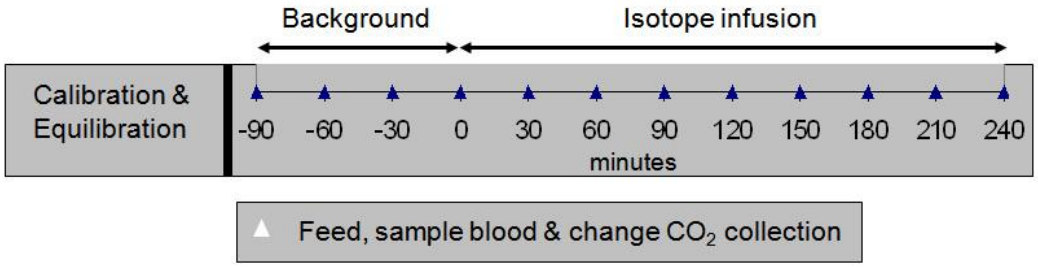
[2] Originally, eight sows were selected for the experiment and were each assigned six graded dietary lysine intakes, for a total of 48 diets. However, one sow aborted her litter after the initial respiration experiment and was removed from the experiment.

fed one-half of their daily feed allowance twice daily, except on study days (see 6.2.3). Individual feed allowances were determined based on body weight and P2 backfat depth at breeding (Table 6.4). Nipple drinkers provided free access to water.

6.2.3 Isotope Delivery and Feeding

On study days, sows were fed a small meal ($1/13^{th}$ of their daily ration) after they were secured in a respiration chamber. The hourly feeding regimens of Moehn et al (2004a,b) were modified so that sows received 11 meals equivalent to $1/26^{th}$ of their daily ration every 30 minutes during the 5.5 hour study period. Comparability of this protocol to once daily feeding was previously verified (Chapter 3.0). The first three meals did not contain labeled phenylalanine and were fed to measure individual $^{13}CO_2$ abundance background profiles (Jones et al, 1985). The oral dose of 2.0 $mg \cdot kg^{-1} \cdot h^{-1}$ L-[1-^{13}C]phenylalanine (Sigma-Aldrich Canada Ltd., Oakville, Ontario) was mixed into individual meals from a stock solution of 20 mg/g. A priming dose of 1.75 times the hourly dose of L-[1-^{13}C]phenylalanine was added to the first dosed meal (Moehn et al, 2004a). The constant oral dose of 2.0 $mg \cdot kg^{-1} \cdot h^{-1}$ L-[1-^{13}C]phenylalanine was previously shown to produce $^{13}CO_2$ sufficiently above the natural background ^{13}C enrichment in breath CO_2 for subjects in the fed state from a diet with similar ^{13}C content for accurate measurement by isotope ratio mass spectrometry (IRMS) (Jones et al, 1985).

Figure 6.1 – Experimental feeding and blood and CO_2 sampling protocol



6.2.4 Indirect calorimetry

Before each study, the S108 oxygen (O_2), S153 carbon dioxide (CO_2), and S126 methane (CH_4) analyzers (Qubit Systems, Kingston, Ontario) were calibrated to zero with N_2 for the O_2 and CO_2 analyzers and a gas of known [O_2] (approximately 21%) and the balance N_2 for the CH_4 analyzers. The upper limits of the analyzers were calibrated with a gas of known [O_2] (approximately 21%), [CO_2] (approximately 1.5%), and [CH_4] (approximately 100 ppm) and the balance N_2 (approximately 77.5%) (Praxair, Edmonton, Alberta). The O_2, CO_2, and CH_4 concentrations were collected by C409 data acquisition system as an average of 200 samples in one-minute intervals during the study (Qubit Systems, Kingston, Ontario). The gas exchange of the animals was determined by comparison to the ambient air values as recorded immediately before and after the study periods. The analytical values for each of the calibration gases was recorded immediately before and after the study and used to correct for drift of the analyzers, if necessary.

Two independent open-circuit indirect calorimetry chambers were each fitted with a 10cm diameter capped PVC tube which allowed feed to be dropped into the feeder and a nipple drinker for *ad libitum* fresh water intake. Access to

the animals was through a clear plexiglass window on the top of each chamber. The calorimetry chambers were designed with two air inlets each consisting of 2.5 cm diameter PVC pipe the length of the chamber with holes drilled approximately every 30cm and capped at the opposite end. Ambient air was drawn into the chamber through these inlet pipes by vacuum displacement as air was withdrawn from the chamber. Air flow out of the chambers of 250 L/min was required to maintain [CO_2] below 1.0% (Chapter 3.0). The animals were directed into the chambers through a rear door that was closed to isolate air flow to the inlet and outlet pipes. The volume of air from the outlet of each box was measured by separate gas meters (model AC630, Canadian Meter Company, Cambridge, Ontario) and manually recorded every 30 minutes. A sub-sample (250 ml/min) from each chamber was dried over a column of drierite before being split and part of the air flow directed to the analyzers and the remaining air flow to the CO_2 collection (see 6.2.5.1).

6.2.5 Sample collection

6.2.5.1 Quantitative expired CO_2 collection

A sub-sample of the air flow from the respiration chambers flowed through midget bubblers (Fisher Scientific, Mississauga, ON or VWR International, Edmonton, AB) suspended in 1 *N* NaOH solution at a rate of ~80 mL/min. A single midget bubbler in ~11 mL of $NaOH_{(aq)}$ was shown to provide complete and quantitative trapping of CO_2 (Chapter 3.0). The solution was changed every 30 min. Charged solution was transferred equally into two evacuated, glass, 16 x 100 mm, serum collection tubes (Vacutainer, Oakville, Ontario) and frozen at -20 ˚C

until analysis.

6.2.5.2 Blood collection

Blood samples of 5 mL sample size were collected every 30 min through the subcutaneous injection port (Chapter 3.0) and transferred into glass or plastic 13 x 100 tubes containing lithium heparin (Vacutainer, Oakville, Ontario) before centrifugation at ~2000 x g for 15 min. Plasma was harvested and stored frozen at -20 °C until analysis. Grab samples of feces (at least 200 g) were taken on the day of respiration before the change of diet, stored frozen at -20 °C, and then freeze dried before further analysis. Small random samples of the mixed diets were collected frequently following mixing during the preparation of individual meals. The small samples were pooled as representing a particular diet before and were stored at room temperature before further analysis.

6.2.6 Chemical analyses

6.2.6.1 Sample grinding

Feed and freeze dried fecal samples were ground in a commercial coffee grinder before analysis to reduce particle size.

6.2.6.2 Bomb calorimetry

Approximately 1g ground samples were weighed in duplicate into tared metal cups. The cup was then placed into the holder in the top of the bomb (LECO Corporation, St. Joseph, Michigan). A 10cm length of platinum wire was situated in the slits on the posts of the top of the bomb, just touching the sample in the cup. The top was put onto the bottom of bomb and the ring screwed down to seal the bomb. The bomb was charged with O_2 to 450 psi. The computer was

previously readied by clearing old data. The weight of the current sample was entered, 'Enter' was pressed and the computer sequence began by lowering the bomb into the water chamber. After the sample in the bomb had been completely oxidized, the computer sequence ended and the bomb was raised from the water chamber. Excess O_2 was released from the bomb and the bomb was opened. After visually confirming complete oxidation of the sample, distilled H_2O with Methyl Orange indicator was used to wash the interior and top of the bomb into a beaker. The washing was complete when there was no longer a colour change of the rinse solution. The solution in the beaker was titrated with 0.8 *N* $Na_2CO_{3(aq)}$ to an orange endpoint. The volume of titrant and the length of wire burned (i.e. 10cm – length unburned) was entered to correct for acid formation during combustion, assumed to be nitric acid, and the caloric contribution from the burned wire, respectively. The 'Enter' was pressed twice and the caloric content (kcal/g) was then printed out by the computer.

6.2.6.3 Acid insoluble ash content

Approximately 2 g samples of ground feed in quadruplicate or 0.5 g samples in duplicate of ground, dried feces were weighed into weighed and labelled 175x12mm glass test tubes. The test tubes were held upright in a 600 mL Pyrex beaker then placed into a 500 ºC oven overnight. The ashed samples were cooled to room temperature before 1.0 ml of 4 *N* $HCl_{(aq)}$ was added to each tube and vortexed. An additional 4 ml of 4 *N* $HCl_{(aq)}$ was then added to each tube. Marbles were placed on top of each of the tubes and heated at 120 ºC overnight. After cooling to room temperature, the sample tubes were centrifuged at 3000

rpm for 10 minutes. The supernatant was removed by vacuum before 5 ml of ddH_2O was added to each tube and vortexed. The tubes were then centrifuged at 3000 rpm for 10 minutes before the supernatant was again removed by vacuum. This washing step was repeated for a total of 3 times. After the final washing, the sample tubes were placed in a 90 ºC oven overnight. Once the samples were completely dry, they were placed into a 500 ºC oven for 24 hours. The ashed samples were removed and cooled to room temperature in a dessicator. Final weights of the tubes were recorded.

6.2.6.4 Nitrogen and carbon contents

Approximately 100 mg of previously ground samples were weighed in duplicate into foil cups. The cups were folded closed around the sample, forming a tear-drop shape. The samples were then loaded into the automatic sample carousel. Nitrogen and carbon contents of the samples were determined by complete combustion. NO_x and CO_2 gas contents were measured by infrared radiation in separate cells of the analyzer (LECO Corporation, St. Joseph, Michigan). The percent nitrogen and carbon was calculated from the previously entered sample weights by the computer.

6.2.7 Plasma free lysine concentration

Previously frozen plasma was thawed at room temperature before plasma amino acid concentrations were measured by reverse-phase HPLC using phenylisothiocyanate derivatives with norleucine added as internal standard, as previously described (Bidlingmeyer et al, 1984; Murch et al, 1996).

6.2.8 Calculations

6.2.8.1 Acid insoluble ash

Acid insoluble ash (AIA) content was calculated as:

$$\frac{\text{Final tube weight} - \text{Initial tube weight}}{\text{Sample weight}} \times 100 = \% \text{ AIA}$$

6.2.8.2 Digestibility

The digestibility of individual dietary components and energy was determined using Celite® as an indigestible marker and analysis for acid insoluble ash (AIA) (McCarthy et al, 1977). The following formula using the AIA contents of feed (AIA_{feed}) and feces (AIA_{feces}) was used to calculate the nutrient digestibility of individual nutrients:

$$\text{Nutrient digestibility (\%)} = 1 - \frac{(AIA_{feed} * Nutrient_{feces})}{(AIA_{feces} * Nutrient_{feed})}$$

6.2.8.3 Volumes of gases

Litres of gases consumed and produced were calculated using the difference between recorded gas exchange values during the experiment and the room air values recorded immediately before and after the experimental period. The values recorded for each minute were corrected for drift of the analyzers, if statistically significant. The difference between the experimental and room air percent gas composition was multiplied by the air flow out of the respiration chambers for each 30 minute period. Therefore, litres of gas consumed or produced were calculated for each 30 minute period, as follows:

V_{O2} (L/30 min) = total air flow (L)/30 min * (% O_2 room air - % O_2 test period)

V_{CO2} (L/30 min) = total air flow (L)/30 min * (% CO_2 test period - % CO_2 room air)

V_{CH4} (L/30 min) = total air flow (L)/30 min * (% CH_4 test period - % CH_4 room air)

6.2.8.4 Heat production

Heat production was calculated according to Brouwer (1965) from the drift-corrected data, if necessary, collected during open-circuit indirect calorimetry. The formula was abbreviated by omitting the urinary nitrogen term because planned attempts to collect urine were unsatisfactory; thus, urinary nitrogen results are not included. According to Weir (1949), 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. Therefore, the formula used to calculate HP from gas exchange was:

$$HP = (16.175 \times V_{O2}) + (5.02 \times V_{CO2}) - (2.17 \times V_{CH4})$$

where V_{O2}, V_{CO2}, and V_{CH4} represent volumes (L) of O_2 consumed and CO_2 and CH_4 produced, respectively. Daily HP was calculated as the summation of HP for each 30 minute of the 4 h isotope delivery period, divided by the previously determined factor (1.17) to extrapolate to a 24 h value (Chapter 3.0).

6.2.8.5 Respiratory quotient

The respiratory quotient (RQ) was calculated as:

$$RQ = V_{CO2} \text{ produced (L/30 minute period)} / V_{O2} \text{ consumed (L/ 30 minute period)}$$

6.2.9 Isotope analysis

Expired $^{13}CO_2$ enrichment was measured by a continuous flow IRMS (CF-IRMS20/20; PDZ Europa, Cheshire, United Kingdom) (Di Buono et al, 2001). Enrichments of all breath samples collected were expressed as atom percent excess (APE) $^{13}CO_2$ above a reference standard of compressed carbon dioxide gas (Jones et al, 1985).

The enrichment of L-[1-^{13}C]phenylalanine in plasma was analyzed using a triple quadrupole mass analyzer (API4000, Applied Biosystems/MDS SCIEX, Concord, ON) coupled to an Aligent 1100 HPLC system (Aligent, Mississauga, ON) (LC-MSMS), as previously described by Turner et al (2006).

6.2.10 Data analysis

Attainment of isotopic steady state in the metabolic pool was shown by plateaus in $^{13}CO_2$ enrichment in breath and plasma [^{13}C]phenylalanine. Plateaus, slope not significantly different from zero, were achieved by 120 min from the start of isotope infusion and were maintained to the end of the study at 240 min. Breath and plasma isotope enrichment values were determined as atoms percent (AP). The last two of the three background samples at baseline and at least three samples taken during the plateau period were used for further analysis. The coefficients of variation for breath $^{13}CO_2$ enrichment were 0.30 % and 0.35 % for baseline and plateau enrichments, respectively. The mean coefficient of variation for the 41 plateaus in oxidation from early-gestation was 8.1±0.5% and for the 39 plateaus in oxidation from late-gestation was 10.5±0.8%.

Plasma phenylalanine flux was determined at isotopic steady state using the equation of Matthews et al. (1980):

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

where E_i was the enrichment of L-[1-^{13}C]phenylalanine infused (MPE), E_p was the enrichment of plasma phenylalanine above baseline at isotopic plateau (MPE), and I was the rate of 1-[^{13}C]phenylalanine infused ($mg \cdot kg^{-1} \cdot h^{-1}$).

The percent of the phenylalanine dose oxidized (Ox) was calculated as follows:

$Ox = [(V_{CO2}*APE/100)/(22.4*(288.15/273.15)*1000*165.19*(99/100)]/dose*100$

where V_{CO2} is the volume of CO_2 expired during a 30 min period, APE was determined by IRMS analysis (see 6.2.9), 22.4 mol/L*(288.15 K/273.15 K) is the correction for the molar volume of an ideal gas at the non-STP temperature of 288.15 K (equivalent to 15 ˚C[3]), 165.19 g/mol is the molecular weight of phenylalanine, and 99% is the enrichment of the L-[1-^{13}C]phenylalanine, as reported (Sigma-Aldrich Canada Ltd., Oakville, Ontario).

6.2.11 Statistical analysis

Statistical analysis was performed using mixed procedure and breakpoint analysis was performed using the non-linear procedure in SAS version 9.1 (SAS Institute Inc., Cary, North Carolina). The classification variable was lysine intake and individual animals, experimental block, and respiration chamber were treated as random variables. Covariates were identified using the mixed procedure and included only when significant. Model statements were tested using the Kenward-Roger degrees of freedom method. Least square means were compared using the 'pdiff' option. Outliers were identified by regression analysis with option /r to calculate CookD statistics. Outliers were removed from further analysis when individual data was 2 or more Cook's D from the mean. When outlier values were removed, these have been identified in table footnotes. Data are presented as means ± SEM. Values were considered significant at $P < 0.05$; a trend at $P<0.10$.

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[3] The air volume measured by the AC 630 gas meters is temperature corrected to 15 ˚C.

6.3 Results

Calculated dietary lysine intakes ranged from 7.5 to 19.2 g/d in early- and 8.1 to 23.7 g/d in late-gestation (Tables 6.2 & 6.3). Breakpoint analysis of phenylalanine oxidation, independen of body weight, indicated that the dietary lysine requirement of pregnant sows was 10.1 g/d (P=0.024) (Figure 6.2) and 16.5 g/d (P=0.033) (Figure 6.3) in early- and late-gestation, respectively. Temperature of the sample air (t) and body weight (bw) on the day of oxidation were included as covariates in the model for early-gestation (Figure 6.6):

early-gestation (when lysine intake (ilys) was less than 10.1 g/d, P=0.006):

oxidation= (61.0±17) – (0.52±0.61)*ilys – (2.3±0.8)*t + (0.05±0.02)*bw

No significant covariates were found for the model in late-gestation:

late-gestation (when lysine intake was less than 16.5 g/d, P=0.033):

oxidation= (19.9±2.9) – (0.44±0.20)*ilys

However, there was a trend (P=0.07) for the change in body weight from breeding to the end of the early-gestation experimental period (Δbw) to be a significant covariate in the model in late-gestation (Figure 6.5):

late-gestation (when lysine intake was less than 16.5 g/d, P>0.001):

oxidation= (19.9±2.4) – (0.52±0.17)*ilys + (0.10±0.03)*Δbw

Heat production, respiratory quotients, and body weight were not different when classified into similar dietary lysine intake levels, except that BW was greater for the highest dietary lysine intake group in early-gestation (Table 6.12). Heat production was greater and energy balance was lower for sows during late-gestation compared to early-gestation.There were no differences in phenylalanine

kinetics (μmol·kg^{-1}·h^{-1}) (i.e. flux, intake, breakdown, oxidation, or synthesis) when grouped according to similar dietary lysine intake levels in early-gestation (Table 6.13). Flux, breakdown, oxidation, and synthesis were not different by dietary lysine intake level, but phenylalanine intake was lower for dietary lysine intake levels 5 and 6 and the isotope dose (μmol·kg^{-1}·h^{-1}) was lower for dietary lysine intake level 5 compared to level 2 and 3. There were differences in mean body weight between dietary lysine intake levels of sows in late-gestation, where the body weight of sows within levels 5 and 6 were greater than within levels 2 and 3 (Table 6.14). There were no differences identified when the results were expressed independent of body weight (i.e. g or mg/d or h). Day of the experimental period was lower for sows within dietary lysine intake levels 3 and 4 compared to level 6 during early-gestation (Table 6.13) but was not different during late-gestation.

The analyzed nitrogen content of random, representative feed samples (n=5) was 1.79±0.08% and the carbon content was 39.9±0.46%. The gross energy content of the feed was 16.09±0.03 kJ/g. Apparent total tract digestibility of energy (90.6±1.1%), nitrogen (79.7±0.1%), and carbon (80.7±0.9%) were not different between parity nor early- and late-gestation (Table 6.11).

Using dietary lysine intake levels as the classification variable, plasma free amino acid concentrations did not increase as lysine intake increased, except in late-gestation when plasma free lysine concentration was greatest (P=0.003) at the highest level of dietary lysine intake (Tables 6.16 & 6.17). When not classified into levels or by parity in early-gestation, plasma free lysine increased

as lysine intake increased (P=0.04). All other amino acids, including total amino acid concentration, were not affected by lysine intake.

Parity two sows tended to weigh less at breeding (P<0.10, 167.5 vs. 199.4 kg, SEM 9.6) and gained more weight (P=0.03, 78.5 vs. 63.3 kg, SEM 3.7) more quickly (P<0.02) during gestation than parity three sows (700.6 vs. 566 g/d, SEM=32.6) from breeding to parturition (Tables 6.5 & 6.6). Consequently, body weight was not different (P=0.58) by parity at parturition when sows weighed 258.8±8.3 kg (Table 6.7). The interaction of dietary lysine intake and parity on phenylalanine oxidation was not significant, regardless of the units used to express dietary lysine intake (Table 6.18). There was a linear increase in backfat thickness and loin depth between breeding and parturition. Loin eye area increased for the first two-thirds of gestation, but was lower in the final third of gestation. Lean percent also was lower in the final third of gestation (Table 6.8 & Figure 6.6).

The mean number of piglets born alive was 15.3±1.2 from six litters (Table 6.9); litter data from the seventh sow was identified as an outlier for litter data and, therefore, removed due to the small litter size of four piglets (Table 6.10). The average piglet birth weight was 1.4±0.1 kg. Parity two sows weaned more piglets (P=0.03, 10 vs. 8 piglets, SEM=0.5) than parity three sows.

6.4 Discussion

The dietary requirement for lysine in this population of sows was 10.1 g/d and 16.5 g/d in early- and late-gestation, respectively. These lysine requirement values are similar to those reported by Srichana (2006) and GfE (2008). Both

Srichana (2006) and GfE (2008) calculated their dietary lysine requirement estimates on the basis of standardized ileal digestibility of lysine. In the current experiment, the dietary lysine requirements were measured on the basis of total amino acids provided by the diets. Srichana (2006) did not use any synthetic amino acids in the test diets, whereas, in the current experiment, the majority of the dietary lysine was provided as L-lysine·HCl in the summit diets. L-lysine·HCl is assumed to be 100% available, so as the free lysine content of the diet increases, so does the digestibility of the lysine from the diet. In fact, 50% of the dietary lysine was provided by free lysine when dietary intake was greater than 11 g/d. The remainder of the dietary lysine was provided by corn, which has a high reported true ileal digestibility for lysine of 84.9 % for sows (Stein et al, 2001). Therefore, the requirement determined in this study can be taken to approximate the requirement for TID lysine.

Whereas NRC (1998) suggests a constant intake of dietary lysine over the entire gestation, data from Dourmad et al (1994b, 1999a,b), Srichana (2006), Srichana et al (2007), GfE (2008), Kim et al (2009), and this experiment indicate that there are significant differences between the stages of gestation for the dietary lysine requirement. Ji et al (2005) also indicated, based on measured crude protein gains of maternal and fetal tissues during gestation, that different quantities of dietary protein may be required by pregnant gilts during late-gestation. The differences between early- and late-gestation requirements are not surprising considering the differences in fetal and maternal metabolism. In early-gestation, the majority of the nutrients are directed towards repair and regeneration of tissue

lost during the previous lactation, which is highly individual. This is reflected in the trend for differential change in body weight from breeding to the end of the early-gestation experimental period (i.e. day 45 of gestation) to be a significant covariate in the model of oxidation of the indicator amino acid in late-gestation. The coefficient for the change in body weight between breeding and day 45 of gestation explains an additional 20% of the variation in oxidation of the indicator amino acid in late-gestation. Similarily, body weight was a significant covariate in the model of oxidation of the indicator amino acid in early-gestation, but not in late-gestation when sows were of similar body weights. Differences in early-gestation body weight account for 10% of the variation of the oxidation of the indicator amino acid in early-gestation. Relatively, a minority of nutrients are required for growth and development of the uterus, placenta, and fetuses in early-gestation. Gradually, over the course of gestation, increasingly more nutrients are required by the developing fetuses. In fact, Ji et al (2005) supposed the measured reduction in accretion rate of protein for maternal carcass soft tissue during late-gestation in gilts was due to partitioning of nutrients to support accelerated fetal growth. After day 70 of gestation, protein gain of the fetuses increases linearly (McPherson et al, 2004), which is reflected in the greater dietary lysine requirement in late- than in early-gestation.

There were no differences between the apparent total tract digestibility of energy, nitrogen, or carbon comparing early- and late-gestation or parity. We should only expect an improved digestibility of nutrients if the digestive tract is changing during gestation. However, the digestive tract quickly returns to normal

size following weaning and then does not grow until very late in gestation as an adaptation to increased nutrient requirements of gestation and lactation (Ji et al, 2005).

Phenylalanine kinetics are reported as $\mu mol \cdot kg^{-1} \cdot h^{-1}$ to account for differences in body weight of the sows which may, otherwise, be difficult to observe. However, there were no differences in phenylalanine flux, dietary intake, appearance from protein breakdown, removal by oxidation or incorporation into body tissue by protein synthesis in early-gestation (Table 6.13). Expressed on a body weight basis, phenylalanine intake was lower for dietary lysine intake levels 5 and 6 and the isotope dose was lower for dietary lysine intake level 5 versus levels 2 and 3 in late-gestation (Table 6.14). Isotope dose was dependent on body weight (i.e. 2.0 $mg \cdot kg^{-1} \cdot h^{-1}$) and was included in the total dietary intake of phenylalanine. There was a trend toward the sows within levels 5 and 6 having greater body weight than sows within levels 2 and 3, which was likely a function of combining the data into similar lysine intake levels *post hoc*. Therefore, lower phenylalanine intake is probably a function of the greater body weight for levels 5 and 6 compared to levels 2 and 3 for the sows from which phenylalanine flux could be calculated. There is a slight difference in the body weights reported in Tables 6.13 and 6.14 versus Table 6.12. It should be recognized that the data in Table 6.12 includes all of the sows and is most reflective of the population of sows, whereas Table 6.13 and 6.14 only include animals for which phenylalanine flux could be measured. Similarly, differences in body weight of levels 5 and 6 are a result of missing values and not reflective of the experimental design.

Phenylalanine flux was compared between early- and late-gestation using Student's t-test procedure. In all cases, except for dose of the tracer, values were greater in early- than in late-gestation (Table 6.15). In early-gestation, the majority of nutrients are partitioned towards replacement and repair of tissue mobilized during the previous lactation (Etienne et al, 1991). The largest numerical difference was between breakdown during early- and late-gestation, but there was also a concomitant increase in protein synthesis and amino acid oxidation leading to an increase in flux. Flux may be greater in early-gestation compared to late-gestation due to remodeling of the maternal tissue. Repair and replacement of body tissue mobilized from the previous lactation occurs in early- and mid-gestation, before fetal nutrient demands become too great (Etienne et al, 1991). Breakdown is much lower in late-gestation, indicating that protein synthesis is dominant, thus resulting in lean tissue deposition, most likely, of the fetuses.

Total plasma free amino acid concentration was 3 times greater in late-gestation than in early-gestation. This is in stark contrast to Bonsnes (1947) who indicated that maternal plasma amino acid concentrations decrease by 25% very early in pregnancy and return to normal levels shortly after parturition. Bonsnes (1947) collected data from pregnant human females, which have a more permeable placenta than sows; the epitheliochorial type placentae of the pig have a greater number of interhemal layers. Maternal and fetal blood supplies are separated by maternal endothelium, connective tissue, uterine epithelium, chorionic epithelium, connective tissue and endothelium. Human placentae are of

the haemochorial classification where maternal blood reaches fetal chorioallantoic trophectoderm directly and, therefore, increases the efficiency of nutrient transport across the placenta. Hence, reduced efficiency of amino acid transport across the many interhemal layers of the porcine placenta may explain the greater plasma amino acid concentration in sows. Also, the amino acids were included in the late-gestation diet at 185% of the requirement predicted by NRC (1998) which should increase the plasma free amino acid concentrations.

Plasma free lysine concentration increased linearly as dietary lysine intake increased during gestation. With sufficient adaptation time, plasma free amino acids increase in concentration after the dietary requirement has been met and exceeded. However, as observed in Chapter 5.0, the short period between study days when using the IAAO method is not adequate to equilibrate the plasma free amino acid concentrations with the dietary intake of the test amino acid (Mitchell et al, 1968).

Temperature of the sample air was significant and, therefore, included as a covariate in the model for breakpoint analysis of IAAO data for early-gestation. The data acquisition software records the temperature of the expired air sample as a proxy for recording the temperature of the room or chamber. The respiration chambers are housed within a climate controlled building, however, changes in the temperature of the room are frequently beyond the tolerance of the climate control. These temperature changes can impact the temperature inside of the respiration chambers and change the respiration rates of the sows. The early-gestation studies were performed in the fall, when ambient temperatures could

still be above the desired room temperature for efficient cooling of the respiration chambers. Late-gestation studies were performed in the winter so that the temperature was more consistent due to heating. There were no differences in HP or RQ of sows classified into dietary lysine intake levels in early- or late-gestation. The RQ of the sows was consistently near one, indicating mostly dietary carbohydrate metabolism, which can be expected based on the diet formulation with 1% canola oil as the only lipid source and corn and starch as the major energy source ingredients. The mean energy intake was 33.7±0.2 MJ/d from the experimental diets during gestation. Heat production, extrapolated to 24 h values by application of the correction factor (Chapter 3.0), was greater (P=0.04) for sows during late-gestation than during early-gestation. This phenomenon was observed previously (Chapter 4.0) and determined by Samuel (2008) to be related to the increased energy expenditure associated with the weight of the developing piglets. Because energy intake was not different, energy balance was greater (P<0.04) during early- compared to late-gestation (2.6 vs. 0.8, SEM±0.4 MJ/d). In fact, the late-gestation energy balance only tended (P=0.08) to be greater than zero. Therefore sows in late-gestation were marginally in positive energy balance, indicating that dietary lysine was the limiting nutrient in the experimental diets.

The development of insulin resistance in the maternal tissues is a compensatory response to insufficient dietary energy intake. Insulin resistance has two effects: 1) glucose uptake by the maternal tissues is decreased which raises the plasma glucose concentration and increases the availability of glucose for

transport across the placenta and 2) the "protective" action of insulin (e.g. reduced breakdown of protein and lipid) is reduced, thus enabling a catabolic state. Elsley (1971) observed that different patterns of sow feed intake had no effect on litter size or total weight. Anderson (1975) and Hard and Anderson (1979) reported that starved sows made available protein and energy from maternal stores for embryonic and placental development when progesterone and estrogen levels were adequate, clearly demonstrating that sows are capable of maintaining litter size during malnutrition (Baumen and Currie, 1980; Pond et al, 1991). In the long-term, insufficient nutrition of the sow during gestation negatively impacts longevity due to reduced rebreeding success or poor lactation performance (Mahan, 1977; Mahan, 1981; Aherne and Kirkwood, 1985; Dourmad et al, 1994a,b; Gaughan et al, 1995; Young and Aherne, 2005; Anil et al, 2006; Tvrdoň and Marková, 2007). Pettigrew (1981) suggested that feeding fat to sows in late-gestation could improve the metabolic status of sows by reducing or eliminating negative energy balance. Because there are no lipid transporters in the porcine placentae, the addition of energy to the diet would exclusively benefit the sow.

Backfat thickness and loin depth increased linearly ($P<0.01$) between breeding and parturition indicating maternal deposition of both body fat and protein during gestation. Dourmad et al (1997) calculated from the chemical analysis of sows that each 1 mm of P2 backfat corresponds to 1.331 kg of body lipid. Sows in this experiment gained 5 mm of backfat from breeding to day 105, which corresponds to 6.7 kg of maternal lipid gain during gestation. The increase in loin depth occurred within the first 5 weeks after breeding and then remained

constant. Similarly, backfat thickness increased more quickly within the first 5 weeks post-breeding than during the remainder of gestation. Therefore, due to the relative increase in backfat compared to loin depth, lean percent was lower in the final third of gestation. Loin eye area increased for the first two-thirds of gestation, and was then lower in the final third of gestation. Without adjustments to feed intake, sows typically become catabolic in late-gestation, oxidizing body tissue to compensate for the energy restriction (Chapter 4.0). The absence of backfat deposition in the final two thirds of gestation indicate that dietary carbohydrates were extensively oxidized to provide necessary energy; there was no storage of additional energy as backfat, as indicated by the marginally positive energy balance. The mean intake of lysine was 12.9±0.6 g/d in early-gestation and exceeded the newly determined dietary requirement. Mean dietary lysine intake in late-gestation (16.5±0.8 g/d) appears to be equivalent to the newly determined requirement. Therefore, lysine intake during gestation was not limiting.

Parity two sows gained more weight (P=0.03) during gestation than parity three sows. This can partially be explained by the numerically larger litter size (16.7 vs. 14.0 piglets, SEM 1.2) and corresponding conceptus gain (NRC, 1998) (40.2 vs. 34.2 kg, SEM 2.5) of the second parity sows compared to the third parity sows. However, there was no difference between parities in the total birth weight of the litters (P=0.31) or mean individual piglet weight (P=0.88), suggesting that some of the additional gain of parity two sows was maternal gain. This is likely due to differences in maternal growth potential during gestation, where parity three animals are closer to achieving adult body weight and, therefore, achieve

less maternal gain during gestation (Cooper et al, 2001). It may by expected that the greater weight gain of parity two sows should result in an increase in the dietary lysine requirement compared to parity three sows. However, the interaction of parity and dietary lysine intake expressed as g/d, g/BW, or g/$BW^{0.75}$ on phenylalanine oxidation was not significant. Therefore, from the data of the current experiment, there was no effect of parity on oxidation of the indicator amino acid (Table 6.18). Close and Mullan (1996) indicated that sows do not reach maturity until their fourth parity. Interestingly, according to Stalder et al (2004), the average parity at cull in the year 2000 was 3.3. Therefore, on average, the nutritional advantages (i.e. little maternal gain during gestation reducing the overall nutrient requirements) afforded once the sow has achieved adult bodyweight are often missed in swine production.

Productivity of the sows (i.e. litter size (15.3±1.2) and weight at birth (21.2±1.6 kg) or weaning (58.6±2.7)) was not different by parity (Table 6.9), except that parity two sows weaned more piglets than parity three sows. However, this difference included early weaning reductions of litter size for on-going research projects and not due to experimental treatments (Table 6.10). Increasing total litter size and growth rate should, in theory, increase the dietary lysine requirement of gestating sows (NRC, 1998), but, due to the small number of animals in this experiment and the statistical model applied, the effect cannot be tested.

Total gain, predicted post-hoc by actual litter size using NRC (1998) software, was compared with observed weight gain during gestation. All sows,

except one, gained more weight than predicted by NRC (1998) (4.8±4.0 kg, range -13.5 to 15 kg), indicating a shortcoming in the NRC (1998) prediction equations for modern sows. The factorial approach used to predict maternal gain relies on estimates of the maintenance energy requirement (Chapter 3.0) and lysine for maintenance requirement (Chapter 5.0), which are too low. Therefore sows do not achieve the protein gain predicted by NRC (1998). Instead, protein gain of sows may be greater than predicted, due to genetic improvements in lean tissue deposition rates, resulting in greater weight gain than predicted. Also, as shown by Stein et al (2001) sows are capable of greater nutrient digestibility from feedstuffs compared to growing animals. For example, NRC (1998) reported the true ileal digestibility of lysine from corn is 78% for swine, whereas Stein et al (2001) reported a value of 84.9% for sows. Therefore, predicted protein gains based on nutrient digestibility values from NRC (1998) will be lower than actual gains.

Phase feeding at least two diets could improve productivity by more correctly providing the necessary nutrients for the growth of the placenta, the piglets, and the mammary gland. When nutrients are provided in excess of the requirements, they must be excreted. In the case of amino acids, energy is required for nitrogen excretion through the urea cycle. Therefore, the energetic efficiency of the animal is reduced, as observed in Chapter 5.0. Phase-feeding was previously demonstrated to have no impact on the performance of sows but did decrease nitrogen excretion (Dourmad et al, 1999b; Clowes et al, 2003).

6.5 Conclusion

Currently, NRC (1998) recommends feeding 9.7 g/d of SID lysine throughout gestation to maximize N retention. Results from Srichana (2006), Srichana et al (2007), GfE (2008), and the current experiment indicate that: 1) the lysine requirement of modern, pregnant sows is greater than reported by NRC (1998), and 2) the dietary lysine requirement is greater in late- than in early-gestation. Ultimately, phase feeding would provide positive economic returns by reducing feed costs and maximizing lifetime productivity of the sows.

Table 6.1 – Formulation of the base and summit diets for pregnant sows fed graded levels of dietary lysine

Ingredient	Base diet	Summit, early	Summit, late
Corn	875	875	875
Starch	6.05	9.5	0.35
Canola oil	10	10	10
Celite	10	10	10
Soybean meal	30	30	30
Histidine	0	0	0.25
Isoleucine	1.05	1.05	2.1
Lysine	0	6.05	8.4
Cysteine	2.05	2.05	3.2
Methionine	0.2	0.2	0.5
Phenylalanine	0	0	0.15
Tyrosine	0.5	0.5	1.25
Threonine	2.9	2.9	4.45
Tryptophan	0.7	0.7	1.1
Valine	0.55	0.55	1.75
Glutamine	9.5	0	0
Choline chloride	2	2	2
Mineral premix[1]	5	5	5
Vitamin premix[2]	7	7	7
Dicalcium phosphate	21.5	21.5	21.5
Limestone	11	11	11
Sodium chloride	5	5	5

[1]provided per kg of premix: 10,000 mg of Cu; 15,000 mg of Fe; 5,000 mg of Mn; 60 mg of Se; 25,000 mg of Zn; 100 mg of I (DSM Nutritional Products, Hay River, Alberta, Canada).

[2]Provided per kg of premix: 1,500,000 IU of vitamin A; 150,000 IU of vitamin D3; 10,000 IU of Vitamin E; 800 mg of vitamin K; 500 mg of thiamin; 1,000 mg of riboflavin; 300 mg of pyridoxine; 3,000 µg of vitamin B12; 7,500 mg of niacin; 3,000 mg of pantothenic acid; 500 mg of folic acid; 50 g of biotin (DSM Nutritional Products, Hay River, Alberta, Canada).

Table 6.2 – Individual lysine intakes (g/d) of sows fed six graded levels of lysine during early-gestation

Lysine intake below estimate[1]			Lysine intake above estimate[1]		
Lysine intake (g/d)	Sow ID	Parity	Lysine intake (g/d)	Sow ID	Parity
7.4633	43101	2	13.5495	43101	2
7.9191	42104	2	14.4096	42104	2
8.0439	38003	3	14.6592	38003	3
8.0835	37102	3	14.7702	37102	3
8.2172	36705	3	14.9039	36705	3
8.3486	43101	2	15.0987	43101	2
8.351	37903	3	15.1714	37903	3
8.6071	42102	2	15.6022	42102	2
8.9176	42104	2	16.157	42104	2
9.0424	38003	3	16.4067	38003	3
9.1534	37102	3	16.6424	37102	3
9.2338	43101	2	16.6479	43101	2
9.2871	36705	3	16.7762	36705	3
9.4208	37903	3	17.0436	37903	3
9.6434	42102	2	17.4157	42102	2
9.9161	42104	2	17.9045	42104	2
10.041	38003	3	18.1541	38003	3
10.2232	37102	3	18.5147	37102	3
10.357	36705	3	18.6484	36705	3
10.4907	37903	3	18.9159	37903	3
10.6797	42102	2	19.2292	42102	2

[1] Estimated dietary lysine requirement for gestating sows according to NRC (1998).

Table 6.3 – Individual lysine intakes (g/d) of sows fed six graded levels of lysine during late-gestation

Lysine intake below estimate[1]			Lysine intake above estimate[1]		
Lysine intake (g/d)	Sow ID	Parity	Lysine intake (g/d)	Sow ID	Parity
8.0835	37102	3	17.4134	43101	2
8.4844	36705	3	18.8934	42104	2
8.667	42104	2	18.9613	43101	2
8.6789	43101	2	19.1428	38003	3
8.8852	37903	3	19.4412	37102	3
9.0411	38003	3	19.7084	36705	3
9.7713	42102	2	19.9757	37903	3
10.8895	37102	3	20.1256	42102	2
11.0007	43101	2	20.5092	43101	2
11.286	42104	2	20.6394	42104	2
11.2904	36705	3	20.8888	38003	3
11.6601	38003	3	21.3119	37102	3
11.6912	37903	3	21.5791	36705	3
12.4893	42102	2	21.8464	37903	3
13.3225	43101	2	21.9376	42102	2
13.6955	37102	3	22.3853	42104	2
13.9049	42104	2	22.6348	38003	3
14.0964	36705	3	23.1826	37102	3
14.279	38003	3	23.4498	36705	3
14.4973	37903	3	23.717	37903	3
15.2073	42102	2	23.7495	42102	2

[1] Estimated dietary lysine requirement for gestating sows according to NRC (1998).

Table 6.4 – Feed allocation and assignment of individual lysine intakes to sows fed graded levels of dietary lysine in early- and late-gestation

					Predicted lysine requirement[1]	
Sow	Body weight at breeding (kg)	P2 backfat at breeding (mm)	Feed allocation of gestation diet[2] (kg/d)	Feed allocation of test diet[3] (kg/d)	Total lysine (percent of diet)	Total lysine (g/d)
37102	230.0	16.0	2.9	2.55	0.49	12.5
36705	203.0	14.0	2.9	2.55	0.51	13.0
37903	185.0	13.0	2.9	2.55	0.52	13.3
42104	186.5	15.0	2.7	2.38	0.52	12.4
38003	179.5	13.5	2.7	2.38	0.53	12.6
42102	159.0	11.5	2.8	2.47	0.54	13.3
43101	157.0	15.5	2.4	2.11	0.56	11.8

[1]calculated with NRC (1998) software with predicted litter size of 12 piglets

[2]12.5 MJ ME/kg

[3]14.0 MJ ME/kg – test diet feed allocation was calculated to achieve the same energy intake as from the gestation diet

Table 6.5 – Calculated total and daily weight gain between breeding and parturition (kg) of individual sows fed graded levels of dietary lysine during early- and late-gestation

Sow	Body weight at breeding (kg)	Body weight before parturition (kg)	Days between weight measurements	Total body weight gain (kg)	Calculated daily weight gain (kg)	Parity
42102	159	241.5	113	82.5	0.73009	2
43101	157	214.5	103[1]	57.5	0.55825	2
42104	186.5	261	111	74.5	0.67117	2
36705	203	266	111	63	0.56757	3
37102	230	295	114	65	0.57018	3
37903	185	241.5	111	56.5	0.50901	3
38003	179.5	248	111	68.5	0.61712	3

[1]data from sow 43101 was not included because pre-parturition weight was not available. Because significant weight gain occurs very late in gestation (Ji et al, 2005) and is highly individualized, pre-parturition body weight could not be predicted.

Table 6.6 – Total body weight gain and daily gain of sows fed graded levels of dietary lysine during early- and late-gestation, compared by parity

	Parity			
Variable	2	3	SEM	P-value
Total body weight gain (kg)[1]	78.5	63.3	3.7	0.03
Daily weight gain (g)[1]	700.6	566.0	32.6	0.02

[1]data (n=6) from sow 43101 was not included because pre-parturition weight was not available. Because significant weight gain occurs very late in gestation (Ji et al, 2005) and is highly individualized, pre-parturition body weight could not be predicted.

Table 6.7 – Mean body weight and P2 backfat at breeding and before parturition of sows fed graded levels of dietary lysine during early- and late-gestation

Variable	Mean	SEM	P-value[1]
Body weight at breeding (kg)	185.7	9.6	0.10
P2 backfat at breeding (mm)	14.1	0.6	0.93
Feed allocation (kg/d)	2.8	0.1	0.12
Body weight on d 105 (kg)	237.9	6.3	0.13
P2 backfat on d 105 (mm)	18.9	0.7	0.87
Body weight before parturition (kg)	258.8	8.3	0.58
Total body weight gain (kg)	68.3	3.7	0.03
Calculated conceptus gain on d 115 (kg)[2,3]	37.2	2.5	0.27
Calculated daily weight gain (kg)[4]	610.9	32.6	0.02

[1]difference according to classification variable parity. Mean values for differences between parity are shown in Table 6.6.

[2]calculated as 19.8 $g \cdot piglet^{-1} \cdot d^{-1}$, according to NRC (1998).

[3]data (n=6) from sow 36705 was not included due to small litter size.

[4]data (n=6) from sow 43101 was not included because pre-parturition weight was not available. Because significant weight gain occurs very late in gestation (Ji et al, 2005) and is highly individualized, pre-parturition body weight could not be predicted.

Table 6.8 – Body weight, real-time ultrasound measurements, and heat production (kJ/min) of sows fed graded intakes of dietary lysine in two week intervals during gestation

Day of gestation (2-week intervals)	14	28	42	56	70	84	98	SEM	P-value
Body weight (kg)	185.2^{a}	197.1^{b}	208.1^{c}	217.9^{d}	220.3^{d}	227.3^{e}	237.9^{f}	3.7	<0.01
Backfat (mm)	15.4^{a}	17.1^{b}	16.9^{b}	18.0^{bc}	18.3^{bc}	19.0^{c}	18.9^{c}	0.3	<0.01
Loin depth (mm)	47.3^{ab}	48.2^{ab}	49.1^{abc}	50.3^{cd}	50.7^{d}	51.0^{d}	50.1^{cd}	0.5	<0.01
Loin eye area, (cm^2)	34.3^{ab}	35.0^{abd}	35.7^{abd}	36.5^{ab}	38.7^{c}	36.1a^{cd}	36.3^{abd}	0.5	0.02
Lean percent	35.5^{ab}	35.2^{abc}	34.9^{bd}	34.8^{bd}	35.5^{ab}	34.4^{cd}	34.3^{cd}	0.1	0.02

[1]: Measurements began two weeks after breeding

[a,b,c]: values which share a common letter are not different

Table 6.9 – Mean lactation performance data from the subsequent lactation of sows fed graded levels of lysine during early- and late-gestation

Variable[2]	Mean	SEM	P-value[1]
Born alive	15.3	1.2	0.32
Born dead	1.5	0.3	0.99
Born alive weight (kg)	21.2	1.6	0.31
Born dead weight (kg)	1.7	0.5	0.66
Piglet birth weight (kg)	1.4	0.1	0.88
Number of piglets weaned[3]	9.0	0.5	0.03
Weight of litter weaned	58.6	2.7	0.14
Days of lactation	20	0.4	0.99

[1]difference according to classification variable parity

[2]litter data (n=6) for sow 36705 was removed due to the small litter size

[3]this difference was due to early weaning reductions of litter size for on-going research projects and not due to experimental treatments

Table 6.10 – Subsequent lactation performance data for individual sows fed graded levels of lysine during early- and late-gestation

Sow	Born alive	Born dead	Born alive weight (kg)	Born dead weight (kg)	Piglet removals or additions[1]	Number of piglets weaned	Weight of litter weaned	Days of lactation	Parity
42104	16	1	21.7	1	-5 CF	11	70	20	2
42102	14	2	21.9	3.1	-4 CF, -1 EW	9	59.4	19	2
43101	20	0	25.3	-	-1 LO, -9 EW	10	58.9	21	2
37102	16	2	23.5	2.1	-2 LO, -1 E, -1 D, -3 CF, -1 EW	8	52	18	3
36705[2]	4	0	8.3	-	5 CF	9	80.7	20	3
37903	15	0	20.8	-	-9 EW, -1 LO, 3 CF	8	51.8	21	3
38003	11	1	14.1	0.7	-1 LO, -1 D, -1 EW	8	59.7	21	3

[1]piglets were cross-fostered (CF) within 48 h after parturition to equalize litter size; other removals: laid-on (LO), euthanized (E), early wean for research (EW), death (D)

[2]data from this sow was removed from calculations related to productivity due to the small litter size.

Table 6.11 – Nutrient digestibility of gestating sows fed graded levels of dietary lysine during early- and late-gestation

	Apparent total tract nutrient digestibility[1]				
Nutrient	Early-gestation	Late-gestation	Mean	SEM	P-value
Energy	91.8	89.6	90.6	1.1	0.54
Nitrogen	80.5	80.6	79.7	0.4	0.69
Carbon	80.2	80.9	80.7	0.9	0.31

[1]: available data (n=10)

Table 6.12 – Heat production (HP) (MJ/d), respiratory quotient (RQ), and body weight of sows fed graded levels of lysine during early- and late-gestation, classified by similar lysine intake levels

	Dietary lysine intake level	1	2	3	4	5	6	SEM	P-value		
									ANOVA	Linear[1]	Quadratic[1]
Early-gestation[2]	Lysine intake (g/d)	8.1	9.1	10.2	14.7	16.5	18.4	0.8	<0.01	-	-
	HP	38.7	36.4	37.5	34.6	38.1	36.0	0.82	0.38	0.39	0.62
	RQ	0.97	0.98	1.04	0.96	0.92	0.97	0.02	0.55	0.14	0.58
	BW	190[a]	192[a]	188[a]	189[a]	192[a]	196[b]	3.9	**0.04**	-	-
Late-gestation[3]	Lysine intake (g/d)	8.8	11.5	14.2	19.0	21.0	22.9	1.0	<0.01	-	-
	HP	38.2	38.7	41.6	37.9	37.6	38.1	0.63	0.42	0.45	0.2
	RQ	1.01	0.97	0.94	0.99	0.98	0.96	0.01	0.23	0.31	0.17
	BW	220	229	230	230	230	234	3.3	0.63	-	-

[1]: Dietary lysine intakes less than 10 g/d for early- and 16.5 g/d late-gestation, respectively

[2]: available data (n=41)

[3]: available data (n=39)

[a,b,c]: values which share a common letter are not different

Table 6.13 – Phenylalanine kinetics (μmol·kg^{-1}·h^{-1}) from sows fed graded levels of dietary lysine, grouped by similar lysine intake (g/d) levels, during early-gestation

Dietary lysine intake level	1	2	3	4	5	6		
Number of animals[1]	4	4	2	3	4	6	SEM	P-value
Mean lysine intake (g/d)	8.2	9.0	10.1	14.5	16.2	18.4	0.9	<0.01
Flux (μmol·kg^{-1}·h^{-1})	91.0	93.4	75.9	88.6	89.9	81.9	2.7	0.54
Intake (μmol·kg^{-1}·h^{-1})	72.2	72.3	68.5	71.2	71.2	71.6	1.0	0.10
Breakdown (μmol·kg^{-1}·h^{-1})	18.7	21.2	7.4	17.4	18.8	10.3	2.4	0.64
Oxidation (μmol·kg^{-1}·h^{-1})	38.0	33.8	39.7	32.0	36.8	32.8	1.5	0.69
Synthesis (μmol·kg^{-1}·h^{-1})	52.9	59.6	36.2	56.6	53.1	49.1	3.4	0.90
Phe retention (μmol·kg^{-1}·h^{-1})	34.1	38.4	28.8	39.2	34.4	38.8	2.3	0.72
Isotope dose (μmol·kg^{-1}·h^{-1})	11.8	11.6	11.8	11.8	11.5	11.3	0.1	0.43
Body weight (kg)	190.8	187.8	212.0	183.8	196.4	195.5	6.0	0.14
Mean day of experiment	13.0^{b}	12.9^{b}	9.0^{a}	7.9^{a}	14.6^{b}	17.9^{c}	1.1	<0.10

[1]: Data identified as outliers by Cook's D statistic were removed; [a,b,c]: values which share a common letter are not different

Table 6.14 - Phenylalanine kinetics (μmol·kg^{-1}·h^{-1}) from sows fed graded levels of dietary lysine, grouped by similar lysine intake (g/d) levels, during late-gestation

Dietary lysine intake level	1	2	3	4	5	6		
Number of animals[1]	5	5	4	5	3	4	SEM	P-value
Mean lysine intake (g/d)	8.8	11.5	14.2	19.0	21.0	22.9	1.0	<0.01
Flux (μmol·kg^{-1}·h^{-1})	70.3	73.8	77.4	69.5	69.9	68.2	1.7	0.34
Intake (μmol·kg^{-1}·h^{-1})	64.6^{ab}	64.8^{b}	66.0^{b}	63.0^{ab}	60.5^{a}	62.5^{a}	0.7	**0.04**
Breakdown (μmol·kg^{-1}·h^{-1})	5.6	9.0	11.4	6.6	9.3	5.7	1.4	0.71
Oxidation (μmol·kg^{-1}·h^{-1})	32.0	30.5	23.5	24.4	16.3	26.1	1.6	0.10
Synthesis (μmol·kg^{-1}·h^{-1})	38.3	43.3	53.9	45.1	53.6	42.0	2.4	0.14
Phe retention (μmol·kg^{-1}·h^{-1})	32.7	34.3	42.5	38.5	44.3	36.3	1.5	**0.15**
Isotope dose (μmol·kg^{-1}·h^{-1})	11.6^{ab}	11.8^{bc}	11.9^{b}	11.7^{bc}	11.0^{a}	11.4^{ac}	0.1	**0.04**
Body weight (kg)	219.1^{ab}	225.3^{b}	215.9^{b}	231.7^{ab}	233.7^{a}	239.8^{a}	4.3	**0.05**
Mean day of experiment	11.4	11.7	11.1	12.4	15.7	13.3	1.2	0.90

[1]: Data from sows for which phenylalanine kinetics could be calculated; [a,b,c]: values which share a common letter are not different

Table 6.15 – Phenylalanine kinetics ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) from sows fed graded levels of dietary lysine compared between early- and late-gestation by Student's t-test

Variable	EG	LG	SEM	P-value
n	23	29	-	-
Flux ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)	93.8	71.6	2.7	<0.001
Intake ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)	71.9	63.7	0.8	<0.001
Breakdown ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)	22.0	7.9	2.2	0.001
Oxidation ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)	35.2	26.0	1.3	0.001
Synthesis ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)	58.6	45.6	2.7	0.01
Isotope dose ($\mu mol \cdot kg^{-1} \cdot h^{-1}$)	11.7	11.6	0.1	0.46

Table 6.16 – Plasma free amino concentrations (mg/g) of pregnant sows fed graded levels of lysine, grouped by similar lysine intake (g/d) levels, during early-gestation

Level	1	2	3	4	5	6		
Number of animals	7	6	5	5	6	6		
Number of samples	14	12	10	10	12	12	SEM	P-value
Mean lysine intake (g/d)	8.03	9.06	10.16	14.65	16.42	18.36	0.48	<0.01
Glu	2.01	1.94	2.05	1.74	2.00	1.86	0.04	0.41
Ser	1.45	1.43	1.54	1.52	1.50	1.46	0.03	0.55
Gly	8.03	8.67	9.00	10.01	8.75	8.06	0.21	0.51
His	1.32	1.37	1.36	1.23	1.32	1.37	0.02	0.65
Thr	3.34	3.36	3.23	3.40	3.26	3.41	0.06	0.95
Ala	4.49	4.08	4.04	4.29	4.45	4.07	0.10	0.80
Arg	4.45	4.66	4.02	4.38	4.22	4.42	0.11	0.97
Pro	4.33	4.48	4.60	4.26	4.45	4.14	0.08	0.74
Tyr	2.09	2.04	2.20	2.06	2.08	2.11	0.03	0.58
Val	3.65	3.49	3.52	2.91	3.35	3.60	0.07	0.19
Iso	1.84	1.61	1.85	1.64	1.71	1.79	0.04	0.19
Leu	3.50	3.46	3.68	3.51	3.72	3.69	0.05	0.82
Phe	1.65	1.67	1.80	1.61	1.65	1.70	0.02	0.51
Lys	5.62	4.91	5.24	6.26	6.11	7.08	0.24	0.51
SUM	47.8	47.2	46.6	47.7	48.7	48.7	0.61	0.91

Table 6.17 – Plasma free amino concentrations (mg/g) of pregnant sows fed graded levels of lysine, grouped by similar lysine intake (g/d) levels, during late-gestation

Level	1	2	3	4	5	6		
Number of animals	6	5	6	5	5	5		
Number of samples	12	10	12	10	10	10	SEM	P-value
Lysine intake (g/d)	8.85	11.5	14.2	19.0	20.9	22.8	0.6	<0.01
Glu	20.2	28.8	27.7	25.8	32.5	28.3	2.3	0.84
Ser	156	195	180	158	218	194	12	0.78
Gly	47.1	65.7	50.9	47.5	51.5	50.9	3.4	0.77
His	11.9	21.4	12.7	10.7	14.6	14.9	1.5	0.40
Thr	47.7	71.6	49.4	50.4	64.5	51.8	3.6	0.51
Ala	100	125	106	109	149	119	9.3	0.82
Arg	29.7	43.4	39.6	30.7	27.0	38.5	2.6	0.51
Pro	44.2	58.8	43.2	44.5	48.6	46.4	2.8	0.83
Tyr	16.2	21.7	16.4	16.2	19.6	19.1	1.0	0.77
Val	33.4	52.5	40.4	37.3	43.7	43.6	3.0	0.61
Iso	17.4	24.0	23.4	18.9	26.8	24.2	1.8	0.51
Leu	25.7	44.3	33.3	22.6	34.7	36.0	3.3	0.58
Phe	1.80	2.87	1.97	1.73	2.14	2.16	0.15	0.63
Lys	4.75^{a}	10.4^{b}	7.36^{ab}	9.37^{ab}	12.0^{bc}	15.5^{c}	0.71	**0.003**
SUM	557	766	632	582	744	684	43	0.78

^{a,b,c}: values which share a common letter are not different

Table 6.18 – Results from statistical tests for interaction of parity and lysine intake (expressed as g/d, g/BW, and g/$BW^{0.75}$) on phenylalanine oxidation

	Units	Coefficient of the interaction: lysine intake and parity	P-value
Early-gestation	g/d	0.21±0.23	0.41
	g/BW	11.4±53.8	0.84
	g/$BW^{0.75}$	4.2±13.7	0.77
Late-gestation	g/d	0.11±0.2	0.59
	g/BW	5.4±44.4	0.91
	g/$BW^{0.75}$	2.3±11.5	0.85

Figure 6.2 – Non-linear regression break-point analysis of the early-gestation lysine requirement of sows fed graded levels of dietary lysine. Dietary lysine intakes have been grouped into similar levels and the means with x and y standard error bars are shown. The non-linear regression equation was:

oxidation = 64.2 ± 17 – 0.64 ± 0.61*ilys – 2.0 ± 0.8*t, P=0.024

A breakpoint in IAAO was identified at 10.1 g/d dietary lysine intake (ilys) for sows in early-gestation when temperature of the sample air (t, ˚C) was included as a covariate in the non-linear regression model. Expected values were used in the figure. Error bars indicate the standard error associated with the grouping of similar lysine intakes (x-axis) and oxidation of the indicator amino acid (y-axis).

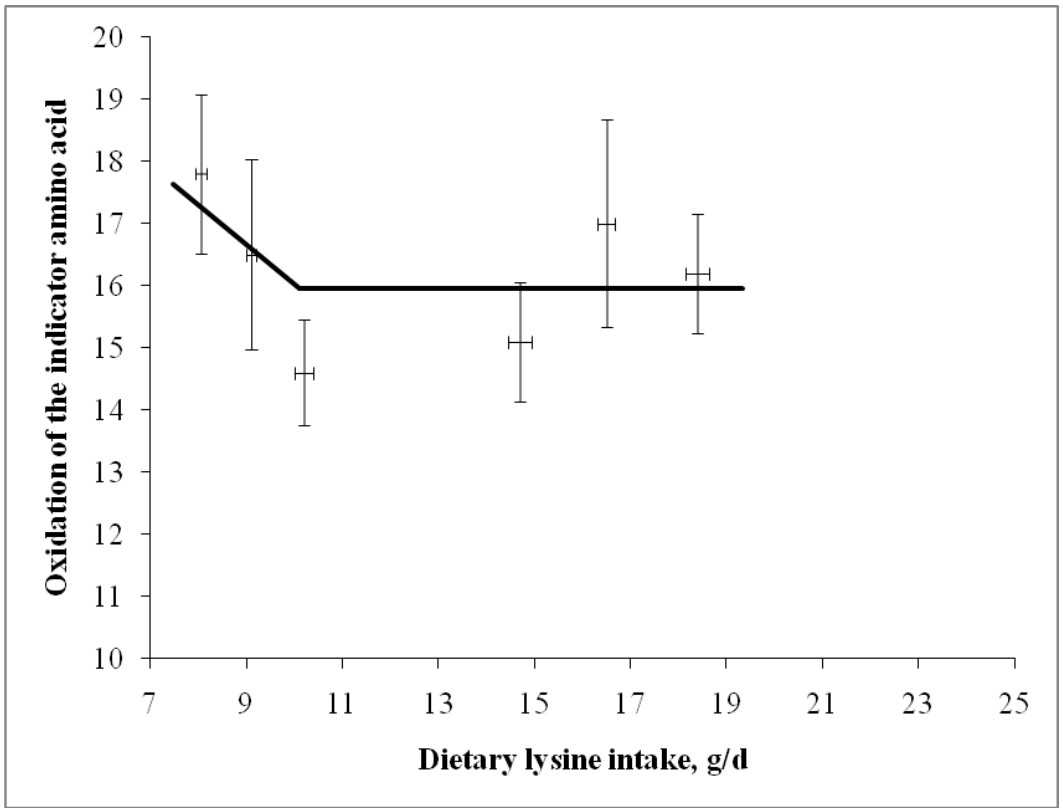


Figure 6.3 – Non-linear regression break-point analysis of the late-gestation lysine requirement of sows fed graded levels of dietary lysine. Dietary lysine intakes have been grouped into similar levels and means with x and y standard error bars are shown. The non-linear regression equation was:

oxidation = 19.9±2.9 - 0.44±0.20*ilys, P=0.033

A breakpoint in IAAO was identified at 16.5 g/d dietary lysine intake (ilys) for sows in late-gestation; no covariates were significant in the non-linear regression model. Error bars indicate the standard error associated with the grouping of similar lysine intakes (x-axis) and oxidation of the indicator amino acid (y-axis).

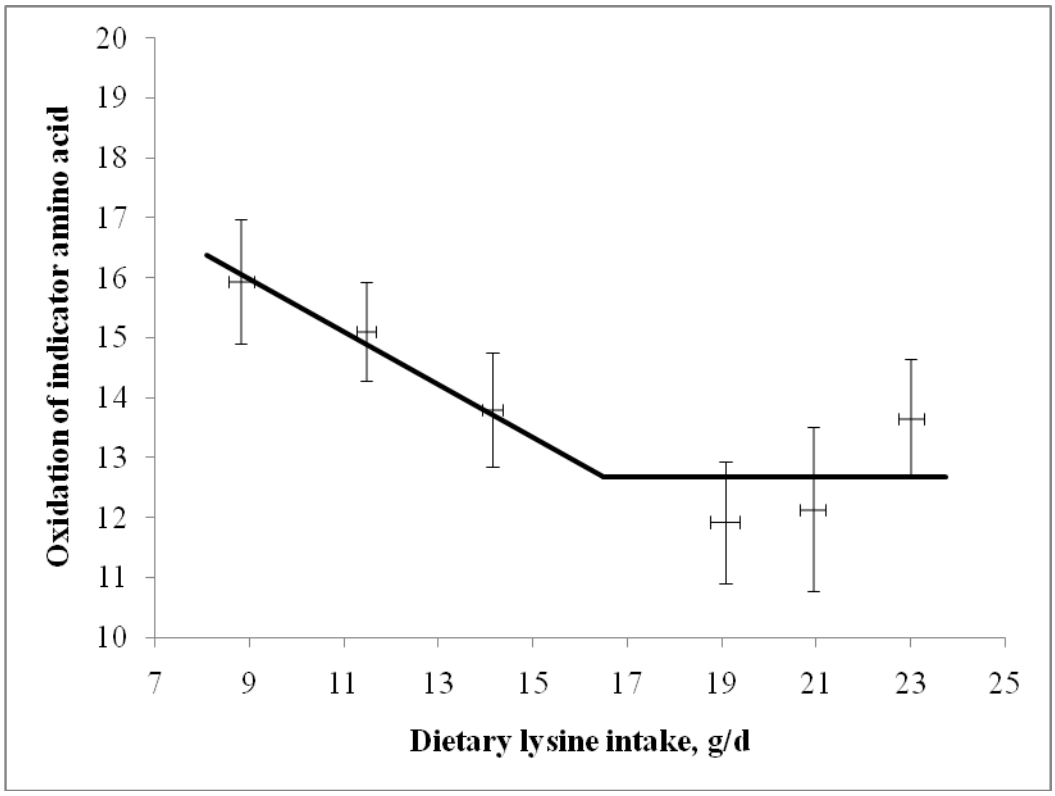


Figure 6.4 – Non-linear regression break-point analysis of the early-gestation lysine requirement of sows fed graded levels of dietary lysine. Dietary lysine intakes have been grouped into similar levels and the means with x and y standard error bars are shown. The non-linear regression equation (P=0.006) was:

oxidation = 61.0 ± 17 – 0.52 ± 0.61*ilys – 2.3 ± 0.8*t + 0.05±0.02*bw

A breakpoint in IAAO was identified at 10.1 g/d dietary lysine intake (ilys) for sows in early-gestation when temperature of the sample air (t, ˚C) and body weight (bw, kg) on the day of oxidation were included as covariates in the non-linear regression model. Expected values were used in the figure. Error bars indicate the standard error associated with the grouping of similar lysine intakes (x-axis) and oxidation of the indicator amino acid (y-axis).

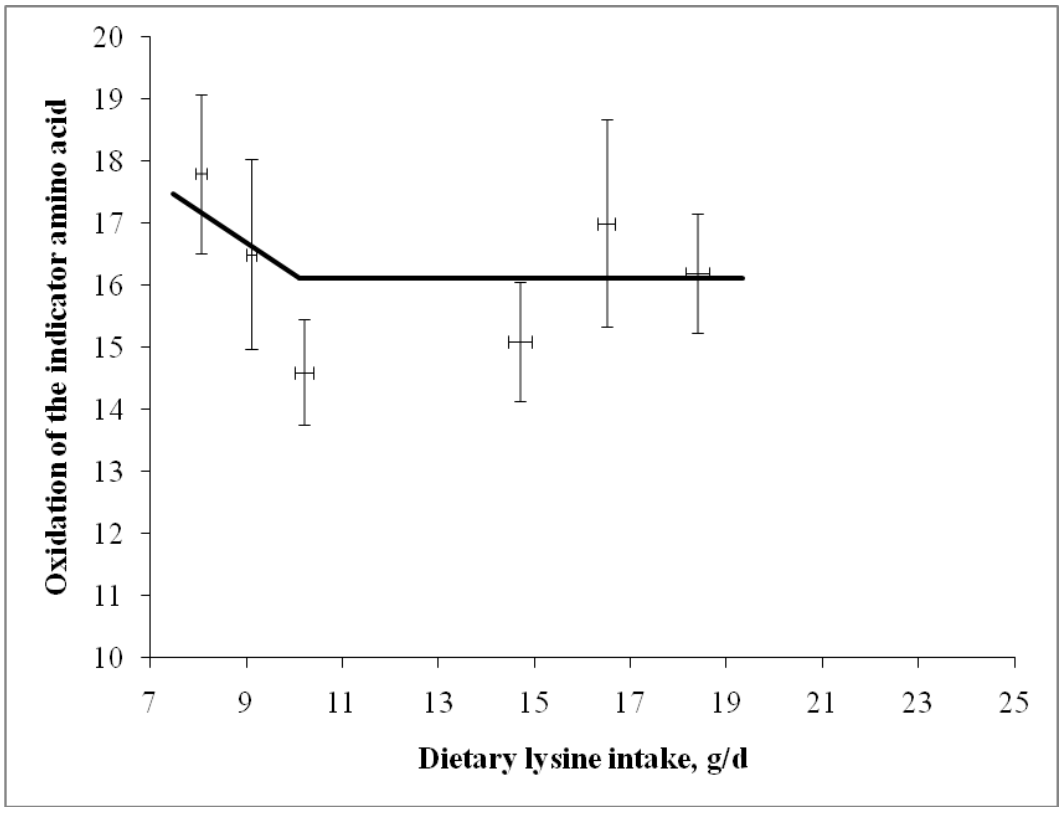


Figure 6.5 – Non-linear regression break-point analysis of the late-gestation lysine requirement of sows fed graded levels of dietary lysine. Dietary lysine intakes have been grouped into similar levels and means with x and y standard error bars are shown. The non-linear regression equation ($P<0.001$) was:

$$\text{oxidation} = 19.9 \pm 2.4 - 0.52 \pm 0.17 * \text{ilys} + 0.10 \pm 0.03 * \Delta\text{bw}$$

A breakpoint in IAAO was identified at 16.5 g/d dietary lysine intake (ilys) for sows in late-gestation when change in body weight (Δbw) from breeding to the end of the early-gestation experimental period was included as a covariate in the non-linear regression model. Error bars indicate the standard error associated with the grouping of similar lysine intakes (x-axis) and oxidation of the indicator amino acid (y-axis).

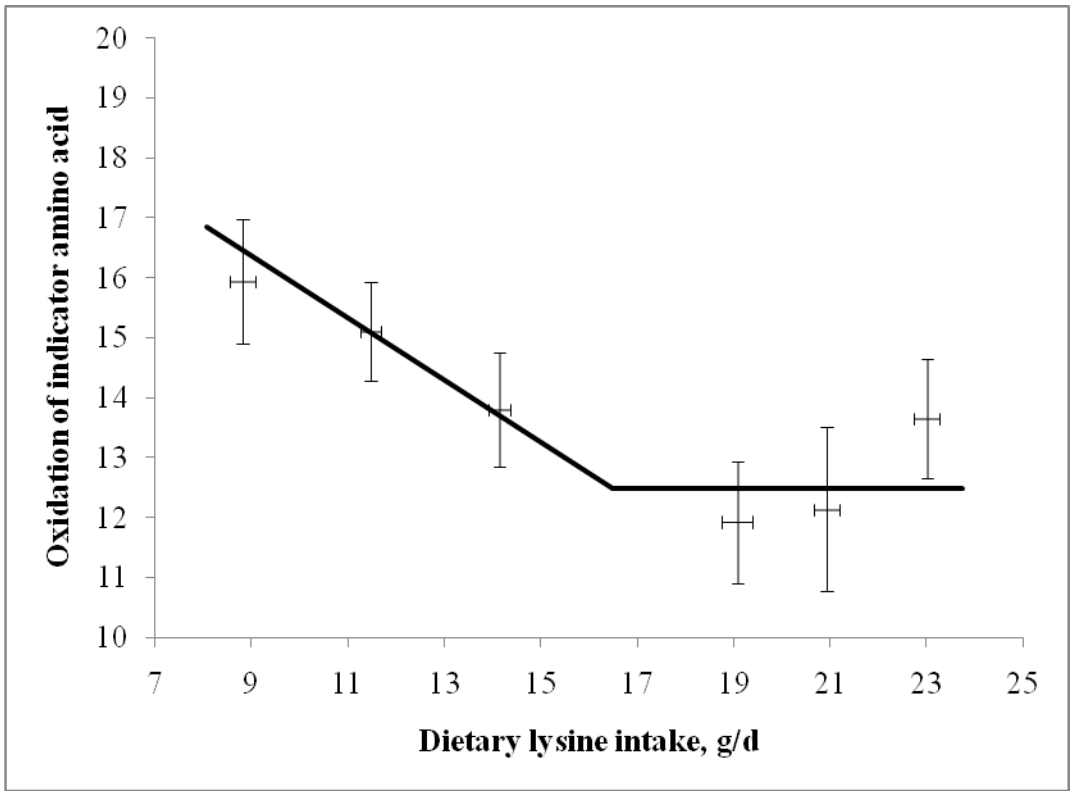
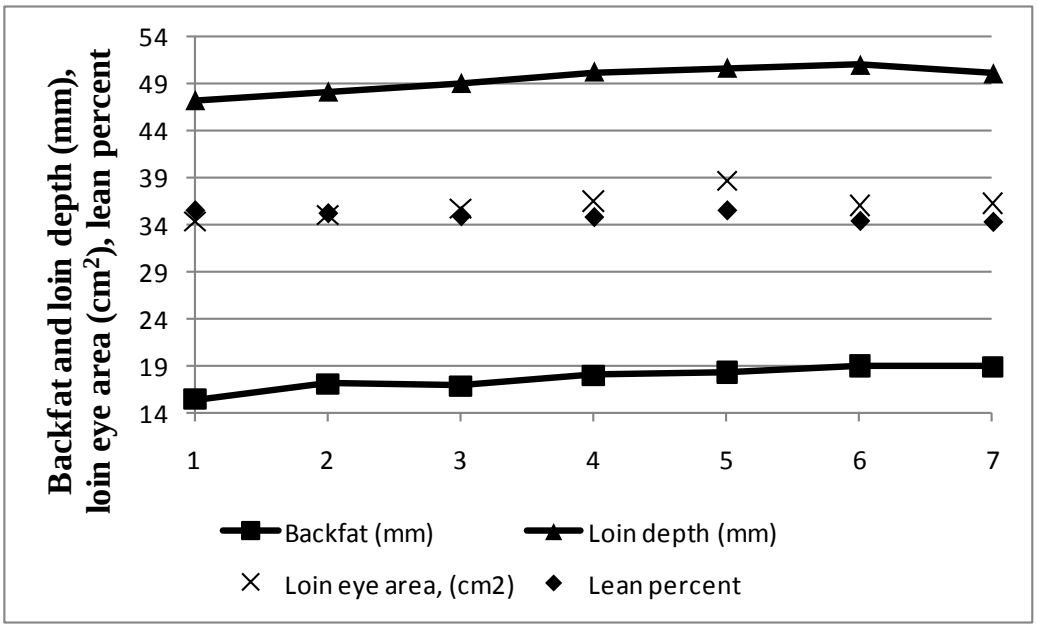


Figure 6.6 – Real-time ultrasound measurements from sows fed graded levels of dietary lysine during early- and late-gestation. Measurements were recorded every two weeks during gestation, between breeding and parturition, beginning on day 14 of gestation.



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7.0 SUMMARY AND FUTURE DIRECTIONS

7.1 Summary

The purpose of this thesis has been to identify nutrient restriction in modern sows. In some instances, as in energy intake restriction of gestating sows during early- and mid-gestation, this is by design. However, the majority of the nutrient insufficiencies identified by this body of work have been due to a lack of knowledge or failure to recognize changes in nutrient requirements due to modern sows or even within a reproductive cycle. The work presented has attempted to deal with the lack of data about energy and protein metabolism in sows at maintenance and during gestation and lactation. Utilizing isotopic techniques and open-circuit calorimetry simultaneously:

1) 24 h studies were performed allowing identification of different metabolic states and the impact of periodicity of feeding and starvation on interactions of energy and protein metabolism were investigated (Chapter 3.0),
2) short-term studies to define amino acid requirements were validated (Chapter 3.0),
3) 24 h studies of the energy and protein metabolism of pregnant and lactating sows indentified periods of nutrient insufficiency and the corresponding interactions of energy and protein metabolism under commercial production management and nutrition (Chapter 4.0), and
4) the dietary lysine requirement for sows at maintenance (Chapter 5.0) and during early- and late-gestation (Chapter 6.0) was determined for a

population of modern, high productivity sows.

7.2 General discussion

The factorial approach to estimating nutrient requirements of sows proposed by NRC (1998) must be based on appropriate measurements of nutrient requirements for each of the factors in order to be correct. Protein and energy nutrient requirements can be predicted by the factorial approach using maintenance as the basis, with additional requirements for growth or reproduction. However, the estimates used in NRC (1998) for the ME_m of sows are based on data that includes growing and early-weaned pigs and was extrapolated to sows. Similarly, data for the dietary lysine requirement for maintenance was extrapolated from data from growing pigs (Fuller et al, 1989). Pettigrew (1993) tried to approximate the dietary lysine requirement of sows by applying a digestibility coefficient of 80%, which is typical for sow diets.

There are important contributions of this thesis to the factorial approach to estimates of nutrient requirements of sows. In the third study (Chapter 4.0) the dietary lysine requirement for maintenance (49 $mg/kg^{0.75}$) was determined to be greater than the current estimate (36 $mg/kg^{0.75}$) utilized in the factorial approach of NRC (1998). In the fourth study, the dietary lysine requirement during gestation was determined to be greater than the current estimates (NRC, 1998) and greater in late- (16.5 g/d) than in early-gestation (10.1 g/d).

The first (Chapter 3.0) and second (Chapter 4.0) studies, report, for the first time, the effect of differential (Chapter 3.0) and current (Chapter 4.0) feeding practices on sow protein and energy metabolism interactions simultaneously

within the same animals.

The main objective of the first study (Chapter 3.0) was to develop simultaneous measurement of heat production and isotope tracer methologies for sows. Indirect calorimetry and isotopic techniques use similar equipment, especially for large animals when only a portion of the total exhausted airflow can be collected for sampling. The remaining airflow must be exhausted at a sufficiently high rate to maintain CO_2 concentration below 1%. Therefore, total airflow must be recorded and multiplied by the measured concentration of the subsample of air. Similarly, quantitative collection of the CO_2 from the subsample must be achieved and the sample must contain sufficient $^{13}CO_2$ enrichment for measurement by isotope ratio mass spectrometry. A tracer dose, twice that of humans, of 1.0 mg/kg/h with a priming dose of 1.0 mg/kg was able to produce $^{13}CO_2$ sufficiently above the natural background $^{13}CO_2$ abundance for measurement (Jones et al, 1985). It was also determined, within the first study, that the mean flux from oral isotope was 3.9 times greater than IV isotope, due to the tracer bypassing the splanchic uptake of the tracer amino acid (Matthews et al, 1999). The correction factor was applied in the calculation of values of whole body protein turnover by IV administration of the tracer, which was necessary for 24 h studies within sows.

The main objective of the second study was to investigate and describe the interactions of protein and energy in sows fed according to currently available recommendations. Sows in early-gestation gained less weight than during mid- and late-gestation. Sows in mid-gestation were anabolic, storing energy as fat for

subsequent mobilization to support late-gestation and lactation. In fact, by day 105 of gestation, sows were catabolic (Samuel, 2008) and the catabolic state continued through lactation, as observed by Mosnier et al (2010). Likely mediated by insulin resistance, nutrients from dietary and maternal body tissue mobilization were directed, except for fatty acids, to the developing fetuses. Fatty acids are not readily transferred across the porcine placenta. Therefore, fatty acids represent a feed energy supplement that can be fed and directed to sow metabolism to increase energy intake (Pettigrew, 1993).

Sows were highly catabolic during lactation and had the same negative energy balance in early- and late-lactation. In fact, additional dietary energy was readily incorporated into increased milk production. Sows in early-lactation were not fed sufficient energy due to step-up feed restriction and sows in late-lactation could not consume enough feed to reduce catabolism of body tissue.

The main objective of the third study was to determine the dietary lysine requirement for maintenance in modern sows. It was determined that the dietary lysine requirement for maintenance was 30 % greater than reported by NRC (1998) at 49 $mg/kg^{0.75}$. The impact of the underestimate of lysine is significant since all other amino acid intakes are determined according to the ideal protein ratio, based on lysine. Based on the ideal amino acid ratio, all other amino acids would need to be increased by 30 percent, the same as lysine (Chapter 5.0). However, work from our lab (Levesque, 2010) and others (Kim et al, 2009) has shown the threonine to lysine ratio to be different in early- versus late-gestation.

In the fourth study the dietary lysine requirement for early- and late-

gestation were found to be different and greater than the average value of 9.7 SID lysine reported by NRC (1998) (Chapter 6.0) at 10.1 and 16.5 g/d, early- and late-gestation respectively. These values agree with data from Srichana (2006) and GfE (2008).

7.2.1 Techniques

Indirect calorimetry and isotope dilution can, and should, be used simultaneously to ascertain interaction of energy and protein metabolism. The system validated and used within these experiments allowed the simultaneous measurement of heat production by indirect calorimetry, quantitative collection of CO_2 production for the measurement of isotopic enrichment for determination of amino acid requirements, and collection of blood samples required for measurement of protein turnover using stable isotopes. Simultaneous measurements are desirable because interactions of protein and energy metabolism can be investigated. Also, the combination of energy and protein balances can provide information about the relative contribution of protein and lipid to weight gain or loss.

7.2.2 Findings

Modern sows have nutrient requirements greater than reported by the most current NRC (1998). Current feeding recommendations (NRC 1998; Aherne et al, 1999) may not be appropriate for optimum productivity of the modern, high producing sow. Modern breeds are a result of constant genetic selection which has resulted in greater growth rates and higher productive and reproductive capacity (Foxcroft, 2008) and thus increased daily nutrient requirements. The increase in

nutrient requirements of modern sows can mostly be associated with increased lean tissue and, therefore, protein turnover (Wenk et al, 1980). Modern sows are leaner than their predecessors which increases their maintenance energy and protein requirements which are directly the result of increased protein turnover. The dietary lysine requirement for maintenance was identified to be at least 46.8 mg/$BW^{0.75}$, (Chapter 5.0) an increase of 30% from NRC (1998). Again, the reason for this is increased lean tissue content and amino acid requirements associated to support greater protein turnover. For example, it has been assumed that the catabolic state of lactation is limited to lactation. However, previous work (Samuel, 2008) clearly shows that sows are catabolic by late-gestation, almost two weeks before lactation.

The dietary lysine requirement for maintenance determined in this thesis provides evidence that the dietary requirement for modern, high productivity sows is greater than suggested by NRC (1998). Current evidence cannot provide an absolute requirement due to the limitations of the data. Clearly, the dietary requirement is greater than 1.09 g of lysine/kg of diet (41.4 mg/$kg^{0.75}$) and lower than 1.38 g of lysine/kg of diet (52.2 mg/$kg^{0.75}$). Statistical analysis using the non-linear mixed procedure (Robbins et al, 2006) determined that a breakpoint in oxidation of the indicator amino acid occurred at 49±11 mg/$kg^{0.75}$(equivalent to 1.29 g of lysine/kg of diet) thus increasing the confidence interval around the requirement estimate. Further experimentation is required to more fully define the dietary lysine requirement for maintenance.

7.3 Future directions

Further indispensable amino acid requirements need to be determined, in order of limitation, to allow directed sow nutrition during gestation. This work, and recent work from our lab (Levesque, 2010) and others (Srichana, 2006; Kim et al, 2009), have identified that lysine and threonine requirements are different between early- and late-gestation and the ratios of amino acids to lysine may change. Work is ongoing to determine the next limiting amino acid. Direct determination of the indispensible amino acid requirements is necessary to calculate appropriate ratios of amino acids to lysine at different stages of gestation. Further characterization of the indispensible amino acid requirements is necessary to determine why the ideal protein ratio does not appear to be consistently valid.

Insufficient energy intake during late-gestation induces sows to become catabolic; the process appears to be mediated by insulin resistance. In the early-lactation period, sows are feed restricted under current step-up feeding recommendations and are, therefore, highly catabolic. Sows begin to approach *ad libitum* feeding by mid-lactation but their reliance on body tissue mobilization for nutrients is not reduced. Instead, due to the continuing influence of the mechanisms of insulin resistance, additional dietary nutrients are directed towards milk production through late-lactation. Modern sows are capable of rebreeding even when significant protein loss has occurred, but the negative effects on longevity (Mahan, 1977; Mahan, 1981; Aherne and Kirkwood, 1985; Dourmad et al, 1994a,b; Gaughan et al, 1995; Young and Aherne, 2005; Anil et al, 2006;

Tvrdoň and Marková, 2007) and overall productivity are expensive (Foxcroft, 2008) and largely uncharacterized. Therefore, it is necessary to investigate if correctly feeding sows energy and protein during lactation can have positive implications for longevity and productivity.

More data is required about the differences in nutrient availability from feedstuffs for sows. It is the combination of a dietary requirement value and a value for nutrient availability that determine the diet formulation. Another limitation to the nutrient requirements of sows is the lack of true ileal digestibility data. For example, Stein et al (2001) reported the true ileal digestibility for lysine from corn as 84.9 % for sows compared to the true ileal digestibility of lysine from corn for swine of 78.8% (NRC, 1998). The difference (7%) could have a significant impact on targeted diet formulation. Recent work in our lab (Levesque, 2010) determined the bioavailability of threonine from limited feedstuffs and compared the bioavailability between growing animals and sows. However, extensive research is required to determine bioavailability of all the amino acids in all the major feedstuffs for swine. Specifically, as it relates to sow nutrition, data about the bioavailability of the amino acids from feedstuffs in sows is necessary. Work should also include determination of the energy availability from different feedstuffs by sows to allow formulation of diets using the net energy system. From bioavailability data for energy and amino acids from feedstuffs, targeted diet formulations can be prepared which should increase productivity and reduce costs by reducing excess nutrient inclusion.

Separation of the maternal and fetal metabolism to determine the relative

impacts of each on the overall nutrient requirements is a goal of future work. Currently, we cannot separate maternal and intrauterine tissue partial efficiencies for energy or protein utilization, which may provide information to increase the efficiency of pork production by allowing targeted delivery of nutrition. Future development of the long-term, in-dwelling catheterization of sows may provide for separation of fetal and maternal metabolism through catheterization of uterine vessels separate from the systemic circulation of the sow. Application of tracer balance techniques, including measures of arterio-venous difference across the gravid uterus, will provide information about the uptake of nutrients by the uterus. It may also be possible, using isotopes to measure energy expenditure, that whole-body and gravid uterus energy expenditure can be measured separately.

7.4 Conclusions

Although this research has increased our knowledge of sow nutrition, there is still a great deal to be determined. As sow production units continue to increase in size, there will be greater financial incentives to improve sow nutrition, reduce the cost of feeding sows, and increase the longevity and long-term health of sows. In anticipation of these trends, emphasis on sow nutrition should be increased.

7.4 Literature cited

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8.0 APPENDICES

8.1 Appendix A – Nitrogen injection details

After calibrating the sensors, stable background readings were recorded for each of the calibration gases and room air. Nitrogen was started pumping into respiration chamber inlets. The flow of nitrogen was set to reduce the measured O_2 concentration by approximately 0.5%. Once new stable readings were recorded for at least five minutes, the gas meter readings for the air flow out of the chamber and the nitrogen flow into the chamber were recorded. The nitrogen was allowed to flow for approximately 30 min before the gas meter readings and total flow time were recorded. The process was then repeated for the other chamber. The percent recovery was calculated by comparing the measured reduction with the predicted, based on the total volume of N_2 injected, reduction in measured O_2 concentration. Stable background readings were recorded for each of the calibration gases and room air.

8.2 Appendix B – CO_2 recovery details

During calibration of the sensors, stir plates were placed into each of the respiration chambers with beakers containing ~3500 mL of water and a stir bar. Stable background readings were recorded for each of the calibration gases and room air. An extension set was linked to a 60 ml syringe of HCl held in a syringe pump. 168.0 g of $NaCO_3$ was dissolved in the water with stirring. The boxes were closed and the syringe pumps were set to deliver 525 mL/hour. Once five minutes of stable room air readings were recorded, the syringe pumps were turned on and the gas meter readings were recorded for each chamber. Once 3 times the molar equivalent of HCl had been added and no more bubbles were being evolved from the beaker, the syringe pumps were stopped and the CO_2 analyzer concentrations were allowed to return to room air values recorded earlier. Final air flow meter readings were recorded for each chamber. Stable background readings were recorded for each of the calibration gases and room air.

8.3 Appendix C – Calculation of correction factor for flux from IV isotope

The experiments described in Chapters 3.0 and 4.0 of this thesis were performed sequentially and utilized a number of the same animals in the non-pregnant, pregnant, and then lactating states. Originally, the experiments were designed to use oral delivery of isotope. However, after the first two sows did not consume their large meal on time during the experimental period, the delivery of oral isotope was abandoned in favour of IV isotope which allowed for more consistent delivery of the isotope. During subsequent analysis of the data, strange results (i.e. negative breakdown) emerged, especially during lactation. Therefore, at the recommendation of the supervisory committee, a correction factor was calculated which would increase flux values calculated from IV administration of isotope for factors including bypassing splanchnic metabolism (see Chapter 3.0 for discussion). Relevant data and calculation of the correction factor is shown.

Table 8.1 –Mean plasma α-KIC enrichment (MPE) of non-pregnant sows fed feeding levels 1.0 and 2.0 by individual pig

Sow ID	Feeding level	Number of observations	Mean plasma α-KIC enrichment (MPE)	SEM
2102	1	14	9.08	0.25
	2	15	11.80	0.50
2201	1	13	17.06	0.68
	2	14	16.88	0.73
2205	1	16	13.35	0.56
	2	14	25.33	1.28
2301	1	24	4.33	0.39
	2	16	3.47	0.52
3703	1	22	3.95	0.38
	2	17	3.18	0.34

Table 8.2 - Mean plasma α-KIC enrichment (MPE) of non-pregnant sows fed feeding levels 1.0 and 2.0 by feeding level

Feeding level	1.0	2.0		
n	5	5	SEM	P-value
Mean plasma α-KIC enrichment (MPE)	9.55	12.13	2.35	0.3564

Table 8.3 - Mean plasma α-KIC enrichment (MPE) of non-pregnant sows by route of isotope administration, oral versus IV

Route of isotope administration	Oral	IV		
n	4	6	SEM	P-value
Mean plasma α-KIC enrichment (MPE)	3.73	15.6	2.4	0.0023

Therefore, the correction factor was calculated as:

$$\text{Factor} = \frac{\text{mean plasma α-KIC enrichment IV isotope}}{\text{mean plasma α-KIC enrichment oral isotope}}$$

$$\text{Factor} = \frac{15.6}{3.73} = 4.2$$

Therefore, the correction factor was calculated to be 4.2 from the mean plasma mean plasma α-KIC enrichment over 24 h.

The correction factor was also calculated comparing the mean plasma α-KIC enrichments during the nibbling states from oral and IV isotope delivery because this identified physiological state should represent an isotopic steady state for both oral and IV isotope application. Therefore, the correction factor was calculated as:

$$\text{Factor} = \frac{\text{mean plasma } \alpha\text{-KIC enrichment IV isotope during nibbling}}{\text{mean plasma } \alpha\text{-KIC enrichment oral isotope during nibbling}}$$

$$\text{Factor} = \frac{15.3}{4.33} = 3.5$$

Because the values do not appear to be different, the arithmetic mean of the values was used as the correction factor. Therefore, the factor applied to correct flux values calculated from IV isotope delivery was 3.9 (Chapter 3.0 & 4.0).