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Defining Amino Acid Requirements of Pregnant Sows: Challenges and Opportunities

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DEFINING AMINO ACID REQUIREMENTS OF PREGNANT SOWS: CHALLENGES AND OPPORTUNITIES

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

AA	amino acid(s)
ADG	average daily gain
AlF_{gwth}	allantoic fluid growth
AlF_{Pd}	allantoic fluid protein deposition
AmF_{gwth}	amniotic fluid growth
AmF_{Pd}	amniotic fluid protein deposition
BW	body weight
CP	crude protein
$Early\text{-}Mid_{AA\ profile}$	amino acid profile of the protein deposited during the first 80 days of gestation
Eq.	equation(s)
Fig.	figure(s)
F_{gwth}	fetus growth
F_{Pd}	fetus protein deposition
His	histidine
IAAO	indicator amino acid oxidation
Ile	isoleucine
$Late_{AA\ profile}$	amino acid profile of the protein deposited after day 90 of gestation
Leu	leucine
Lys	lysine
MB_{gwth}	maternal body growth

MB_{Pd}	maternal body protein deposition
MG_{gwth}	mammary gland growth
MG_{Pd}	mammary gland protein deposition
Met	methionine
NRC	National Research Council
Pd	protein deposition
Phe	phenylalanine
Pl_{gwth}	placenta growth
Pl_{Pd}	placenta protein deposition
Pm	protein for metabolism and tissues other than lean tissues
$Pm_{coeff.}$	percentage whole body metabolic protein required for metabolism
RMSE	root mean squared error
Tau	taurine
Thr	threonine
Trp	tryptophan
U_{gwth}	uterus growth
U_{Pd}	uterus protein deposition
WB	whole body
WB_{gwth}	whole body growth
WB_{mp}	whole body metabolic protein
WB_{Pd}	whole body protein deposition

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ABSTRACT

DEFINING AMINO ACID REQUIREMENTS OF PREGNANT SOWS:

CHALLENGES AND OPPORTUNITIES

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The efficient use of protein in animal production is dependent on the protein supply and its constituent amino acids (AA) in relation to the animal's needs. Excess AA are deaminated, and the resulting nitrogen (N) is excreted, whereas suboptimal AA intake reduces animal performance. Both increased nutrient excretion and decreased animal performance reduce the overall efficiency of the production unit. In sows, AA requirements should be adequate for optimizing reproductive performance, as measured, for example, by the number of pigs produced per sow per year while limiting N excretion. There is a desire to feed pregnant sows AA levels that meet both physiological needs and environmental considerations. Current methods for estimating AA requirements, however, are geared toward reducing N excretion, rather than optimizing reproductive performance. Minimizing N excretion increases N utilization efficiency but decreases overall meat production efficiency if these dietary levels do not maximize pregnant sow reproductive performance. Thus, the overall objective of this dissertation is to increase our understanding on the dynamics of N excretion and retention across gestation and to determine dietary AA levels that optimize reproductive performance. To accomplish these objectives a series of in silico and in vivo studies were performed and are outlined in four research chapters (Chapter 2, 3, 4, and 5).

In Chapter 2, a mechanistic model was developed to quantify and characterize the N and AA deposition in the different tissue pools that make up the pregnant sow as well as their interaction in terms of nutrient competition: placenta, allantoic fluid, amniotic fluid, fetus, uterus, mammary gland, and maternal body were considered. Growth curves for each tissue were developed, as well as curves describing retention of the 10 essential AA and N for each tissue. The growth and nutrient retention curves were developed as a function of the model's inputs: parity, body weight (BW) at breeding, litter size, average piglet birth weight, and number of available teats. All functions were combined in an algorithm that dynamically distributed the dietary N and AA to the different tissues based on the inputs of the model. The model characterized a trend in maternal N retention that could not be previously explained, although identified as time-dependent protein deposition (Pd), by the NRC (2012) gestating sow model. The trend in N retention described by the time-dependent protein pool appears to be due to a drop in daily N retention, particularly during early gestation, rather than increased N retention, as previously thought, implying that current energy and/or AA requirements for early pregnancy should be reviewed.

In Chapter 3, a dose-response curve defined as the linear-logistic model was developed based on experimental data from pregnant gilts at d 50 of gestation to describe the response of N retention to graded levels of the AA lysine (Lys). The linear-logistic model describes two inflection points defined as: 1) N retention max (NRmax) and 2) N retention minimum (NRmin). Based on the hypothesis that Lys intakes corresponding to NRmax maximized maternal body growth and Lys intakes corresponding to NRmin maximized the reproductive performance of pregnant sows, a meta-analysis was

performed. The standardized ileal digestible (SID) Lys requirements for sows throughout gestation were calculated using the linear-logistic model and observations from Chapter 2. The meta-analysis confirmed that SID Lys levels corresponding to NRmax and NRmin maximized maternal growth and reproductive performance, respectively. Because current AA requirements are more likely to represent NRmax, current AA requirements appear to optimize maternal lean tissue deposition rather than conceptus development. Optimal reproductive performance appears to occur at AA levels above current recommendations.

In Chapter 4, a study was performed to test model outputs from Chapters 2 and 3 and investigate the effects of SID methionine (Met) intakes greater than current requirements on the metabolic status of gilts at breeding age. The metabolic status of the animals was evaluated using plasma AA concentration, with a particular emphasis on plasma taurine (Tau) which is a non-proteogenic AA that is biosynthesized from Met and regulates a variety of functions in the body. In addition, the dynamics of N retention at potential SID Met intakes that maximize Tau biosynthesis were investigated. The results of this study showed that SID Met intakes corresponding to 230% of current requirements maximized Tau biosynthesis and, thus, potentially Tau-related metabolic functions, and resulted in a decrease in whole-body N retention. Optimal metabolic status appears to occur at AA levels above current recommendations.

In Chapter 5, a pilot study was performed to study the effects of SID Met intake levels corresponding to 230% of the requirement for gilts during the first 90 days of gestation on sow BW gain. A total of 39 sows were provided either a control or a high Met diet. Sows in the control group gained in average 1.67 kg more than those in the high Met group (P=0.070). The reduced BW gain in the high Met group may be explained by

a decrease in the priority for maternal body deposition and an increase in reproductive function and metabolic status, as predicted in Chapter 3 and 4. However, due to a disease outbreak in the research herd, the effects of dietary SID Met on litter size could not be determined and the previous claim could not be fully supported.

In summary, the developed mechanistic model enhanced understanding of the dynamics of N retention across gestation. Observations made in all four research chapters suggest that current AA requirements are underestimated when metabolic status and reproductive function are considered. In Chapter 3, 4 and 5 it is suggested that at optimal AA intake for metabolic and reproductive status there is a decrease in the efficiency of N utilization (i.e. increased N excretion). As a result, it is advised that AA requirements based on maximal N efficiency may be limiting sow performance. The linear-logistic model is proposed as an analytic tool for the estimation of dietary AA requirements that maximize reproductive performance and metabolic status. The SID Lys requirements estimated using the linear-logistic model and the SID Met requirements empirically estimated using plasma Tau measurements have the potential to improve pregnant sow reproductive performance.

CHAPTER 1

AMINO ACID REQUIREMENTS IN PREGNANT SOWS: GAPS IN KNOWLEDGE AND OPPORTUNITIES FOR RESEARCH

INTRODUCTION

Feeding pigs diets with an amino acid (AA) balance that corresponds to the animal's requirements is critical for the efficiency of the swine industry. In sows, AA requirements should be adequate for optimizing reproductive efficiency as measured, for example, by the number of pigs produced per sow per year. However, rather than reproductive performance, AA requirements during gestation have been based on methods such as protein synthesis measurements [often nitrogen (N) balance], ideal AA patterns for protein deposition (Pd), and plasma AA concentrations (NRC, 2012). While these methods are thought to be optimal for estimating AA doses for optimal lean tissue deposition and, thus, adequate for estimating AA requirements in growing pigs, they may be insufficient for estimating AA requirements that maximize reproductive performance.

The current literature review analyzes the adequacy and limitations of the most commonly used methods for the estimation of AA requirements in pregnant sows. The assumptions used in each method are first examined and then the biological accuracy of the estimated requirements with each method is evaluated.

LITERATURE REVIEW

Conceptual models

Understanding and quantifying the use of AA by an animal is a difficult task. Living organisms are complex systems with many levels of organization (e.g., organ,

tissue, cell), each of which depend on AAs for performing metabolic functions such as protein synthesis, cell signaling, RNA and DNA synthesis, and protein degradation (Wu, 2010). All these systems are linked and interdependent, but they are also controlled by the brain, which is influenced by external factors. Attempting to understand and predict whole body AA use by studying all possible connections and relationships between the components of an animal and its surroundings would be exhausting and error-prone, especially because many interactions remain unknown. For this purpose, conceptual models are preferred.

A conceptual model is a representation of a system. The intention of a conceptual model is to convey the fundamental principles and functionality of a system (Robinson, 2008). In the field of nutrition, conceptual models are used to investigate physiological responses to nutrient supply. The ideal protein concept (Mitchell, 1964) and the concept of partitioning nutrients based on a hierarchy of need (Hammond, 1944; Hammond, 1952) are examples of conceptual models widely used in animal nutrition because they considerably simplify practical animal feeding (Lewis and Southern, 2000; van Milgen and Dourmad, 2015).

A conceptual model, while intended to simplify a complex system, must also abstract an appropriate simplification of reality (Pidd, 1997). However, the simplest model is frequently preferred (i.e. the parsimony principle), even though the simplest model may not be the most biologically accurate model, particularly when the goal is to understand complex biological systems (Coelho et al., 2019). The concept of the hierarchy of nutrient use, for example, was developed by Hammond (1944) using deductive reasoning and no measures of its biological accuracy were reported. Indeed,

recent research indicates that, while the simplified reality described by the Hammond's model is sufficient for estimating AA requirements in growing pigs, it may not adequately represent AA use by pregnant animals.

Concept of the hierarchy of nutrient use

The concept of nutrient partitioning was developed by Hammond (1944) to describe the use of nutrients among tissues based on their priority of need, with nutrients being used first for functions and tissues essential for the individual's survival (maintenance), then for those required for the production and survival of the animal's offspring (reproduction), animal growth (bone and skeletal muscle deposition), and finally adipose tissue deposition. Hammond prioritized nutrient utilization so that tissues with the highest metabolic rate receive first or greater priority of nutrient use than less metabolically active tissues (Hammond, 1944; Hammond, 1952). According to Hammond's thesis, less metabolically active tissues had the least impact on coordinating body functions and animal survival, so the body assigned them to receive fewer and fewer nutrients as nutrients became limited (Moberg and Mench, 2000). However, Hammond's thesis is contradicted by the concept of functional AA. The concept of functional AA was theorized by Wu (2010) and considers the fact that AA, besides serving as building blocks of proteins, "regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms" (Wu, 2013). The concept of functional AA suggest that AA doses required for optimizing animal physiologic functions important for reproduction are greater than those for maximal growth (Chalvon-Demersay, 2021). The sections that follow examine the

empirical evidence to determine whether Hammond's or Wu's perspective is better suited to describing AA use by pregnant animals.

Skeletal tissue deposition vs maintenance related functions

The conceptual model of the hierarchy of nutrient use assumes that maintenance-related functions, which are linked to an individual's survival and include the functioning of tissues such as the gastrointestinal tract and other organs, are prioritized over skeletal tissue deposition. Nevertheless, multiple authors have demonstrated that protein synthesis requirements are a key regulator in the metabolism of essential AA, with bioavailable AA levels above those required for maximum protein synthesis being catabolized or excreted (Waterlow, 1984, 1990; Young and Marchini, 1990; Campbell et al., 2001; Shimomura et al., 2001; Cole et al., 2012; Robinson et al., 2016).

Young and Marchini (1990) and Robinson et al. (2016) found that at dietary Met intakes lower than those required for maximal protein synthesis, the rate at which Met enters transmethylation reactions is reduced compared to the rate at which Met enters protein synthesis pathways. Transmethylation reactions are important for the development and function of the gastrointestinal system (Riedijk et al., 2007; Chen et al., 2014), as well as the biosynthesis of metabolites such as Tau, which regulates a variety of functions in the reproductive system, central nervous system, renal system, among other important physiological functions (Norberg et al., 1998; Ripps and Shen, 2012; Tang et al., 2018; Mu et al., 2019; Wen et al., 2019). Similarly, when protein intake is lower than what is required for maximum protein synthesis, catabolism of branched chain AA is reduced, and protein synthesis prioritized (Young and Marchini, 1990; Shimomura et al., 2001). Nevertheless, the catabolism of branched chain AA is important for the proper

functioning and development of the central nervous system (Cole et al., 2012), placenta (Holm et al., 2017), mammary gland (Lei et al., 2012), and small intestine (Chen et al., 2009; Sun et al., 2015). According to the preceding observations, protein synthesis appears to be prioritized, and rates of AA catabolism increase after the AA requirements for protein synthesis are met. It is important to consider that AA catabolism differs from AA excretion in that AA catabolism is required to perform metabolic functions essential for the body's proper functioning (Young and Marchini, 1990). Thus, adequate rates of AA catabolism required for optimizing metabolic status seems to occur at levels above those required for maximum protein synthesis. Bhargava et al. (1970) published intriguing data that illustrated this phenomenon.

Bhargava et al. (1970) reported that when chicks challenged with the Newcastle virus were fed diets with increasing levels of valine (Val), both growth and antibody titers improved; however antibody titers increased further after the animal had reached a maximum growth (Fig. 1-1). These results suggested that there are differences in Val needs to achieve the maximal response of the given variable. Specifically, maximum lean tissue deposition was achieved at lower Val intakes compared to the Val that was required for maximum immune response. This seems to indicate that Val intakes that maximize growth are insufficient for maximizing immune response. As a result, as Val intake increased, chicks achieved optimal growth first (higher priority), and then optimal metabolic functions such as immune response (lower priority).

Similarly, Wu (2014) reported that higher dietary arginine (Arg) levels than those recommended by the NRC (2012) for optimal protein synthesis were suggested to improve pig growth performance, milk production, and embryonic/fetal survival. In

addition, Pampuch et al. (2006) found that while tryptophan (Trp) dietary levels for optimal sow milk protein synthesis "might lead to apparently optimal performance of the sows, metabolic criteria indicate physiological stress and the inadequacy of supply, which probably lead to a long-term performance impairment". According to the findings of Pampuch et al. (2006), dietary Trp levels for optimal milk protein synthesis are insufficient for the lactating sow's optimal metabolic status, as measured as blood serotonin levels. According to the previously described empirical data, lean tissue deposition appears to have a higher priority for nutrient use than metabolic related functions and reproduction, which seems to be advantageous from an ecological standpoint.

The main defensive strategies against predation for wild boar are hiding and running away (Sodeikat and Pohlmeyer, 2003). Increased lean body mass plays a fundamental role in escaping predation as it is positively correlated to running speed (Christiansen, 2002; Hirt et al., 2017). Increased protein synthesis is also important for reproduction. Experiments conducted using domestic pigs and wild boars have shown that heavier animals occupy the highest social ranks in the herd (Puppe and Tuchscherer, 1999; Újváry et al., 2012) and more dominant wild sows have been found to have higher chance of breeding (Fernández-Llario and Mateos-Quesada, 2005; Bergqvist et al., 2018). Thus, increased lean tissue deposition seems to increase the chances to escape predation and reproduction.

Moreover, hyperreactive immune response may be associated to increased predation risk. For instance, coughing which is a protective reflex regulated by the neural and immune system (Song and Chang, 2015), may be put swine species in danger while hiding.

According to Van den Bergh et al. (2012) the probability of survival may increase when an organism is able to suppress cough while hiding away from a predator. In this sense, hyperreactive immune systems may reduce the capacity of a wild boar to suppress cough while hiding and, therefore, increasing predation risk. Also, because a trade-off between growth and immune function exists (van der Most et al., 2011; Rauw, 2012), hyperreactive immune systems may compete with lean tissue deposition resulting in animals with less lean body mass and therefore reduced chances of escaping predation. Consequently, it seems advantageous to maximize immune response after the animal has reached its maximum scape potential.

It is also possible that animals maximize metabolic functions such as the immune response when food availability is high because the predation risk decreases. In a rich habitat with easy access to food, wild boar limit their movement to a smaller area, reducing the risk of predation, whereas in a poor nutritional environment, wild boar move more in search of food and water, increasing the risk of predation (Fernández-Llario, 2004; Morelle et al., 2015). Thus, it is possible that because at low nutrient availability the animal increases mobility (and the associated predation risk) lean body mass is prioritized over metabolic functions such as immune response. Overall, prioritizing lean tissue deposition at low AA intakes may increase survivability in wild boar, a condition that, while redundant, may persist in the domesticated pig.

The previous observations suggest that lean tissue deposition has a greater hierarchy of AA utilization than metabolic functions and that these metabolic functions are optimized at AA intakes greater than those for maximum lean tissue deposition. In addition, a large body of literature shows that essential AA supplementation above levels

for optimal growth and protein synthesis improved metabolic functions (Andersen et al., 2016; Wu et al., 2017; Le Floc'h et al., 2018; Ma et al., 2019; Tang et al., 2019; Columbus, 2020; Chalvon-Demersay et al., 2021; Cynober and Chalvon-Demersay, 2021; Liu et al., 2021; Rodrigues et al., 2021). These observations support Wu and other authors' conclusion that dietary AA levels above the level required for optimal protein synthesis benefit overall animal health and metabolic functions such as embryonic/fetal survival and gut function (Wu, 2014; Robinson et al., 2016; Chalvon-Demersay, 2021; Chalvon-Demersay et al., 2021). On the other hand, there is little evidence to support Hammond's proposed hierarchy of use conceptual model. Given that the methods used in estimating AA requirements in pregnant sows are directly dependent on Hammond model assumptions, it would be significant if the Hammond model incorrectly described the physiological responses of animals to AA availability, which will be discussed in the following section.

Empirical estimation of the AA requirements in the context of the Hammonds model

The NRC (2016) recommends using the following criteria for the design of studies aimed at determining AA requirements:

1. The use of a test AA-deficient basal diet
2. Other than the test AA, the basal diet contains adequate levels of nutrients.
3. The application of at least four graded levels of the AA in question.
4. Appropriate experiment duration in relation to the response criterion
5. An adequate statistical model to describe the animal's response to the AA supply and to calculate the AA requirement.

In pregnant sows, the response criterion for AA requirement studies has typically been measures of N retention or protein synthesis (NRC, 2012). The simplest model for such a response is linear-plateau broken-line regression (Oldham, 1987). this model assume that an increase in AA is converted to a product with a limiting efficiency (linear phase) and, beyond a certain point, there is no further response as the AA supply exceeds the ability of the animal to use additional AA (plateau phase). Under the linear-plateau broken-line approach, the breakpoint is considered as the AA requirement. In addition to the linear-plateau model, the quadratic-plateau broken-line regression model has been used to estimate AA requirements (van Milgen and Dourmad, 2015). Although the use of these models results in different AA requirements, the AA requirement has been defined in both cases as the minimum AA intake that maximizes N retention (van Milgen and Dourmad, 2015).

Measures of N retention, according to the hierarchy of nutrient use conceptual model proposed by Hammond, are sufficient for estimating AA requirements in pregnant sows because it is assumed that at optimal AA doses for maximum lean tissue deposition the AA required for higher priority functions such as reproduction have already been met. Nonetheless, different studies indicate that measures of N balance do not correlate with a physiological state that is optimal for metabolic status and reproduction. For example, experiments in humans have shown that adult men consuming an Arg-free diet can maintain a stable N balance for 9 days, but both the number and vitality of their sperm cells are decreased by 90% (Wu, 2010). Similarly, Young and Marchini (1990) found that the human body is capable to maintain leucine (Leu) balance and AA homeostasis during a 3-week experimental period via a reduction in important physiological functions such

as protein turnover. Based on the body's ability to maintain AA balance that results simultaneously in significant losses in some important metabolic functions (Young and Marchini, 1990), Marchini et al. (1993) concluded that “the findings from the N-balance data, as anticipated, are equivocal and further emphasize the limitations of the technique for purposes of establishing the AA requirements of healthy adult subjects”.

This physiological adaptation that results in maintaining the N balance at the cost of reducing important metabolic functions have been defined as accommodation (Waterlow, 1968; Young and Marchini, 1990; Morse et al., 2001; Hays et al., 2009). An accommodation state provides survival benefits. For example, during anorexia, N excretion and protein requirements are reduced (Iizaka et al., 2012). Anorexia which is linked to immune response in pathogen-challenged pigs (Kyriazakis and Doeschl-Wilson, 2009) may be an accommodation process that reduces protein requirements, possibly by reducing metabolic functions other than immune response. As stated by Young and Marchini (1990), although an accommodation state favors survival of the individual, it simultaneously results in significant losses in some important functions that may compromise long-term health. Therefore, N-balance estimates may be insufficient for estimating AA doses that maximize physiological functions, particularly because N-balance may remain relatively constant during the plateau phase in the broken-line approach even at suboptimal AA intakes. Thus, it is presumed that AA doses that maximize whole-body metabolic status lies at intake levels above the breakpoint for N retention. This also implies that empirically estimated AA requirements using the N balance technique are underestimated when metabolic criteria are considered; hence the need to review the empirical methods for estimating AA requirements. Besides empirical

methods, essential AAs requirements have also been calculated using other methods such as the application of the ideal protein concept.

Ideal protein concept

A fundamental conceptual model used in swine nutrition is the ideal protein concept. Mitchell (1964) proposed the ideal protein concept model more than 50 years ago, referring to a situation in which all essential AA are co-limiting for performance, so that AA supply exactly matches AA requirement. Under the ideal protein concept model, essential AA requirements are expressed in terms of the lysine (Lys) requirement because Lys is typically the first-limiting AA in diets for pigs (van Milgen and Dourmad, 2015). The Lys requirement is usually estimated empirically using protein synthesis measures (NRC, 2012) and the estimates of AA requirements for other essential AA are often derived from the animal's tissue AA composition. Estimates of the essential AA profile in ideal protein for growing pigs have been derived from an examination of pig tissue composition and estimates of the AA profile in ideal protein for lactating sows have been derived from the composition of sow's milk (Lewis and Southern, 2000). Similarly, the AA profile of ideal protein during pregnancy has been based on the main tissues that make up the whole body of the pregnant sow (NRC, 2012). However, not all tissues have been considered for these estimations. For example, during early gestation, pregnant sows produce uterine fluids which are the primary source of nutrition for porcine embryos (Amoroso, 1952). Although uterine fluids represent only a small proportion of total body tissue, they appear to require high levels of certain essential AA. The concentration of metabolites such as Tau, which is biosynthesized from cysteine (Cys) and Met (Ripps and Shen, 2012), is found in uterine fluids at a concentration 65 times greater than that of Lys

at d 5 after estrus (Li et al., 2007). It is therefore possible that fluid tissues such as uterine fluids demand important amounts of sulfur AA for Tau biosynthesis, which has not been considered in the AA profile of the ideal protein for gestating sows. The NRC (2012) gestating sow model considers essential AA and N used for functions other than tissue deposition as inefficiencies. However, the AA profile needed for these inefficiencies is unknown. The lack of knowledge about the AA profile needed for performing physiological functions other than Pd [i.e. inefficiencies according to NRC (2012)] is one of the main weaknesses of the ideal protein concept.

Another important weakness of the use of the ideal protein concept based on the composition of animal tissues is that tissue turnover rates are not considered. It is known that different tissues have different protein turnover rates. For example, the total mass turnover in the human body is 80 ± 20 g/d, of which 41% corresponds to gastrointestinal epithelial cells, 24 % to erythrocytes, and 1.1% to myocytes or muscle cells (Sender and Milo, 2021). Consequently, an adult human replaces approximately 32 g of gastrointestinal epithelial cells, 19 g of erythrocytes, and 1 g of muscle cells every day. This means that, despite accounting for a smaller proportion of the human body, blood and the gastrointestinal tract require quantitatively more AA for maintenance than muscle cells. According to the previous observations, one of the main limitations of estimating AA requirements based on the relative contribution of each tissue to whole body AA composition is that turnover rates are not considered and, thus, AA requirements are likely to be underestimated.

Accuracy of AA requirements using the ideal protein concept

To analyze the difference between calculated and empirically observed data, a comparison of calculated leucine (Leu) requirements using the ideal protein concept and empirical data on reproductive performance at graded levels of Leu supply is used. According to the NRC (2012) gestating sow model, the optimal dietary standardized ileal digestible (SID) Leu to SID Lys ratio for a pregnant gilt across gestation is 0.92 on average. However, the results published by Wang et al. (2018) suggest that this ratio may not be adequate for optimal reproductive performance. Wang et al. (2018) provided diets with graded levels of Leu to pregnant sows from d 70 of gestation to farrowing. Although Wang et al. (2018) reported Leu levels on total basis, for the sake of this comparison, Leu on a SID basis was calculated according to NRC (2012) ingredient composition and digestibility values. The four experimental diets provided 36, 48, 60 and 71 g SID Leu/d and all provided 22.2 g SID Lys/d. The SID Leu to SID Lys ratios in the experimental diets were 1.6, 2.1, 2.7, and 3.2. Wang et al. (2018) only performed ANOVA analysis on their study; however, a reanalysis of their data showed a significant quadratic relationship between SID Leu intake vs birth weight, and plasma Leu. Quadratic relationships were maximized at ~ 54 g SID Leu/d. Similarly, a quadratic relationship between Leu intake and stillborn and mummified piglets ($P<0.05$) was minimized at ~ 54 g SID Leu/d. Thus, based on Wang et al. (2018), the optimal SID Leu to SID Lys ratio was calculated to be 2.4. These observations indicate that SID Leu requirements based on the composition of the tissues that comprise the pregnant sow may be significantly underestimated. The SID Leu requirement based on reproductive performance exceeds current recommendations by 140%; a difference that may be explained by the functional role that Leu serves in

different organs. Experiments in growing pigs have shown that the small intestine alone retains 50% of the Leu absorbed (Li et al., 2008) and experiments in humans have shown that Leu is the essential AA used in the greatest amounts by the placenta (Holm et al., 2017). It appears that organs such as the gastrointestinal tract and the placenta, despite constituting a relatively small proportion of the total body, require high amounts of Leu for maintenance possibly due to high turnover rates. These are dynamics that conceptual models based on tissue composition cannot capture. In addition, Wang et al. (2018) concluded that the diet providing 48 g SID Leu/d (i.e. 0.40% dietary Leu supplementation; SID Leu to SID Lys ratio of 2.2) "significantly increased the relative weight of the small intestine in newborn piglet". According to various authors, reduced gastrointestinal development is the main cause of the slower growth and higher neonatal mortality noted for light-born piglets (Jiang et al., 2009; Wang et al., 2010; Lanferdini et al., 2018). Therefore, gastrointestinal development may be a better predictor of piglet survivability than other variables such as birth weight (Lanferdini et al., 2018) and, thus, a SID Leu to SID Lys of 2.2 may potentially maximize newborn piglet robustness.

Based on the previous observations, the empirically determined optimal SID Leu to SID Lys ratio that maximizes piglet birth weight and physiological development seems to be around 240% of the optimal SID Leu to SID Lys ratio estimated using the ideal protein concept based on tissue composition. An ideal AA ratio that takes into account not only tissue composition but also AA turnover and physiological criteria would be more appropriate for estimating AA requirements that maximize pregnant sow reproductive performance. Therefore, we suggest that in addition to the structural role of the AA, the functional aspect of the AA need be considered. Considering only tissue

composition is one of the primary current limitations of the ideal protein concept for estimating AA requirements during pregnancy.

The plasma AA technique

The plasma AA technique is an empirical method for estimating AA requirements that, like the other methods described, has limitations. Using the plasma AA technique, AA requirement is defined as the point where additional dietary AA supply causes an increase in plasma AA levels, based on the assumption that the body lacks a storage compartment for free AA (France and Kebreab, 2008). Under the plasma AA technique, increased plasma AA levels have been regarded as waste and potentially harmful, based on the observation that excess amounts of AA accumulate in the body causing rare disorders such as phenylketonuria (France and Kebreab, 2008). However, data reported by Wang et al. (2018) suggest that Leu intakes that maximized piglet birth weight and gastrointestinal development during gestation also resulted in maximum plasma Leu. Other studies have also shown that increases in plasma AA is associated with increased conceptus development (Li et al., 2014; Che et al., 2019; Xia et al., 2019) and increased AA availability for immune response in growing pigs (Le Floc'h et al., 2018). Therefore, AA requirements estimated with the plasma AA technique are likely to be underestimated. Based on the results from Wang et al. (2018), the plasma AA technique can be used to estimate AA requirements that maximize reproductive performance, but not as the intake where additional dietary AA supply causes an increase in plasma AA levels, but rather as the intake that maximizes plasma AA.

The model validation concept

Science attempts to build knowledge about the natural world. However, in order for this knowledge to be objectively true, personal biases, emotions, and false beliefs must be eliminated (Daston and Galison, 2021). Nevertheless, biases are part of the human nature and are also considered a natural part of scientific research (Martin, 1979). Thus, minimizing scientific bias is an important consideration in developing objective truths. The model validation concept is a tool that could help to reduce bias in science including AA requirements research. Because conceptual models are frequently developed using deductive reasoning, their biological accuracy must be empirically validated (i.e. confirmed) before they can be considered objective truths and used to design experiments and generate knowledge.

The deductive approach was the one used by Hammond (1944) for the development of the hierarchy of nutrient use and by Mitchell (1964) for the development of the ideal protein concept. Deductive reasoning is the process of deducing a logical conclusion from one or more statements or premises (Sternberg et al., 2012). According to Dickstein (1980), deductive reasoning is susceptible to multiple biases such as over-generalization, extrapolation, addition of information, and erroneous integration of information reasoning. In the case of the Hammond model, observations made considering individual organs and tissues were integrated in an approach that was merely possible, but not necessitated by the given information. The assumption that organs with greater metabolic rates must be prioritized for animal survival was, although logical, not empirically demonstrated. Similarly, the ideal protein conceptual model based on tissue composition assumes that the body requires an ideal AA profile that is primarily

determined by whole-body protein composition; however, this assumption has not been empirically demonstrated to maximize physiological functions such as reproduction. Furthermore, although logical, the assumption that increased plasma AA has negative physiological consequences because the body lacks a storage compartment for free AA and that excess amounts of AA accumulate in the body in rare disorders (France and Kebreab, 2008) is contradicted by empirical evidence (Li et al., 2014; Wang et al., 2018; Che et al., 2019; Xia et al., 2019). Thus, the deductive approach infers effects from given causes, but, as in the previous examples, the inferred effects are not always empirically validated. In this sense, the deductive approach is a powerful method for hypothesis construction, but it should not be considered a scientific method as the conclusions made using this approach are possible but not objectively true. Conceptual model assumptions must be empirically validated before being used in AA requirements research.

Application of the model validation concept in AA requirements research

As previously mentioned, mammals models have shown that the body prioritizes protein synthesis over biological reactions such as transmethylation (Young and Marchini, 1990; Robinson et al., 2016). Because transmethylation reactions are important for the biosynthesis of essential metabolites for fetal development such as Tau (Holm et al., 2018) and the donated methyl group resulting from transmethylation reactions is required in a number of metabolic pathways (Riedijk et al., 2007; Tesseraud et al., 2008; Marcinkiewicz and Kontny, 2014), measures of protein synthesis do not seem to be sufficient for maximizing metabolic health. In addition, as previously discussed, measures of protein synthesis have been considered as inadequate for estimating AA requirements when physiological functions including reproduction are considered (Young

and Marchini, 1990; Marchini et al., 1993; Pampuch et al., 2006; Wu, 2014; Robinson et al., 2016). It appears critical to conduct research that measures variables related to reproductive function or metabolic criteria to determine AA requirements in pregnant sows. Because a constant N balance can be maintained by reducing important physiological functions (Young and Marchini, 1990), AA requirements estimated solely on protein synthesis measures may not meet the desired goal of maximizing animal performance; thus, the method used to estimate AA requirements must be ensured to improve the desired parameters.

Other methods, such as using nutrient balances other than N, could be used to estimate AA requirements in pregnant sows. Phosphorus, for example, is deposited in the fetus at a 10-fold higher concentration than in the maternal body (NRC, 2012) and, thus, it may be a proxy for fetal development. However, a phosphorus balance may result in AA levels that maximize bone and skeletal tissue growth but not necessarily fetal physiological development or sow metabolic status. Thus, the use of phosphorus balance must be validated to ensure that it can be used for the indirect estimation of AA intakes that result in optimal sow and piglet performance. Another alternative for the use of N balance for the estimation of AA requirements is the study of the dynamics of N retention at dietary AA levels for optimal sow reproductive performance. Furthermore, methods such as gene expression could be used to investigate physiological adaptations to various levels of AA intake and, as a result, could be used to estimate AA requirements in sows.

Estimation of AA requirements based on metabolic biomarkers

As stated previously, empirical estimation of AA requirements using methods such as N balance or plasma AA has traditionally failed to include physiological criteria

that may indicate the optimal metabolic status of the animal required for maximizing functions such as reproduction (Wu, 2010), gut health (Chen et al., 2014; Robinson et al., 2016), and immune status (Le Floc'h et al., 2018). It is, therefore, likely that current AA requirement methods underestimate the AA required for optimizing sow performance. Pampuch et al. (2006) found that that the dietary Trp levels that maximized metabolic status (measured as blood serotonin) exceeded optimal levels for protein synthesis by 37% in lactating sows. According to Pampuch et al. (2006), traditional methods based on protein synthesis measures may increase nutrient efficiency in the short term while negatively impacting long-term performance. It is thus recommended that different biomarkers be considered when estimating AA requirements, particularly during gestation, because sow longevity is a performance variable that should be maximized in practical commercial livestock production.

Plasma Tau concentration has been proposed as a marker for fetal wellbeing in sheep, with increased maternal plasma Tau levels resulting in increased fetal AA concentrations and reduced fetal hypoxia-induced inflammation (De Boo and Harding, 2007). In addition, urinary Tau has been proposed as a potential biochemical marker of total body protein status (Waterfield et al., 1991) and there are several studies on Tau as a biomarker in cardiovascular and inflammatory disease in humans as revised by Schuller-Levis and Park (2006). Also, Tau has been suggested as a marker of increased cell proliferation (Beckonert et al., 2003).

Taurine is an essential AA during gestation (Petters and Wells, 1971; Holm et al., 2018) and may serve as a marker of fetal and embryo development. At d 5 after estrus, Tau is the most abundant AA after glycine in porcine uterine fluids, with a concentration

190 fold that of Met, 100 fold that of Trp, 60 fold that of Lys, and 50 fold that of Thr, indicating the importance of Tau for early embryo development (Li et al., 2007). During mid gestation, Tau also seems to play a fundamental role. Experiments in multiple species have revealed that amniotic fluid contains stem cells (Fauza, 2004; Chen et al., 2011; Loukogeorgakis and De Coppi, 2017) and experiments in humans and mice have reported that the proportion of amniotic stem cells peaks during mid gestation and decreases thereafter (Ditadi et al., 2009; Schiavo et al., 2015; Loukogeorgakis and De Coppi, 2017). Because amniotic stem cells can differentiate into bone, fat, cartilage, muscle, hematopoietic, endothelial, hepatic, and neuronal tissues (Loukogeorgakis and De Coppi, 2017), it is speculated that the peak production of amniotic stem cells during mid gestation occurs to support the rapid fetal growth that occurs during late gestation (Bagci et al., 2016). Because Tau plays an important role for the proliferation of different kinds of stem cells (Ripps and Shen, 2012; Li et al., 2017; Mashyakhy et al., 2021), it is likely that Tau is also important for amniotic stem cell proliferation. Besides, amniotic fluid is rich in Tau which is found in greater quantity in amniotic fluid than in maternal blood (Underwood et al., 2005). Because Tau is considered essential for gestation (Holm et al., 2018), it is expected that there will be a link between Tau bioavailability and fetal growth. In this regard, Jung and Choi (2019) discovered a positive correlation between maternal Tau intake during late pregnancy and birth length in humans, implying that Tau bioavailability may serve as a marker of fetal growth. In sows, Tau supplementation during late gestation improved piglet birth weight and growth performance due to improved intestinal morphology and barrier function, as well as lowering oxidative stress (Xu et al., 2019).

Because Tau is biosynthesized from sulfur AAs (Met and Cys) via transmethylation reactions that are maximized at AA intake levels higher than those required for optimal protein synthesis (Robinson et al., 2016), estimating sulfur AA requirements must include measures of Tau bioavailability if sow and progeny performance is to be optimized. Because vitamin B^{12} and folate are required to maintain the rates of transmethylation reactions (Carmel et al., 2003), measures of Tau biosynthesis may also be considered for estimating the requirements for these two vitamins.

Moreover, the bioavailability of metabolites derived from essential AAs can be used as biomarkers to estimate AA requirements. Carnitine, which is a conditionally essential nutrient (Alesci, 2004), is biosynthesized from Lys (Young and Marchini, 1990) and plays an important role in reproduction. Carnitine supplementation has been linked to significant improvements in pregnancy rate and total sperm motility in patients suffering from male infertility (Zhou et al., 2007), oocyte growth and maturation in vitro (Wu et al., 2011; Dunning and Robker, 2012), and piglet litter weight and mean birth weight increased in carnitine-supplemented sows (Eder et al., 2001). Furthermore, creatine which is synthesized from Arg (Young and Marchini, 1990), has been proposed as an essential component of bioenergetics for successful reproduction (Muccini et al., 2021), and has been shown to improve fetal and neonatal morbidity and mortality in high-risk human pregnancies (Dickinson et al., 2014). Thus, considering physiological responses such as metabolite biosynthesis and quantifying them as biomarkers may aid in estimating AA requirements that improve animal health and performance.

CONCLUSIONS

The use of conceptual models helps to make sense of complex dynamic systems; however, they can lead to biased conclusions if the conceptual model's assumptions are not well understood. Because empirical methods for estimating AA requirements in pregnant sows rely on conceptual model assumptions, biologically inaccurate conceptual model assumptions would result in inaccurate AA requirements. According to the Hammond conceptual model's assumptions, AA intakes that maximize N-balance measures are considered adequate for pregnant sows, but empirical data suggest otherwise. An increased N balance can be achieved by suppressing physiological functions (i.e. accommodation); thus, attempting to maximize N efficiency without considering reproductive performance will likely result in suboptimal sow performance, resulting in an overall reduction in the efficiency of the production unit. Furthermore, the application of the ideal protein concept based primarily on sow tissue composition does not account for all tissues produced (e.g., uterine fluids), nor does it account for important biological functions that influence the dynamics of AA and N utilization, such as protein turnover. As a result, the traditional application of the ideal protein concept for estimating AA requirements in sows should be used with caution. Moreover, some important assumptions have been made, such as that increased plasma AA levels are wasteful and potentially harmful. Empirical evidence suggests that high plasma levels of essential AAs are not excessive, but rather appear to improve reproductive efficiency.; Nevertheless, this hypothesis must be tested for all essential AAs. It seems that traditional methods for the estimation of AA requirements have significant limitations that need to be investigated further. The current literature review identified gaps in the assumptions used

in conceptual models for estimating AA requirements in pregnant sows. Revising these assumptions and conducting studies that contribute to accurately describing physiological responses necessary for estimating optimal AA needs during pregnancy is a significant opportunity for research development.

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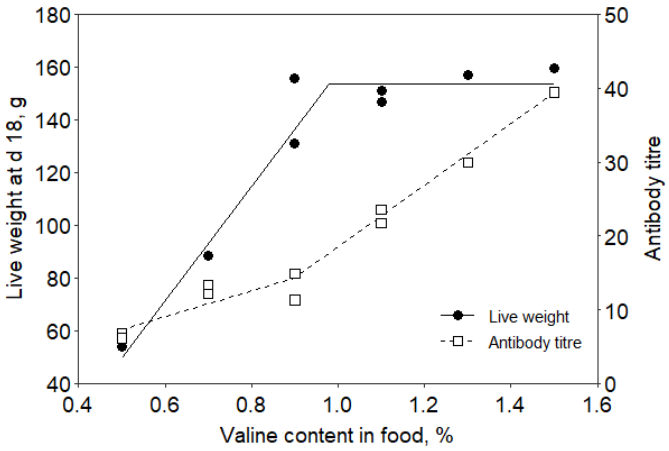

Figure 1-1. The responses in live weight and antibody titers to increasing dietary valine in chicks exposed with a Newcastle virus during an 18-day period; adapted from Bhargava et al. (1970) and France and Kebreab (2008).

CHAPTER 2

A MODEL OF GROWTH, PROTEIN, AND AMINO ACID DEPOSITION IN THE PREGNANT SOW

ABSTRACT

The current model quantifies the growth, Pd and AA deposition in the sow across gestation by modelling the growth, Pd and AA deposition in the key tissues that make up the pregnant sow: placenta, allantoic fluid, amniotic fluid, fetus, uterus, mammary gland, and maternal body. A total of 13 scientific articles published between 1977 and 2020 were selected for the development of the model. The data used in this modelling approach were obtained from published scientific articles reporting weights, crude protein (CP) and essential AA composition of the previously mentioned tissues. Growth, Pd, and essential AA deposition curves were developed with nonparametric statistics using splines regression. Validation of the whole body growth model showed a strong agreement between observed and predicted growth (r^2 = 0.92, root mean square error = 3.2 kg). The proposed model offers descriptive insights into the growth and Pd during gestation. The apparent increase in maternal Pd previously defined as time-dependent Pd is better defined as an increase in fluid and pregnancy-related tissues coincident with lower maternal Pd between d 40 and 70 in combination with decreased Pd during early gestation relative to pre breeding and d 68 Pd levels. The proposed model quantifies the negative maternal Pd that occurs in late gestation and indicates Leu demands for pregnancy-related tissues may drive the mobilization of maternal tissue towards the conceptus. The model demonstrates that pregnancy-related tissues not only increase in weight but also in protein density as gestation progresses where previous models assumed

a constant CP content in all protein pools. In addition, the proposed model shows that the AA profile of the protein deposited changes dynamically throughout pregnancy, especially from d 80 through farrowing. The current model has identified areas of specific investigation that are needed to develop advanced models of AA requirements to better predict maternal and fetal needs during gestation.

INTRODUCTION

Although mathematical models are simplified representations of complex processes, they are a useful tool for expressing theories and advancing understanding. Mathematical models have been used to predict AA requirements across gestation for different populations of sows. The accuracy of these predictions depends on the data and assumptions on which the model has been developed. The most recently published mathematical models for sows are InraPorc (2008) and NRC (2012). However, recent animal studies have shown that AA recommendations by the NRC (2012) for the gestating sow model are suboptimal for Arg (Liu et al., 2012; Wu et al., 2012; Nuntapaitoon et al., 2018; Hong et al., 2020; Luise et al., 2020), Leu (Wang et al., 2018), Met (Bin et al., 2018; Xia et al., 2019), Threonine (Thr; Shi et al., 2018), and Val (Gao et al., 2019; Che et al., 2020), which suggests that current estimations need to be reviewed.

In silico studies (i.e. research performed on computer or via computer simulation), represent a relatively new avenue of investigation in the field of pig production. In silico studies have the potential to reduce the time and cost of producing knowledge without the ethical considerations and lack of control associated with in vivo experiments (Barh et al., 2020). These models are intended to complement, rather than replace, experimental research.In fact, experimental research is needed to develop and validate in silico models.

The main goal of in silico modeling is a deep understanding of the function of the system being studied (Barh et al., 2020).

An in silico model was developed to characterize the growth, Pd and essential AA deposition of the different tissues that make up the pregnant sow, as well as the relationships between tissues, in order to better characterize the dynamics of tissue deposition across gestation. Characterization of tissue deposition dynamics during gestation will aid in identification of key gaps in knowledge allowing development of targeted empirical studies to better characterize protein and AA demands, ultimately building more precise models of AA requirements during pregnancy.

MATERIALS AND METHODS

Model description

The modelling approach utilized for this work followed that defined by France and Kebreab (2008) where "a mechanistic model is constructed by looking at the structure of the system under investigation, dividing it into its key components and analyzing the behavior of the whole system in terms of its individual components and their interactions with one another". The present model analyzes the growth, Pd and AA deposition in the sow across gestation by modeling the growth, Pd and AA deposition in the key tissues that make up the pregnant sow, as well as the interactions between the key tissues of the model, using the algorithm described below (Eq. 2) and in the Supplementary Flowchart. Placenta, allantoic fluid, amniotic fluid, fetus, uterus, mammary gland, and maternal body were the key components (tissues) considered in the current approach. The growth, Pd and AA deposition across gestation were calculated for each of the key tissues using empirical models. Empirical models are models in which

experimental data are used directly to quantify relationships in a tissue or other levels of organization (e.g. cell, organ, animal) and are based on observation and experimentation rather than preconceived biological theory (France and Kebreab, 2008). Empirical models are often curve-fitting approaches (France and Kebreab, 2008) and, because no mathematical functions have yet been developed that could describe the growth of certain pregnancy related tissues or the deposition of AA and protein in them, curve-fitting was performed using spline regression. Splines regression is a piecewise regression technique that, through a combination of linear/polynomial functions, fits the data. Each spline curve is thus composed of many segments, each segment having its own function. Because the algebraic representation of each spline curve would consist of a complex system of equations, splines are usually presented in numerical (tables) or graphical forms. In the present study, each spline consists of a vector containing the growth or nutrient deposition for each of the 114 days of gestation. Hereafter, vector variables (splines or variables resulting from operations performed with splines) are denoted in functions with an arrow above the variable name.

Although each spline is static, when combined with additional calculations or model inputs the curve becomes dynamic and responsive to the parameterization of the model. For example, the growth curve of the placenta (Eq. 2-1) is calculated as the placenta growth spline (Fig. 2-1A) times the number of fetal pigs (litter size). Because the placenta growth spline was calculated per fetal pig (amount of placenta needed per individual fetus), when combined with the parameterized litter size, the whole placenta growth is estimated.

$$\overrightarrow{\text{Placenta}}_{\text{growth}} = \overrightarrow{\text{Placenta}}_{\text{growth spline}} \times \text{Litter Size} \quad \text{Eq. 2-1}$$

Data obtained from selected research papers provided multiple timepoints throughout gestation; however, no one dataset was 100% complete, thus educated guesses about the possible trend of the splines were made. For example, often data points from a few weeks after breeding and several days before farrowing were missing. In this case, the data trend was extended. CurveExpert Professional (version 2.6.5) software was used for the development of all splines and all the calculations were made in R (version 3.6.3) and RStudio (version 1.3.959).

The data used in this modelling approach were obtained from published scientific articles reporting wet weights, crude protein (CP) and essential AA composition of the previously mentioned key tissues. A total of 13 scientific articles published between 1977 and 2020 were selected for the development of the model (Supplementary Table 1). The specific articles used are reported within the relevant model description sections. Model predictions are estimated based on model inputs of parity, body weight (BW) at breeding, litter size, average piglet birth weight, and number of available teats.

Pd and tissue growth

The placenta growth spline (Fig. 2-1A) was developed based on Knight et al. (1977) who reported changes in placental weight per fetal pig at 11 timepoints across gestation. Allantoic and amniotic fluid growth splines (Fig. 2-1B and Fig. 2-1C) were also developed based on Knight et al. (1977). Fetal growth spline was developed based on Wu et al. (1999) (Fig. 2-1D). Mammary gland growth spline (Fig. 2-1E) was developed based on Ji et al. (2006) and uterus growth spline (Fig. 2-1F) was calculated based on Jang et al. (2017).

Protein deposition splines for placenta and uterus were developed based on Jang et al. (2017) as g of CP/100 g of placenta wet weight. The Pd spline for allantoic and amniotic fluid were developed from Knight et al. (1977) as mg of CP/g of fluid. The Pd spline for fetus was calculated based on Wu et al. (1999) as g of CP/100 g of fetus wet weight. Mammary gland Pd spline was calculated from Ji et al. (2006) as g of CP/100 g of mammary gland wet weight. The Pd splines of the fetus and placenta are shown in Fig. 2-2A and Fig. 2-2B, respectively. By combining growth splines together with Pd splines and parameterizing the model with observed farrowing performance, Pd in placenta, fluids, fetus, uterus, and mammary gland were estimated.

Maternal body Pd was estimated as whole body (WB) Pd minus Pd in placenta, fluids, fetus, uterus, mammary gland, and protein used for metabolism and other functions commonly associated with maintenance (Pm) according to Eq 2. The Pm was defined as the protein retained by soft tissues not including lean tissue as well as protein used in metabolism such as the biosynthesis of non-proteogenic AAs (e.g., Tau and carnitine) and inorganic compounds (e.g., nitric oxide from Arg metabolism). The WB Pd for parity 1 sows was calculated based on the results reported by Miller et al. (2016) who provided sows with two feeding levels (high and low) supplying 15% above and 15% below estimated energy requirements (Fig. 2-3A). The average WB Pd between high and low fed sows was assumed to represent WB Pd at energy requirement based on the observation that WB Pd is positively related to feed intake, with high and low fed sows exhibiting a similar pattern of WB Pd but varying in magnitude depending on intake (Fig. 2-3A). Because the proposed model studies the growth, Pd, and AA deposition in

pregnant sows fed following NRC (2012) energy recommendations, the calculated average WB Pd was used.

For the WB Pd for parities 2, 3 and 4+ the WB Pd for parity 1 sows was adjusted based on the results reported by Miller et al. (2017) and Ramirez-Camba et al. (2020) and are shown in Fig. 2-3B. The WB Pd at breeding for all parities were estimated based on NRC (2012) gestating sow model equations. The Pm was calculated as a percentage ($Pm_{coeff.}$) of WB metabolic protein (Eq. 2-3). The WB metabolic protein was defined as the metabolic protein at breeding (BW^0.75 * 0.1511) plus Pd across gestation [the coefficient of 0.1511 corresponds to the CP content in the carcass of medium-lean genotype sow fed 0.55 % Lys diets as reported by Friesen et al. (1994)]. The $Pm_{coeff.}$ was estimated to be 0.0042 or 0.42% of the WB metabolic protein because this coefficient minimized the mean squared error between the model predicted WB growth and the observed WB growth reported by Buis (2016).

$$\overrightarrow{MB}_{Pd} = \overrightarrow{WB}_{Pd} - \overrightarrow{Pl}_{Pd} - \overrightarrow{AlF}_{Pd} - \overrightarrow{AmF}_{Pd} - \overrightarrow{F}_{Pd} - \overrightarrow{U}_{Pd} - \overrightarrow{MG}_{Pd} - \overrightarrow{Pm} \quad \text{Eq. 2-2}$$

MB_{Pd} = Maternal body protein deposition

WB_{Pd}= Whole body protein deposition

Pl_{Pd} = Placenta protein deposition

AlF_{Pd}=Allantoic fluid protein deposition

AmF_{Pd}= Amniotic fluid protein deposition

F_{Pd} = Fetus protein deposition

U_{Pd}= Uterus protein deposition

MG_{Pd}= Mammary gland protein deposition

Pm = Protein for metabolism and tissues other than lean tissue

$$\overrightarrow{Pm} = \overrightarrow{WB}_{mp} \times Pm_{coeff.} \quad \text{Eq. 2-3}$$

Pm = Protein for metabolism and tissues other than lean tissue

WB_{mp} = Whole body metabolic protein

$Pm_{coeff.}$ = Percentage of WB_{mp} required for Pm

In addition, maternal body growth (MB_{gwth}) was estimated by dividing maternal Pd by maternal CP content (Eq. 2-4). Maternal growth together with placenta, fluids, fetus, uterus, and mammary gland growth allow the calculation of WB growth (Eq. 2-5).

$$\overrightarrow{Mb}_{gwth} = \overrightarrow{Mb}_{Pd} / 0.1511 \quad \text{Eq. 2-4}$$

$$\overrightarrow{WB}_{gwth} = \overrightarrow{Pl}_{gwth} + \overrightarrow{AlF}_{gwth} + \overrightarrow{AmF}_{gwth} + \overrightarrow{F}_{gwth} + \overrightarrow{U}_{gwth} + \overrightarrow{MG}_{gwth} + \overrightarrow{MB}_{gwth} \quad \text{Eq. 2-5}$$

WB_{gwth}= Whole body growth

Pl_{gwth} = Placenta growth

AlF_{gwth} =Allantoic fluid growth

AmF_{gwth} = Amniotic fluid growth

F_{gwth} = Fetus growth

U_{gwth} = Uterus growth

MG_{gwth} = Mammary gland growth

MB_{gwth} = Maternal body growth

Essential AA deposition

The model calculates the daily WB deposition for Arg, Cys, histidine (His), isoleucine (Ile), Leu, Lys, Met, phenylalanine (Phe), Thr, Trp, and Val by adding the daily deposition of each AA in all seven tissues considered by the model (placenta, allantoic fluid, amniotic fluid, fetus, uterus, mammary gland, and maternal body). The

daily AA deposition in the placenta, allantoic fluid, amniotic fluid, fetus, uterus, and maternal body was calculated by multiplying their daily weight gain by the AA concentration in the tissue (g AA/g wet weight). The AA concentration in allantoic and amniotic fluids were calculated from the results of Wu et al. (1998); the authors reported AA concentrations at d 40 and 60 of gestation, these values were averaged for representing single AA concentration at d 50. In the absence of data specific to the pregnant sow, the AA concentration in the maternal BW gained was assumed to be the same as the AA concentration in 146 kg pig carcasses from Mahan and Shields Jr (1998). In addition, because empirical studies showed that the AA concentration in the placenta, fetus and uterus vary throughout gestation, splines that described these changes were developed. For example, according to Wu et al. (1999) the Lys concentration in the fetus changes from 3.42 ± 0.05 mg/g wet weight at d 60 to 5.77 ± 0.06 mg/g wet weight at d 114 of gestation. Thus, the splines that described the daily AA concentrations in the placenta and uterus were calculated based on Jang et al. (2017) for all essential AA except for tyrosine because it was not reported. The spline that described the daily AA concentrations in the fetus was developed based on Wu et al. (1999). Lastly, daily AA deposition in the mammary gland was calculated by multiplying the daily Pd by the AA concentration in the mammary protein reported by Kim et al. (1999) at d 5 of lactation. The flowchart and equations used in the present modelling approach are shown in the Supplementary Flowchart.

RESULTS AND DISCUSSION

General structure of the model

The current mechanistic model studies the growth, Pd and AA deposition of the gestating sow using empirical models that quantify growth, Pd, and AA deposition in each of the key tissues that make up the pregnant sow and the interactions between the key tissues are addressed by the algorithm described in the model development section and the Supplementary Flowchart. The interactions performed by the model algorithm allow it to dynamically react to the inputs of the model adjusting BW, Pd and AA deposition to the empirically observed competition for nutrients between the maternal body and the conceptus (Miller et al., 2016). The model distributes the retained dietary protein among tissues, with increased litter size and weight resulting in decreased maternal body Pd and vice versa. This dynamics of protein and AA use has not been addressed by previous models. The equations that represent the daily Pd of the placenta and fluids, uterus, and mammary gland in the NRC (2012) gestating sow model, for example, are fixed regardless of litter size, litter weight, parity, or sow BW, while the equations that represent maternal Pd are independent of litter size and litter weight inputs or estimations of placenta and fluids, uterus, or mammary gland. Considering competition for nutrients between tissues adds a level of complexity that will aid our understanding of the growth and development that occurs during pregnancy.

The algorithm of the current mechanistic model predicts partitions between conceptus and maternal body for different populations of sows. However, in order to capture the dynamics of growth, Pd and the deposition of 11 AA in the 7 key components (tissues) of the mechanistic model and the interactions between them, the mechanistic

model contains more than 100 functions, including recursive and conditional functions (Supplementary Flowchart). Because small variations in the model algorithm would produce different outputs, the model may be erroneously replicated. To reduce the possibility of incorrectly replicating the model and to facilitate user interaction, an interactive version of the current model is available upon request.

Model assumptions

Although the model contains an extra layer of complexity in comparison to previous models (interaction between key components or competition for nutrients among tissues) it is still a simplified representation of a more complex system: the gestating sow. As stated by France and Kebreab (2008), mathematical models are simplifications, not duplications of reality, therefore, mathematical models provide us with representations that we can use but cannot account for all possible factors involved in the system. The current model accounts for a few factors, includes assumptions based on limited data, and does not claim to be predictive in all scenarios.

There are assumptions that are implied in the model development section. For example, the AA composition of tissue growth is assumed to be the same as the composition of the tissue itself, something that may or may not be true for some or all tissues. Also, as noted previously, due to a lack of data it was assumed that the composition of the maternal BW gain is similar to that of 146 kg pig carcasses. In addition, the model was developed by using and combining data from multiple studies, under the assumption that the composition of the tissues is similar among different populations of sows, something that may or may not be true. Specifically, studies were performed in different years, with different genetics, and in animals fed different diets.

Despite its limitations, we believe the proposed model is a useful tool for making quantitative and qualitative predictions about a pregnant sow's growth and development dynamics. Furthermore, this model's assumptions are similar to those of previous models, including the NRC (2012) gestating sow model, so we believe they are not overly speculative.

Splines regression

As stated in the model development section, spline regression was used because no mathematical functions have yet been developed that could describe the growth of certain pregnancy related tissues or the deposition of protein and AA therein. It is widely known that a problem associated to fitting data with high degree polynomial functions such as spline regression is overfitting (Hawkins, 2004). When a model becomes overfitted, the data noise (unexplained variability) is included in the model, so the model accurately reflects the data from which it was developed but is unable to fit well to new data (Hawkins, 2004). Models that overfit the data from which they were built have low predictive power, which is not the case with the current model, as detailed in the next section. The use of mathematical models simpler than splines regression reduces the possibility of capturing unexplained variability and reduces the possibility of adequately describing biological responses such as, for example, the dynamics of fluid retention, as indicated in the next paragraph.

The growth splines with the highest degree of nonlinearity in the current model describe the growth of allantoic fluid, amniotic fluid, and the placenta (Fig. 2-1). Interestingly, the spline that describes amniotic fluid growth in this study closely resembles the growth of amniotic fluid observed in humans (Igbinidu et al., 2013). The

spline that describe the growth of the allantoic fluid in the current study closely resembles the allantoic fluid volume observed in pigs by Goldstein et al. (1980). Because the allantoic and amniotic fluids are contained within the placenta, a related growth of the placenta is expected, as depicted by the spline in Fig. 2-1A. These fluid retention dynamics that match empirical observations, but not described by previous models, are essential for explaining other biological phenomena, such as the previously unexplained whole-body Pd described as a time-dependent protein by the NRC (2012) gestating sow model, which is discussed further down. As a result, while spline regression functions are considered to capture unexplained variability in the existing model, they also capture biological responses and dynamics that have not been previously described.

Challenging the model

According to Murray-Smith (2015), "model validation" is the process of evaluating a model's accuracy and predictive abilities by comparing model predictions to a real-world and unknown dataset. To validate the model, comparison between observed versus model-predicted weight gains during gestation were made. The daily gilt weights reported in Thomas et al. (2018a) were contrasted with model-predicted BW (Fig. 2-4). An average litter size of 14.5 piglets and an average birth weight of 1.25 kg was assumed based on Thomas et al. (2018b) and Thomas (2019) which reported farrowing performance of sows from the same barn in the same period. Thomas et al. (2018a) reported weights beginning at week 2 of gestation; authors stated weights from week 1 were considered inaccurate, thus, based on the data from week 2 an initial BW of 151 kg was estimated. The model root mean squared error (RMSE), which measures the model's average error in predicting the outcome for an observation was 3.2 kg, and the coefficient

of determination (r^2), which is a statistical measure of how well the model approximated the observed data points, was 0.92. This indicates the model prediction of BW gain fits well with empirical data not used in model development.

The NRC (2012) reports average sow BW at breeding, gestation weight gain, and litter size by parity compiled from 8 published studies (Mahan, 1998; Cooper et al., 2001; Van der Peet-Scherwing et al., 2003; Musser et al., 2004; Young et al., 2004; Dourmad et al., 2006; Gill, 2006; Veum et al., 2008). Table 2-1 shows observed gestation weight gains by parity as reported by NRC (2012) versus our model-predicted gestation weight gains. The difference between our model predicted gain and actual reported gain were <5% for all parities. Based on the RMSE, the r^2 and the small differences between observed and model-predicted weight gains the developed model is a fair representation of the growth of the different tissues that make up the prolific gestating sow.

Growth and Pd

As previously shown in Fig. 2-2A, the proportional CP content (percentage of CP relative to wet weight) in the fetus dynamically changes over time increasing from 5.2% at d 60 to 10.9% at d 114 (Wu et al., 1999) and the proportional CP content in the placenta follow a similar trend as fetal Pd increasing from 3.7% at d 58 to 6.7% at d 108 (Fig. 2-2B; Jang et al., 2017). Similarly, the proportional CP content of the mammary gland increases from 5.8% at d 60 to 15.2% at d 112 (Ji et al., 2006). Previous models did not consider the dynamic increase in CP content over time and the CP content in protein pools were averaged to represent a single value across gestation (NRC, 2012). This consideration is important because it shows that pregnancy-related tissues not only increase in weight but also in protein density as gestation progresses.

In addition, the CP differences and metabolic rates among tissues suggest that conceptus development is an inefficient process in terms of N retention compared to maternal tissue deposition. Our calculations suggest that at d 80 of gestation, the CP content in the pregnancy related tissues of an average sow is 5.5 % [placenta: 5.05%; allantoic fluid: 0.83%; amniotic fluid: 0.10%; fetus: 6.67%; uterus 9.93%; mammary gland: 7.54% CP (Knight et al., 1977; Wu et al., 1999; Ji et al., 2006; Jang et al., 2017)], in contrast to the 15% crude protein in the maternal body (Friesen et al., 1994). Therefore, pregnancy related tissues have low protein content relative to maternal tissue but higher turnover rates (Alenzi, 2004; Krisher and Prather, 2012), as well as higher demands for essential AA catabolism for the biosynthesis of metabolites required for conceptus development (Huxtable, 1992; Holm et al., 2017; Holm et al., 2018; Mu et al., 2019; Xu et al., 2019). Thus, depositing maternal lean tissue seems to be a more efficient process in terms of protein synthesis and N retention than depositing pregnancy related tissues. Thus, if a dietary strategy induces the sow to divert more nutrients to conceptus development rather than maternal tissue deposition, reproductive performance improves while N retention and/or efficiency may decrease. This finding has potential implications for methods used to establish individual AA requirements during pregnancy (i.e. maximal N retention or efficiency).

The growth (Fig. 2-5) and Pd (Fig. 2-6) of the whole body of the pregnant sow were determined by estimating growth and Pd in key tissues considered by the current model. The relative contribution of maternal and pregnancy-related tissues to weight gain and Pd changes as pregnancy progresses and as parity increases. The current model shows that maternal body Pd is not linear or constant, as supported by Miller et al.

(2016), and may even be negative during late gestation (maternal body mobilization), depending on the growth of the products of conception. Predictions of the model suggest that for an average sow, irrespective of parity, fed a common gestation diet providing 12 g SID Lys / d and following NRC (2012) AA ratios relative to Lys and energy recommendations, and with a litter of 11 piglets, there is no mobilization or maternal lean tissue gain in late gestation (d 95 to farrowing). Litters larger than 11 piglets would increase the mobilization of maternal tissue and less than 11 piglets would allow maternal lean tissue deposition.

As previously stated, the dynamic variations in growth and Pd represented by spline regression models in the current study help explain a previously unknown increase in Pd during the first 55 days of gestation referred to as time-dependent Pd by the NRC (2012) gestating sow model. Our results suggest that this apparent increase in maternal Pd described by NRC (2012) as time-dependent Pd occur for two reasons. First, the decrease in the proportional Pd observed between d 40 to d 70 in the fetus (Fig. 2-2A), placenta (Fig. 2-2B), and a decrease in WB Pd (Fig. 2-3) coincides with an increase in placenta (Fig. 2-1A), allantoic fluid (Fig. 2-1B) and amniotic fluid growth (Fig. 2-1C). Therefore, it seems that when fluids deposition is increased, there is a decrease in Pd, without a decrease in the rate of WB weight gain. This temporary shift away from Pd may also be related to a shift in energy storage. Samuel (2008) reported that during mid gestation pregnant sows have a primary need for energy storage rather than AA. During mid gestation, placental glycogen stores increase, which is assumed to ensure that the fetal glucose supply is maintained at times of maximum demand such as during late gestation (Barash and Shafrir, 1990; Coan et al., 2006; Tunster et al., 2020)

Second, non-pregnant gilts at breeding age retain similar CP levels as pregnant gilts (parity 1 sows) at d 68 of gestation (Miller et al., 2019) implying that a reduction in Pd occurs during the early stages of gestation. This hypothesis is supported by the findings of Hoving et al. (2011) and Hoving et al. (2012), who found that increased feed intake (+30%) improved BW gain (and thus Pd) during the first month of gestation in parity 1 and 2 sows. According to a systematic review performed by Leal et al. (2019), increased feed intake during early gestation improves body condition while having no deleterious consequences on embryo survival in parity 1 sows. Thus, our results indicate that the apparent increase in maternal Pd described by NRC (2012) as time-dependent Pd is better defined as decrease in WB Pd between d 40 and 70 along with an increase in fluid deposition and a decrease in Pd during early gestation relative to pre breeding and d 68 Pd levels (Fig. 2-7). It is unknown whether the decrease in daily Pd from d 40 to 70 can be lessened by nutrition or whether mitigating the decrease has long-term benefit to sow reproductive performance or fetal development. Nevertheless, current feeding strategies may be inadequate for early gestation according to the reduced Pd described by the present model and other models such as the NRC (2012) gestating sow model and the INRAporc model (van Milgen and Dourmad, 2015). Reduced Pd results in increased N excretion, lowering the efficiency of the production system, which suggest the need for more accurate requirements during early gestation.

AA deposition

Similar to the changes in proportional CP content described in the previous section, the proportional AA content in the pregnancy related tissues changes over time. For example, Lys content in the fetus increases from 3.4 mg per g wet weight at d 60 to

5.8 mg per g wet weight at d 114, according to the results of Wu et al. (1999) with a similar pattern occurring for all essential AA. A similar trend is shown for all essential AA in the placenta (Jang et al., 2017). The dynamics of AA deposition over time were captured by the different splines developed for each of the tissues considered in the present mechanistic model. The deposition of the 11 AA in the 7 tissues considered in the current model can be explored in the model's interactive web-based version.

By aggregating the AA deposited in each of the key tissues, AA deposition in the pregnant sow was estimated. The calculated WB AA deposition shows two differentiable AA deposition profiles (Fig. 2-8A). From breeding to d 80 of gestation, a relatively constant AA profile is observed and primarily represents maternal tissue AA deposition (Early-Mid$_{\text{AA profile}}$). A different AA profile is observed from d 81 to farrowing and primarily represents AA deposition in the products of conception and mammary gland (Late$_{\text{AA profile}}$). The Early-Mid$_{\text{AA profile}}$ shows that Lys is the AA deposited in larger proportions followed by Leu and Arg, while in the Late$_{\text{AA profile}}$ Lys is the third most abundant AA after Leu and Arg.

As shown by the model estimates of Pd, the Late$_{\text{AA profile}}$ could be met by dietary or mobilized AAs because, depending on conceptus growth, protein mobilization may, or may not, occur in late gestation. Because Leu is the AA that is deposited in greater amounts during late gestation, Leu demand for pregnancy-related tissues may drive the mobilization of maternal tissue towards the conceptus. According to Wu et al. (1999) Leu is the essential AA deposited in greatest quantities during late gestation in the fetus. Similarly, Jang et al. (2017) reported that Leu is the AA deposited in greatest quantities in placenta and uterus and Kim et al. (1999) showed that Leu is the essential AA

deposited in greatest quantities in the mammary gland. Hence, current model predictions point to a likely need for more accurate Leu requirements during gestation.

The fetal growth, Pd and AA deposition in the current model was calculated based on Wu et al. (1999) because they reported data until d 114, contrary to Jang et al. (2017) who reported fetal data just until d 108. However, the data of Jang et al. (2017) data may better represent the composition of the piglets under current conditions and genetics because it is more recent. The Arg to Lys ratio in the fetal pig reported by Jang et al. (2017) is 1.25 in contrast to the Arg to Lys ratio reported by Wu et al. (1999) of 1.13, which suggest that the Arg predictions in the present model may be underestimated. In addition, as previously shown in Fig. 2-8, Arg is a more important AA for fetal protein synthesis in late gestation than Lys in terms of magnitude, and a potential dietary limiting factor for optimal fetal development. Thus, more accurate Arg requirements for optimizing fetal development throughout gestation are needed.

Besides Leu and Arg, the AA ratios relative to Lys are higher in the $Late_{AA\ profile}$ compared to the Early-$Mid_{AA\ profile}$ for Met, Cys, Phe, Thr, and Val, lower for His and Ile and constant for Trp (Supplementary Table 2) compared to NRC (2012) model predictions. These dynamic changes in AA ratios seems to be related to the composition of the tissues being deposited: mainly maternal body during early and mid-gestation and fetal skeletal tissue, fetal organs, placenta, uterus, and mammary gland during late gestation and supports the need for further investigation into stage-specific AA requirements.

Model limitations and future research

It is important to note that in silico studies are not a replacement for experimental research, and model predictions should be used cautiously and empirically confirmed. Also mathematical models cannot accurately predict or describe biological phenomena for which there is a lack of quantitative data. For example, sow protein intake has the potential to alter the fetal AA composition by affecting the relative fetal organ weight (% of birth weight). Thus, if the sow has greater bioavailability of specific AAs, these AAs can be used for building specific tissues. For example, Wang et al. (2018) showed that Leu supplementation during late gestation increased small intestine relative weight at birth. As a result, different dietary AA levels may result in different fetal AA compositions. Because the relative small intestine weight, for example, may be a better predictor of piglet survival than birth weight (Leenhouwers et al., 2002; Lanferdini et al., 2018), empirical studies investigating effect of maternal dietary AA levels on relative fetal organ weights or other biological traits associated with piglet growth and survivability are warranted. As more quantitative data becomes available, model predictions can be improved.

In this sense, the quality of the model predictions is determined by the data used for model development; models based on research from sows fed suboptimal diets would result in suboptimal AA deposition estimates. It cannot be known whether the diets provided in the studies used to develop the present model were optimal for fetal development, thus our predictions of AA deposition may reflect the conditions under which the various experiments were conducted more so than optimal levels of AA deposition. However, despite its limitations, we believe the present model provides

valuable information on the growth and development of tissues that make up the WB of the pregnant sow, which can be used to develop future research aimed at better defining AA requirements under a range of conditions; ultimately improving sow reproductive efficiency.

Conclusions

In conclusion, the proposed model combines current knowledge of growth and protein and AA deposition during pregnancy; its growth predictions fit well with empirical data, providing additional qualitative information on the processes that occur throughout pregnancy. The current model better characterizes maternal body Pd referred to as time-dependent Pd, which previously could not be associated with energy intake or reproductive tissues. The trend in Pd described by the time-dependent protein pool appears to be due to an increase in fluid and placental tissue and lower daily maternal Pd during early gestation implying that current energy and/or AA requirements for early pregnancy should be reviewed. The proposed model characterizes negative maternal Pd that can occur in late gestation. In addition, the proposed model shows that the AA profile of protein deposited changes dynamically throughout pregnancy, especially from d 80 through farrowing due to shifts in relative contribution of maternal and pregnancy-related tissues and differences in AA composition of maternal body and products of conception as pregnancy progresses and parity increases. In particular, that Leu demands for pregnancy-related tissues may drive mobilization of maternal tissue in late gestation. The current model has identified areas of specific investigation that are needed to develop advanced models of AA requirements to better predict maternal and fetal needs during gestation.

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Table 2-1. Observed versus model-predicted gestation weight gain.

Parity	1	2	3	4+
Observed performance[1]				
Body weight at breeding , kg	135.4	158.3	196.4	184.8
Gestation weight gain, kg	67.4	56.3	46.4	42.4
Litter size, n	10.7	10.8	11.4	11.1
Model-predicted performance[2]				
Gestation weight gain, kg	65.16	53.53	47.47	43.15
Gestation weight gain difference[3]**, %**	3.3%	4.9%	2.3%	1.8%

[1] Observed performance values summarized by NRC (2012).
[2] Average piglet birth weight was assumed to be 1.4 kg across all parities; litter size was parameterized as observed performance and 14 available teats were assumed for all parities.
[3] Absolute value of the percent difference between observed and model-predicted performance.

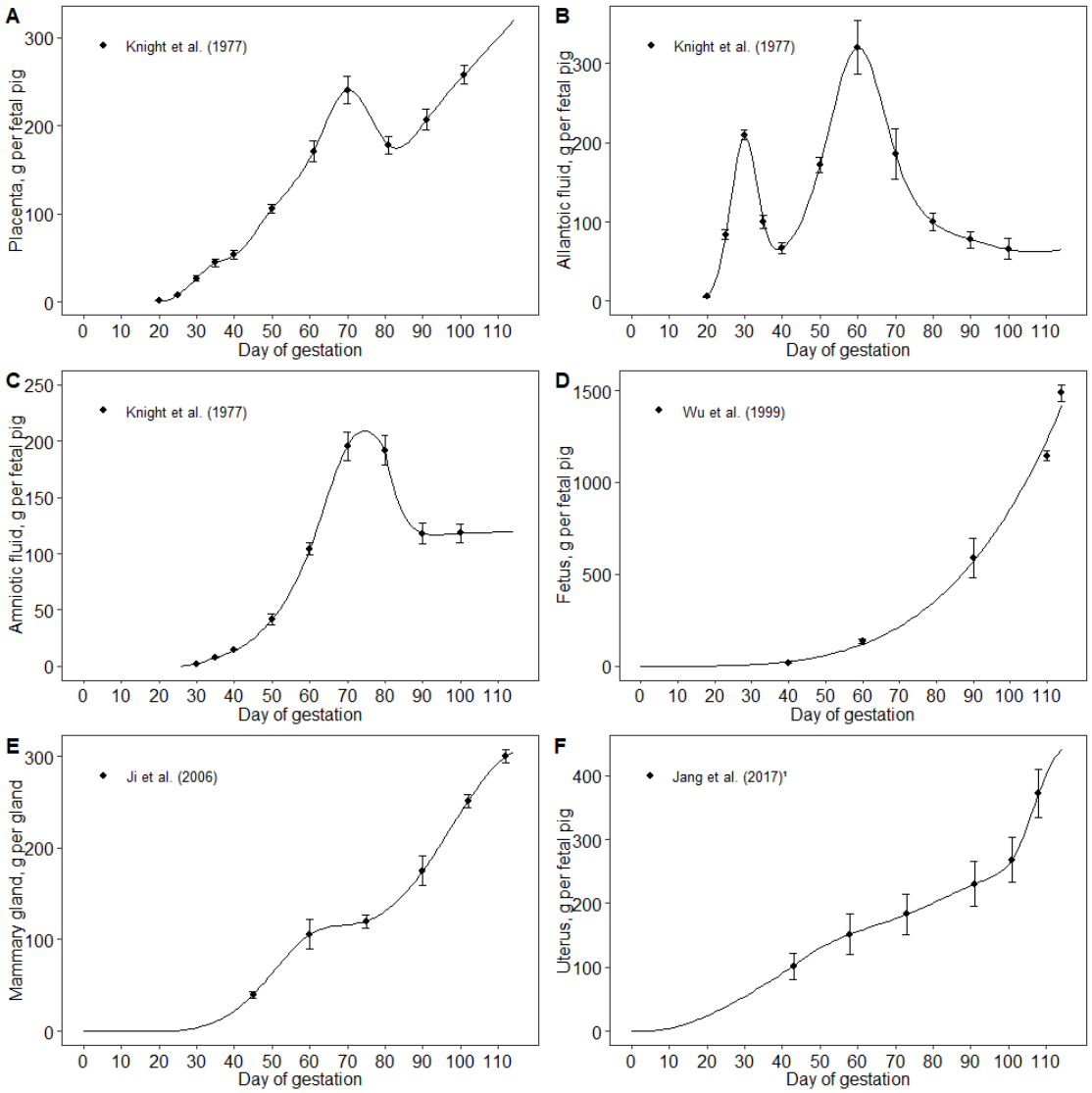


Figure 2-1. Wet weight growth spline curves developed for placenta (A), allantoic fluid (B), amniotic fluid (C), fetus (D), mammary gland (E) and uterus(F) of gestating sows.

[1] non-gravid uterus weight was obtained from Ji et al. (2005). Uterus weight per fetal pig was estimating by dividing total uterus weight gain by litter size per slaughtered group. Litter size for Jang et al. (2017) was reported in Ma et al. (2014).

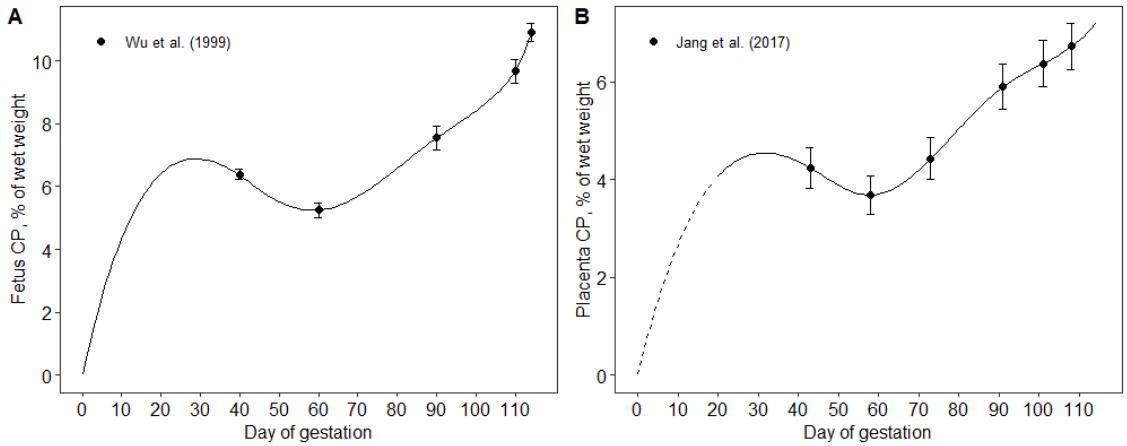


Figure 2-2. Crude protein (CP) spline curves developed for the fetus (A) and placenta (B). The splines represent the CP in the tissues as a percentage of wet weight. Because placental growth begins at d 20, the CP values displayed in dotted lines were not used in the calculations. A similar pattern can be seen in CP changes between the fetus and placenta.

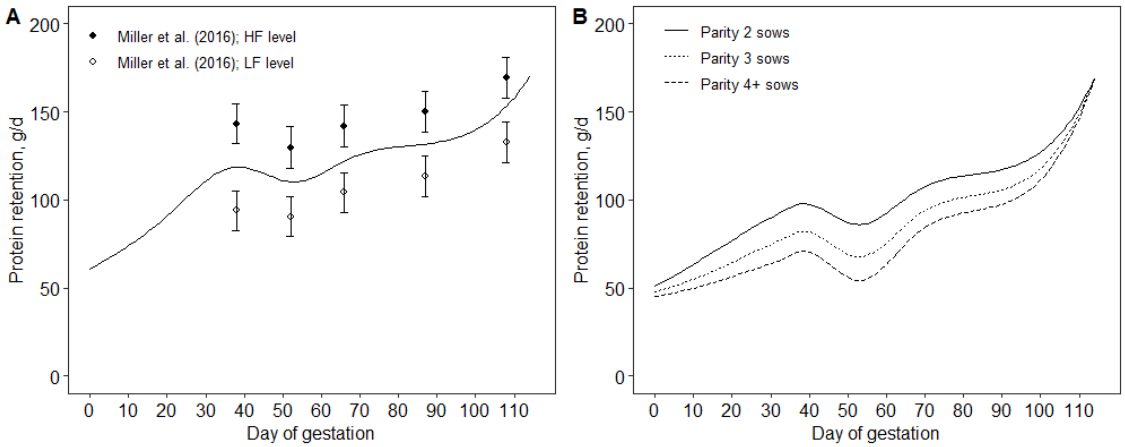


Figure 2-3. Whole body protein deposition spline for gilts (A), and multiparous sows (B). As gilts were provided with two feeding levels (HF: high feeding and LF: low feeding) providing 15% above and 15% below estimated energy requirements according to NRC (2012), the averages between these two feeding levels groups were used to represent protein deposition at the energy requirement. For multiparous sows the whole body protein deposition spline for gilts was adjusted based on the results reported by Miller et al. (2017) and Ramirez-Camba et al. (2020). Protein deposition at breeding was estimated based on NRC (2012) gestating sow model equations.

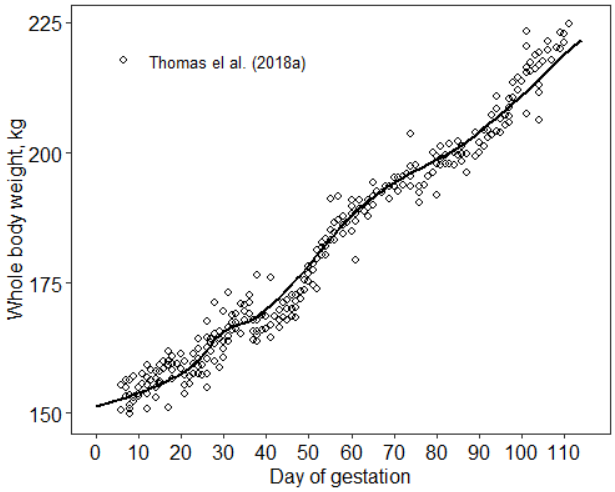


Figure 2-4. Observed body weights (○) versus model predicted weight (solid line) across gestation. The model was parameterized with litter size of 14.9, average birth weight of 1.4 kg, 14 available teats, and 151 kg of body weight at breeding for predicting the growth reported by Thomas et al. (2018a).

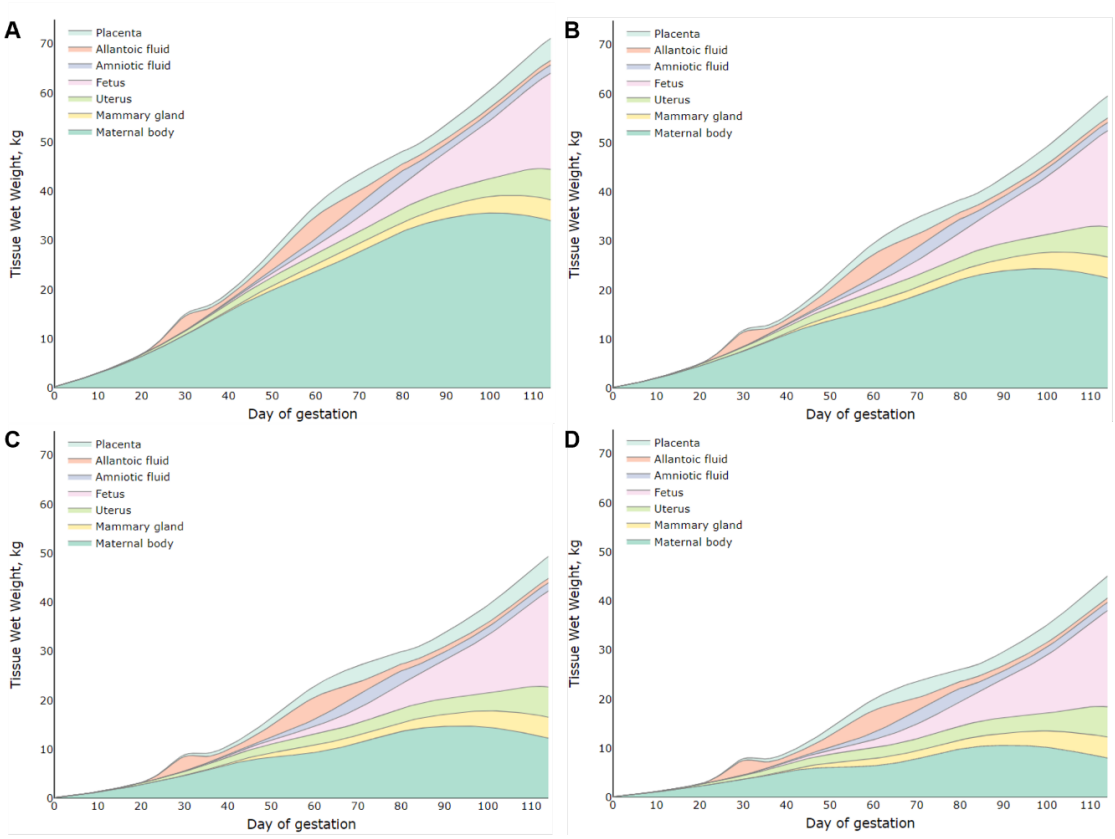


Figure 2-5. Cumulative weight gain for parity 1 (A), parity 2 (B), parity 3 (C) and parity 4 or greater sows (D) during gestation according to the proposed model. The model was parameterized as litter size = 14, average birth weight = 1.4 kg, 14 available teats, and body weight at breeding for parity 1 = 135 kg; parity 2 = 158 kg; parity 3 = 196 kg and for parity 4 or greater = 185 kg.

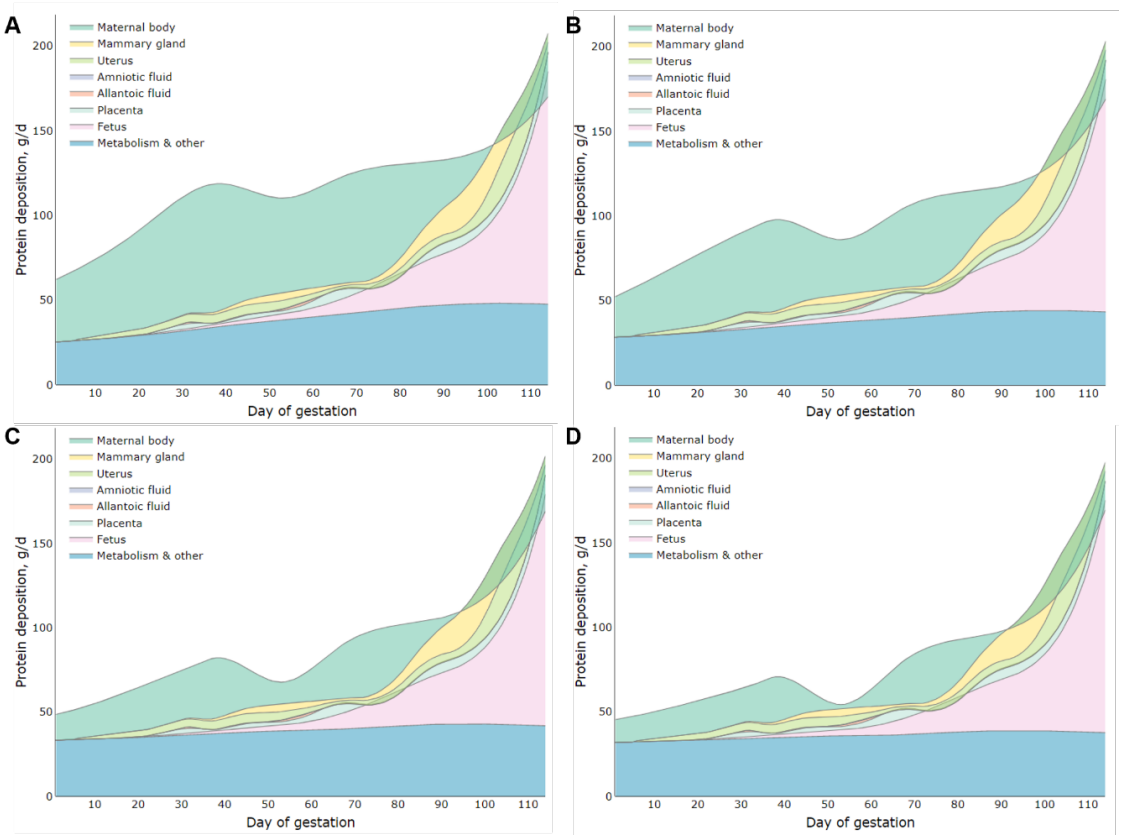


Figure 2-6. Daily protein deposition for parity 1 (A), parity 2 (B), parity 3 (C), and parity 4 + sows (D) during gestation according to the proposed model. The overlapped area in late gestation indicates a negative maternal body protein deposition, that is, maternal body protein mobilized for deposition into the products of conception. The model was parameterized as litter size = 14, average birth weight = 1.4 kg, 14 available teats, and body weight at breeding for parity 1 = 135 kg; parity 2 = 158 kg; parity 3 = 196 kg and for parity 4 or greater = 185 kg.

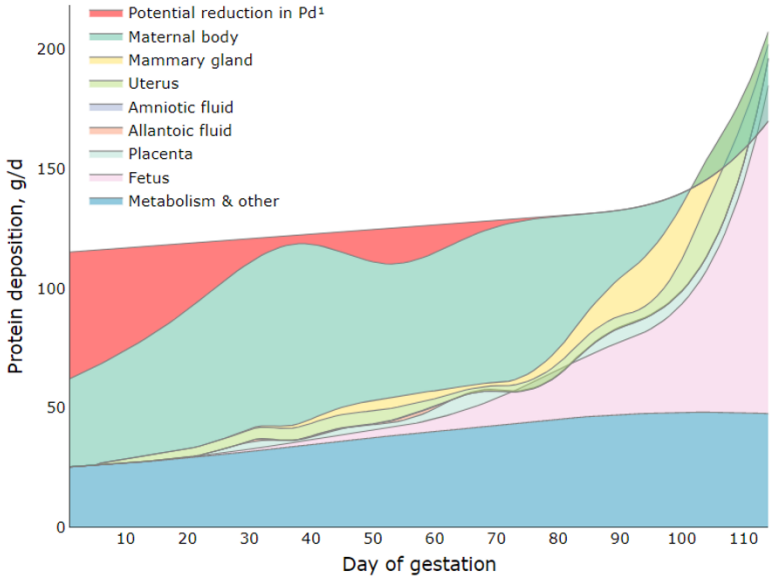


Figure 2-7. Daily protein deposition for a parity 1 sow showing a potential drop in Pd during early and mid-gestation. The model was parameterized as litter size = 14, average birth weight = 1.4 kg, 14 available teats, and body weight at breeding = 135 kg.

[1] According to Miller et al. (2019) pre-breeding Pd is similar to d 68 Pd, hence a drop in Pd during early and mid-gestation is presumed.

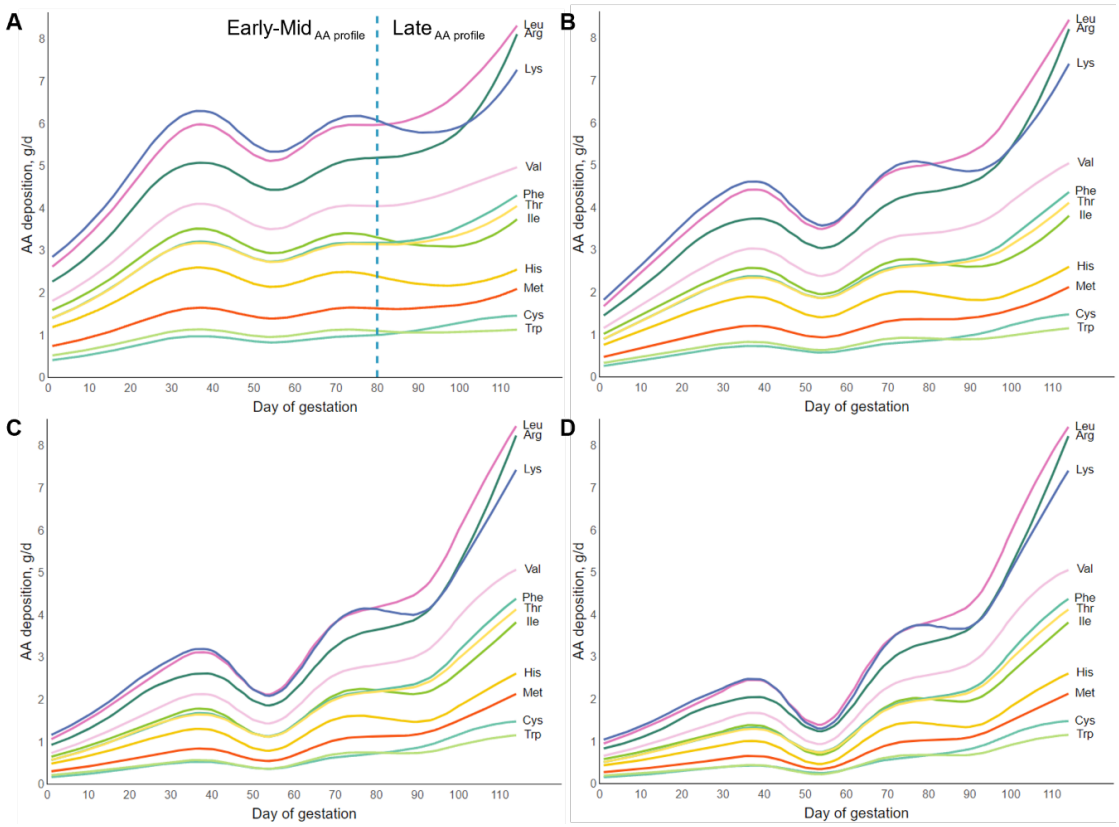
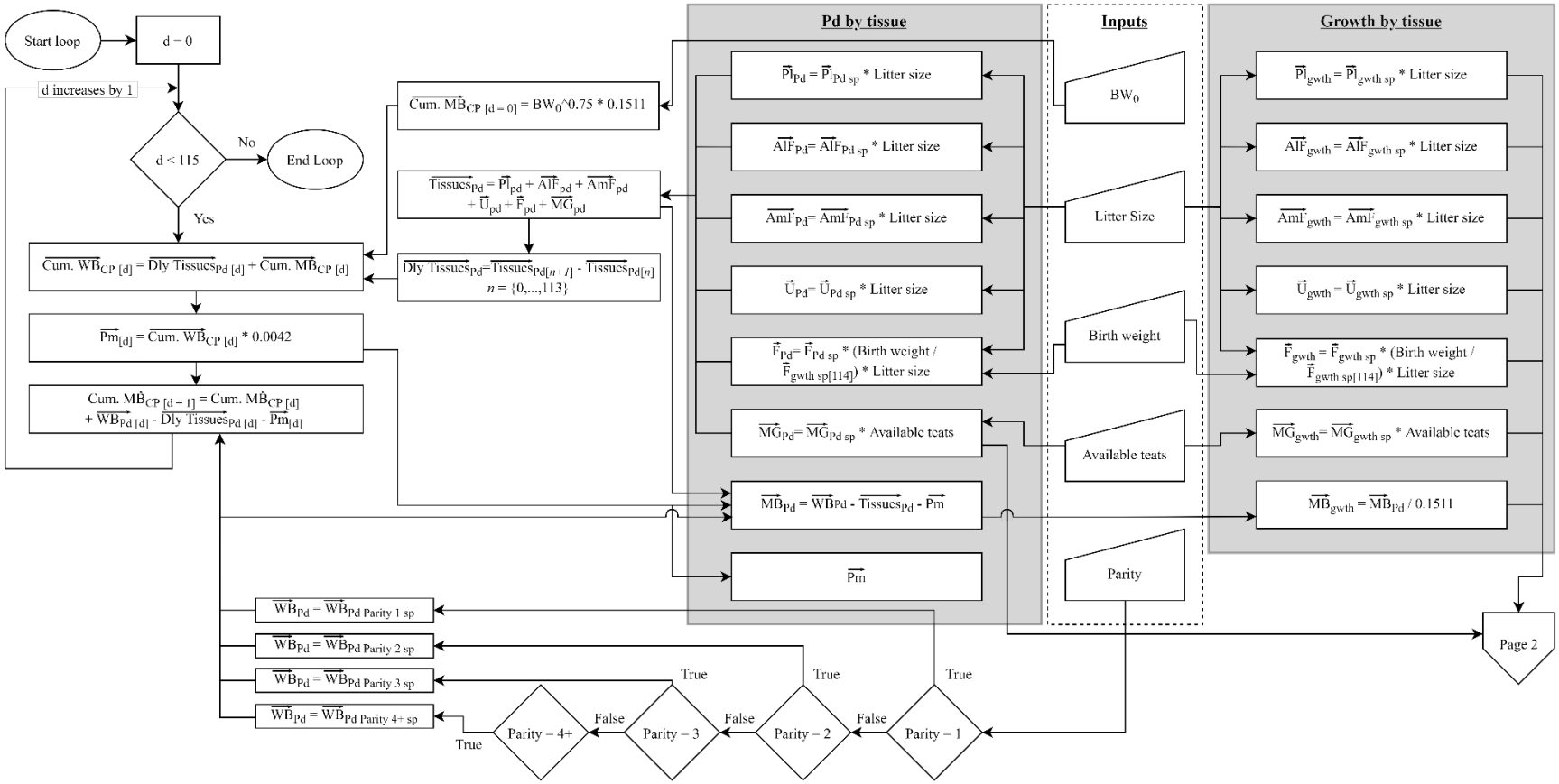


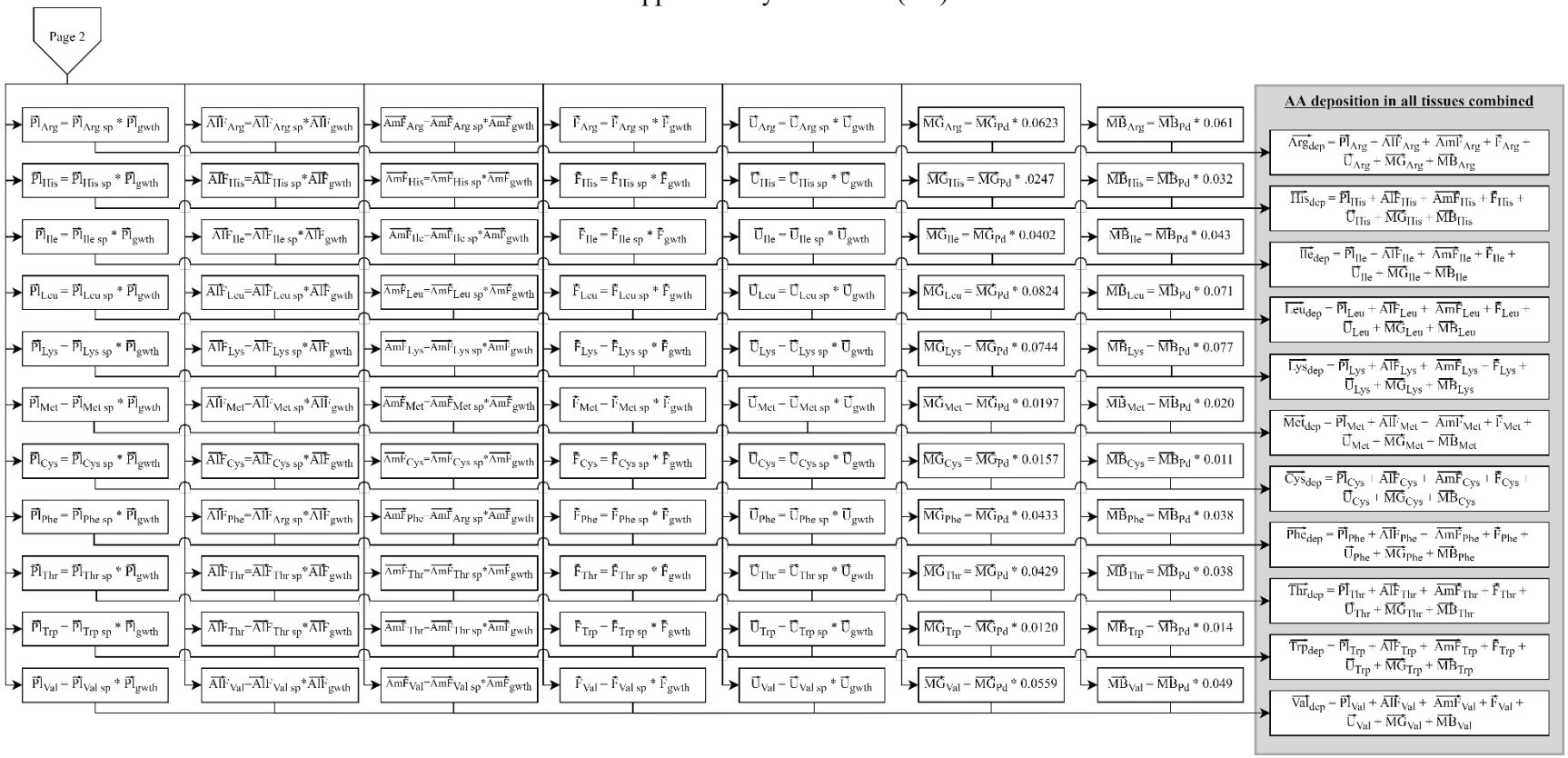
Figure 2-8. Model estimations of the essential amino acid deposition shows that during the first 80 days of gestation a relatively constant amino acid profile is deposited in the whole body of the female while after d 80 the deposited amino acid profile dynamically changes until farrowing (A). Estimations of the essential amino acid deposition for parity 1 (A), parity 2 (B), parity 3 (C), and parity 4 + sows (D) during gestation are shown. The model was parameterized as litter size = 14, average birth weight = 1.4 kg, 14 available teats, and body weight at breeding for parity 1 = 135 kg; parity 2 = 158 kg; parity 3 = 196 kg and for parity 4 or greater = 185 kg. Maternal tissue mobilization that may occur in late gestation was not considered, that is, amino acids mobilized from maternal tissue were not subtracted from the values shown.

Early-Mid$_{\text{AA profile}}$: Amino acid profile of the protein deposited during the first 80 days of gestation; Late$_{\text{AA profile}}$: Amino acid profile of the protein deposited after d 80 of gestation.

Supplementary Flowchart (1/2)



Supplementary Flowchart (2/2)



AlF: allantoic fluid; AmF: amniotic fluid; Arg: arginine; BW: body weight; BW_0: body weight at breeding; CP: crude protein; Cum.: cumulative; Cys: cysteine; dep: deposition; Dly: daily; F: fetus; gwth: growth; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; MB: maternal body; Met: methionine; MG: mammary gland; Pd: protein deposition; Phe: phenylalanine; Pl: placenta; Pm: protein for metabolism and tissues other than lean tissue; sp: spline; Thr: threonine; Trp: tryptophan; U: uterus; Val: valine; WB: whole body.

Supplementary Table 1. List of pubished studies for development of the growth, protein deposition and AA deposition model of pregnancy in sows.

Paper	Parameter	Data points available
	Model development	
Knight et al. (1977)	Placenta growth spline	d 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100
	Allantoic fluid growth and Pd spline	d 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100
	Amniotic fluid growth and Pd spline	d 30, 35, 40, 50, 60, 70, 80, 90, 100
Mahan and Shields et al. (1998)	Maternal body AA deposition	at 146 kg of body weight
Wu et al. (1998)	Allantoic and amniotic fluid AA deposition	d 40, 60
Kim et al. (1999)	Mammary gland AA deposition	d 5 of lactation
Wu et al. (1999)	Fetal growth, Pd and AA deposition splines	d 40, 60, 90, 110, 114
Ji et al. (2005)	Weight of the non-gravid uterus	d 0
Ji et al. (2006)	Mammary growth and Pd spline	d 45, 60, 75, 90, 102, 112
NRC (2012)	Whole body Pd splines (parity 1, 2, 3, 4)	d 0
Buis et al. (2016)	Percentage of metabolic protein used for metabolism	d 5, 12, 19, 26, 33, 40, 47, 54, 61, 68, 75, 82, 89, 96, 103
Miller et al. (2016)	Whole body Pd (parity 1) spline	d 38, 53, 67, 87, 108
Jang et al. (2017)	Uterus growth and Pd spline	d 43, 58, 73, 91, 101, 108
	Placenta growth and Pd spline	d 43, 58, 73, 91, 101, 108
	Placenta and Uterus AA deposition splines[1]	d 43, 58, 73, 91, 101, 108
Miller et al. (2017)	Whole body Pd splines (parity 2, 3, 4)	d 38, 53, 67, 87, 109
Ramirez-Camba et al. (2020)	Whole body Pd splines (parity 2, 3, 4)	d 50, 77, 105
	Model validation	
Mahan (1998); Cooper et al. (2001); Van der Peet-Schwering et al. (2003); Gill (2006); Dourmad et al. (2008)	Observed weight change for parity 1 sows[2]	d 0, 114
Mahan (1998); Cooper et al. (2001); Van der Peet-Schwering et al. (2003); Veum et al. (2009)	Observed weight change for parity 2 sows[2]	d 0, 114
Mahan (1998); Young et al. (2004); Van der Peet-Schwering et al. (2003); Veum et al. (2009)	Observed weight change for parity 3 sows[2]	d 0, 114
Mahan (1998); Musser et al. (2004); Veum et al. (2009)	Observed weight change for parity 4 sows[2]	d 0, 114
Thomas et al. (2018a)	Observed gilt weighs across gestation	d 8 to 114
Thomas (2019)	Performance variables	d 0

[1] Except for tyrosine

[2] Summarized by NRC (2012)

Supplementary Table 2. Model predicted AA deposition for pregnant sows.

Parity[1]	1				2				3				4+			
Day of gestation	40	60	90	110	40	60	90	110	40	60	90	110	40	60	90	110
	Standardized ileal digestible AA whole body deposition, g/d															
Lysine	6.25	5.48	5.79	6.73	4.58	3.89	4.86	6.72	3.16	2.53	4.02	6.75	2.44	1.84	3.71	6.76
Arginine	5.05	4.55	5.33	7.25	3.73	3.29	4.59	7.25	2.60	2.21	3.92	7.27	2.03	1.66	3.67	7.28
Histidine	2.57	2.23	2.21	2.40	1.87	1.57	1.82	2.39	1.28	1.01	1.47	2.40	0.98	0.72	1.34	2.41
Isoleucine	3.48	3.04	3.12	3.46	2.55	2.15	2.60	3.46	1.75	1.39	2.13	3.47	1.35	1.01	1.96	3.48
Leucine	5.94	5.34	6.16	7.80	4.40	3.87	5.30	7.79	3.09	2.62	4.52	7.82	2.42	1.98	4.23	7.83
Methionine	1.63	1.45	1.64	1.94	1.20	1.03	1.39	1.94	0.83	0.68	1.18	1.94	0.64	0.50	1.09	1.95
Cysteine	0.96	0.86	1.11	1.43	0.72	0.63	0.98	1.43	0.52	0.44	0.86	1.44	0.42	0.34	0.81	1.44
Phenylalanine	3.19	2.86	3.26	4.06	2.36	2.07	2.80	4.06	1.66	1.40	2.39	4.07	1.30	1.06	2.23	4.08
Threonine	3.16	2.83	3.19	3.80	2.33	2.04	2.73	3.80	1.63	1.37	2.31	3.81	1.27	1.03	2.16	3.82
Tryptophan	1.12	1.00	1.06	1.11	0.82	0.71	0.89	1.11	0.56	0.46	0.74	1.11	0.43	0.34	0.68	1.11
Valine	4.07	3.65	4.16	4.83	3.01	2.64	3.57	4.82	2.11	1.77	3.04	4.84	1.65	1.33	2.84	4.85

[1] The model was parameterized as litter size = 14, average birth weight = 1.4 kg, 14 available teats, and body weight at breeding for parity 1 = 135 kg; parity 2 = 158 kg; parity 3 = 196 kg and for parity 4 or greater = 185 kg.

CHAPTER 3

A NEW PERSPECTIVE ON LYSINE REQUIREMENTS IN PREGNANT SOWS THROUGH DATA-DRIVEN MODELLING.

ABSTRACT

Feeding pigs diets with an AA balance that corresponds to the animal's requirements is critical for the efficiency of the swine industry. In sows, AA requirements should be adequate for optimizing reproductive efficiency, as measured, for example, by the number of pigs produced per sow per year. However, AA requirements have traditionally been defined as the minimum AA intake that maximizes protein retention (NRC, 2012). Nevertheless, it is not clear whether the AA requirement at maximal protein retention in fact optimizes AA demands for both maternal and fetal protein. Thus, the current study seeks to identify dietary Lys levels that optimize maternal and fetal Pd. The SID Lys levels predicted to increase fetal tissue deposition are proposed as the optimal SID Lys requirement for reproduction. To investigate the dynamics of protein retention in relation to AA supply, two dose-response curves were developed using data from studies using the N-balance method and the indicator AA oxidation (IAAO) technique on pregnant sows. The developed dose-response curves enabled the calculation of SID Lys requirements, which are described by the following equation: $22.56 – 0.362 \times day + 1.46E\text{-}03 \times day^2 + 2.36E\text{-}05 \times day^3$. To validate that the calculated SID Lys requirements are adequate to optimize the sow's reproductive performance throughout gestation, a meta-analysis was conducted. Using the Rapid Review methodology, studies that investigated the effects of Lys intake on litter size, piglet birth weight, and sow BW were chosen. The meta-analysis included 17 articles published between 2004 and 2020.

The meta-analysis indicated that sow BW gain was maximized at Lys intakes corresponding to 70% of the requirement, while piglet birth weight and litter size were maximized at 100% of the Lys requirement. These findings imply that the linear-logistic model can be used to estimate dietary AA levels that maximize either lean tissue deposition or reproductive efficiency. Because current AA requirements during pregnancy are calculated as the minimum AA intake that maximizes protein retention, current Lys requirements are more likely to represent 70% of the requirement for optimal reproductive efficiency. Thus, current AA requirements appear to optimize maternal lean tissue deposition rather than conceptus development. The developed dose-response curves are presented as a tool for the estimation of AA levels that optimize physiological functions such as reproduction. The proposed Lys requirements have the potential to improve reproductive efficiency; however, further research is needed to further support this conclusion.

INTRODUCTION

Feeding sows diets with an AA balance that corresponds to the animal's requirements (i.e. precision feeding) is critical for the efficient use of dietary protein in swine production. With the increasing availability of crystalline AA and the use of technologies such as electronic sow feeders, tailored diets can be provided to individual animals; however, information on the AA requirements of pregnant sows remains limited (NRC, 2012). Hence, a lack of accurate AA requirements can be regarded as a substantial limitation in the application of precision feeding of sows.

The accuracy of AA requirement estimates is determined by various factors, one of which is the statistical method used to calculate it. The linear-plateau and curvilinear-

plateau are broken-line regression models that are the most used in AA requirement estimation (Robbins et al., 2006). While each of these models produces different estimates of AA requirements (van Milgen and Dourmad, 2015), the requirement has been defined in both cases as the lowest AA intake that maximizes the response criterion (usually protein synthesis in sow research). However, this requirement definition has conceptual flaws and limitations. For example, whether the protein synthetized at the defined AA requirement corresponds to the maternal body, the conceptus, or some combination cannot be determined. Therefore, AA requirements that aim to maximize whole body protein synthesis may not be adequate for maximizing fetal protein synthesis.

In fact, multiple authors have reported that protein synthesis is prioritized over certain metabolic functions such as the biosynthesis of metabolites that are essential for optimal fetal development (Waterlow, 1984, 1990; Young and Marchini, 1990; Campbell et al., 2001; Shimomura et al., 2001; Robinson et al., 2016). As a result, dietary AA levels for optimal fetal development could be different than those for maximum whole-body protein synthesis. If this is the case, traditional analysis does not adequately describe the full response to AA supply and may not be appropriate to model AA requirements during gestation.

The current study used a data-driven approach to develop an alternative function to broken-line regression models that not only captures dietary AA levels that maximize protein retention but also AA levels that optimize pregnant sow reproductive performance. The developed function was then used in the calculations of the SID Lys requirements during gestation and a meta-analysis was then performed for the validation of the calculated SID Lys requirements.

Data-driven research

The rise of information technology has had a significant impact on science, causing paradigms to shift. Throughout history, science has evolved from being empirical (describing natural phenomena), to theoretical (using models and generalizations), to computational (simulating complex phenomena), and nowadays data-driven (Hey et al., 2009). Data-driven science is an approach that employs analytical techniques and modes of reasoning to extract scientifically relevant insights (i.e. patterns) from data (Maass et al., 2018). In contrast to theory-driven (or hypothesis-driven) science, data-driven science extracts hypotheses (in the form of patterns or algorithms) from the data rather than creating them based on intuition or preconceived theories. These developed algorithms, once validated, can then be used to describe, or predict the behavior of similar systems.

In this study, the protein retention response pattern to graded dietary levels of AA was extracted from data published by Ramirez-Camba et al. (2020) and tested using other published works to validate that the pattern reflected animal response to AA supply.

MODEL DEVELOPMENT

Algorithm development and validation

The algorithm that described the protein retention to AA supply was developed based on empirical observations of the response of N retention to graded levels of SID Lys in diets fed to gilts at d 50 of gestation using the N balance method (Ramirez-Camba et al., 2020), estimations of SID Lys maintenance requirements and estimations of N excretion of a 140 kg gilt on a N free diet (Fig. 3-1A). It was observed that the response of protein retention to AA intake can be described by Eq. 3-1 which is a linear ($y = a +$

bx) minus a logistic ($y = \frac{c}{1+de^{-fx}}$) function and is referred to as a linear-logistic model hereafter, where *x* represents AA or protein intake, and *y* represents N or protein retention

$$y = a + bx - \frac{c}{1 + de^{-fx}} \quad \text{Eq. 3-1}$$

In comparison to linear-plateau broken line regression which has generally been used in AA requirement research (France and Kebreab, 2008; Elango et al., 2012), the linear-logistic model had a greater goodness of fit in terms of coefficient of determination (R^2) when tested in data from studies measuring protein retention using the N balance method in pigs of different ages (Fig. 3-1B to 3-1D). As a result, the linear-logistic model is believed to adequately describe the response of whole body protein retention to AA supply. Equations and parameter estimates of the comparison between the linear-plateau and linear-logistic models shown in Fig. 3-1B to 3-1D can be found in Supplementary Table 3.

In addition, a function resulting from adding a linear and a logistic model (hereafter referred to as modified linear-logistic model; Eq. 3-2) can be used to describe the dynamics of protein retention in studies using the IAAO technique. The IAAO technique is based on the concept that when the essential amino acid in study is deficient for protein synthesis, then other essential amino acids including the indicator (e.g., 1-^{13}C-Phenylalanine) will be oxidized. This technique is an empirical method for estimating whole-body protein retention in which the oxidation of the indicator is often measured rather than the protein or N retention. The modified linear-logistic model had a greater goodness of fit in terms of R^2 when tested in data from studies using the IAAO technique in growing pigs (Fig. 3-2A) and pregnant sows (Fig. 3-2B), but also in adult humans (Fig. 3-2C) and children (Fig. 3-2D). Data from human studies were used in the analysis

because data from swine studies that provided AA levels above the breakpoint (under a linear-plateau model) were limited.

$$y = a + bx + \frac{c}{1 + de^{-fx}} \quad \text{Eq. 3-2}$$

After validating that the linear-logistic and modified linear-logistic models adequately reflected the pigs' response to AA supply, it was used in the calculations of the SID Lys requirements during pregnancy.

Definition of the SID Lys requirement during gestation

The linear-logistic model describes two inflection points defined as maximum N retention inflection point (NRmax) and minimum N retention infection point in response to SID Lys intake (NRmin; Fig. 3-3A). In addition to enabling the development of the linear-logistic model, the data from Ramirez-Camba et al. (2020) revealed a significant cubic relationship (P=0.016) between SID Lys intake and the number of total piglets born of gilts fed experimental diets between d 48 to 52 of gestation preceded by 7 d of adaptation (Fig. 3-3B). As shown in Fig. 3-3, the piglets born cubic function was maximized at SID Lys intakes corresponding to NRmin. Therefore, the current modeling approach considers SID Lys intakes that correspond to NRmin in a linear-logistic model as the SID Lys requirement, based on the observation that sow reproductive performance (defined as litter size) was optimized at this dietary level. Although observations from a single dataset may not be sufficient to sustain a new AA requirement definition during gestation, subsequent sections and observations provide additional support. For research that used the IAAO technique, the Lys intake corresponding to the inflection point where the indicator oxidation is maximized (or protein retention is minimized) under a modified linear-logistic model is considered as the requirement under the current methodology.

Calculation of the SID Lys requirement during gestation

As shown in Fig. 3-3A, the SID Lys requirement for pregnant gilts at d 50 of gestation (average between d 48 and 52) was considered to be 11.1 g SID Lys/d. In addition, NRmax occurred at 7.8 g SID Lys which corresponds to 70% of the SID Lys requirement. Because of a lack of additional data, it was assumed that NRmax occurs at 70% of the SID Lys requirement throughout pregnancy (Eq. 3-3).

$$SID\ AA\ requirement = \frac{SID\ AA\ intake\ at\ NRmax}{0.70} \qquad \text{Eq. 3-3}$$

Therefore, applying Eq. 3-3 to estimated NRmax values obtained by fitting linear-logistic models on the data reported by Ramirez-Camba et al. (2020) [i.e. the response of N retention to graded levels of SID Lys intake at d 77 (NRmax=9.9) and d 105 (NRmax=18.5)], the SID requirement was calculated to be 14.1 and 26.4 g SID Lys/d, respectively. The data reported by Ramirez-Camba et al. (2020) did not allow a direct estimation of the SID Lys requirement (NRmin) for d 77 and d 105.

Interestingly, the trend formed by the SID Lys requirements calculated herein using a linear-logistic model for d 50, 77, and 105 (11.1, 14.1 and 26.4 g SID Lys/d, respectively) matched the SID Lys requirements calculated by Feyera and Theil (2017) for the transition period (d 112 to 115). Therefore, the SID Lys requirement of 36.2 g SID Lys for d 115, as recommended by Feyera and Theil (2017), was factored into the SID Lys requirements calculations during gestation.

The SID Lys requirement for early gestation was calculated based on Samuel (2011) who reported the response of protein retention to graded levels of Lys in pregnant

sows during early gestation (d 24 to 45) using the IAAO technique. Samuel (2011) dietary Lys content (total basis) was converted to SID basis based on NRC (2012) ingredient digestibility values. By fitting a modified linear-logistic model on the data reported by Samuel (2011), the SID Lys requirement for d 34 was calculated to be 12.75 g/d (Fig. 3-2B).

Then, the calculated SID Lys requirements for d 34, 50, 77, 105 and 115 (12.75, 11.1, 14.1, 26.4 and 36.2 g SID Lys/d, respectively) were fitted with different linear and nonlinear models from which a cubic model produced the best goodness of fit in terms of R^2 (Eq. 3-4 and Fig. 3-4).

$$\textit{SID Lys reqmt., g/d} = 22.56 - 0.362 \times day + 1.46E\text{-}03 \times day^2 + 2.36E\text{-}05 \times day^3 \quad \text{Eq. 3-4}$$

The cubic function that describes the SID Lys requirements (Fig. 3-4) suggests an increase in SID Lys needs during early gestation which is supported by multiple authors that show that increased AA intake during the first weeks of gestation increase luteal tissue mass, embryo survival and the maintenance of pregnancy as reviewed by Langendijk (2021). Despite being based on empirical data, the Lys requirements previously calculated must be validated, that is, it must be verified that these requirements are adequate to optimize the sow's reproductive performance throughout gestation.

Validation of the calculated SID Lys requirements

To determine whether the calculated SID Lys requirements achieved the intended goal of maximizing sow reproductive performance, empirical effects of SID Lys intake

relative to the calculated SID Lys requirements (Eq. 3-4) were evaluated. In other words, the validation analysis sought to determine what happened to litter size and litter weight when sows were given different proportions (percentages) of the calculated SID Lys requirement. For this purpose, data from published scientific studies examining the effect of increased SID Lys intake at various gestational timepoints on litter size, litter weight, and sow BW gain were chosen.

The scientific articles were selected following a Rapid Review approach and the data analyzed using the Meta-Analysis methodology of (Mikolajewicz and Komarova, 2019). The relevant data was extracted from the selected studies, but not conclusions, data interpretation or data analysis performed by the authors. Articles published before October 2021 (search date) and available in the main databases for science research were considered (PubMed, ISI Web of Science, Science Direct, Scopus and SciELO). The eligibility of the studies was determined under the following criteria:

- Only studies written in English were considered.
- Articles published more than 15 years prior to the search date were not considered.
- Data from abstracts, oral presentations, thesis, or dissertations were not considered.
- The selected studies reported Lys content on a SID basis or allow for the estimation of SID Lys content by reporting dietary ingredients. For studies that reported dietary Lys on a total basis, the ileal digestibility of the diet was determined using NRC (2012), and then the dietary SID Lys content was calculated.
- Studies that reported methodological issues, such as unexplained responses caused by factors other than dietary SID Lys intake, were not considered.

The variables considered in the studies that provided increased SID Lys intake levels during mid and late pregnancy (> d 30 of gestation) were piglet birth weight and sow BW (Table 3-1). The variables considered in the studies that provided increased SID Lys intake levels during early gestation (< d 30 of gestation) were litter size and sow BW (Table 3-2). Although dietary interventions after day 30 may influence litter size, it was assumed that differences in litter size were more likely to be detected during early gestation. Similarly, it was assumed that dietary interventions during the first 30 days of pregnancy would have little effect on litter birth weight.

To quantify the effect of increased SID Lys intake on reproductive performance, for each study the increment (or reduction) in piglet birth weight or litter size was calculated. Similarly, to quantify the effect of increased SID Lys intake on sow maternal BW, the daily weight gain increment during the experimental period was calculated.

MODEL VALIDATION

Piglet birth weight and litter size are predicted to be maximized at SID Lys intakes corresponding to the SID Lys requirement (Fig. 3-5A and Fig. 3-5B). In addition, based on the studies represented in Fig. 3-5A at levels close to 70% of the requirement, it is predicted that maternal BW gain is maximized. Furthermore, Fig. 3-5B suggests that during early gestation, SID Lys intakes greater than the requirement are associated with an increase in maternal BW gain and a decrease in litter size. When using a linear-logistic model to describe the potential changes in protein (or N) retention that occur at different proportions of the SID Lys requirement, increases in protein retention are associated with increases in maternal BW gain (Fig. 3-5C). On the other hand, at the SID Lys requirement, where maximum litter size and piglet birth weight are predicted, there

appears to be a reduction in the efficiency of protein utilization. Interestingly, at maximum fetal growth a reduction in the efficiency of protein utilization is expected for reasons that are discussed in the next section.

Biological phenomena described by a linear-logistic response

Multiple authors have shown that at low AA intakes, the rate at which AA enter pathways of protein synthesis is increased relative to the rate at which AA enter pathways associated with certain metabolic processes (Waterlow, 1984, 1990; Young and Marchini, 1990; Campbell et al., 2001; Shimomura et al., 2001; Robinson et al., 2016). Transmethylation reactions, required for the biosynthesis of essential metabolites for fetal development [e.g., Tau; Holm et al. (2018)], are sacrificed to maintain protein synthesis when Met intake levels are low (Young and Marchini, 1990; Robinson et al., 2016). Low intakes of Val and Leu have also been linked to decreased metabolic functions without reductions in protein synthesis (Young and Marchini, 1990; Shimomura et al., 2001). The data reported by Bhargava et al. (1970) and reanalyzed by France and Kebreab (2008) show that when chicks challenged with the Newcastle virus were fed diets with increasing levels of Val, both growth and antibody titres improved, but the antibody titre response increased further after the animal had reached a plateau in its growth (Fig. 3-6). The previous observations suggest that when AA intake is low, protein synthesis takes precedence over metabolic functions, and that certain metabolic functions increase at AA intake levels greater than those for maximum protein synthesis. These findings support the current study's AA requirement definition, in which SID Lys intake levels that maximize reproductive performance are higher than minimum SID Lys intakes for maximum protein retention (traditional AA requirement definition).

The increased and decreased protein retention responses predicted by the linear-logistic model (NRmax and NRmin, respectively) could be related to potential changes in metabolic functions at various AA intake levels. Young et al. (1987) observed that in healthy adult humans, low Leu intake levels led to higher N retention compared to Leu intakes deemed as adequate when considering protein turnover rates. Protein turnover is an inefficient process; for example, in healthy adult humans consuming a well-balanced protein diet, 300 g/d of protein turnover was measured, of which 75–80 g was lost via oxidative pathways and 14 g was lost at the ileal level (Tomé and Bos, 2000). Therefore, increased protein turnover would lead to increased protein excretion. During fetal development, cell proliferation rates increase protein turnover rates (Alenzi, 2004; Krisher and Prather, 2012). Thus, at AA intake levels that maximize fetal development, protein turnover is expected to increase, accompanied by an increase in protein excretion and, as a result, an overall decrease in net protein retention. Therefore, increased protein retention at low AA intake, as described by the linear-logistic model and dubbed NRmax, could be due to a reduction in inherently inefficient metabolic functions like protein turnover, whereas reduced protein retention at the SID Lys requirement (NRmin), could be due to increased metabolic functions like protein turnover associated with fetal growth.

Overall, the evidence presented thus far suggests that current AA requirements for pregnant sows are underestimated. This hypothesis, however, contradicts the findings of several authors who report increased AA intake (e.g., bump feeding) did not improve or had negative impacts on sow performance (Shelton et al., 2009; Gonçalves et al., 2016; Mallmann et al., 2018; Mallmann et al., 2019; de Oliveira Araújo et al., 2020). The

section that follows discusses possible reasons for why AA intake levels above current recommendations have not been beneficial.

Do sows really need more AA during gestation?

Shelton et al. (2009), Gonçalves et al. (2016), Mallmann et al. (2018), and Mallmann et al. (2019) provided levels of SID Lys from very low AA levels (<50% of the SID Lys requirement) to levels close to 70% of the SID Lys requirement or NRmax, respectively (Table 3-1) in late gestation. All of these studies concluded that increased Lys intake in late gestation had no positive effect on piglet birth weight, litter size and sow BW but had negative effects such as increases in mummified fetuses (Mallmann et al., 2018), stillborn piglets (Gonçalves et al., 2016; Mallmann et al., 2019), and decreased colostrum yield (Mallmann et al., 2019). On the other hand, Heo et al. (2008) and Zhang et al. (2011) provided SID Lys from 70% to 100% of the proposed Lys requirement and observed increases in colostrum dry matter and colostrum protein. Heo et al. (2008) reported increased piglet growth rate during lactation (Table 3-1). In addition, Magnabosco et al. (2013) and Seoane et al. (2020) who provided increased SID Lys from levels close to 85% of the SID Lys requirement to 115% and 145% of the SID Lys requirement, respectively, also reported positive effects of increased SID Lys intake (Table 3-1).

As a result, the effects of increased SID Lys intake appear to be dependent on the doses provided in comparison to the control. Thus, very low AA intakes (<50% of the requirement) appear to have a positive effect on reproduction when compared to low AA intakes (~70 percent of the requirement), and levels close to the requirement appear to improve reproductive performance when compared to low AA intakes. As previously

stated, there appears to be a higher priority for protein synthesis in the form of maternal lean tissue deposition at low AA intakes, with associated reductions in physiological functions. As a result, it appears feasible to recommend avoiding levels near 70% of the requirement (or NRmax). However, NRmax more closely resembles current AA requirements, as shown in Fig. 3-1, which suggests that current AA requirements for pregnant sows should be revised.

There were no empirical studies found in the literature that evaluated the effects of SID Lys intake levels corresponding to the proposed requirement versus very low SID Lys intake levels for which a direct comparison could be made. More research is needed to quantify this relationship, but feeding SID Lys levels to the requirement as proposed by the model herein appears to be more adequate than feeding sows very low AA levels for reasons that will be discussed further below. The section that follows discusses how research on wild boars may help to explain why metabolic functions appear to improve at very low AA intakes compared to low AA intakes.

What can wild boar teach us about protein utilization efficiency?

In a rich habitat with easy access to food, wild boar restrict their movement to a smaller area, whereas in a poor nutritional environment, wild boar travel more in search of food and water (Morelle et al., 2015). It is therefore possible that, at low AA intakes, the priority for mobility relative to the priority for reproduction increases. In addition, because there is an increased risk of predation associated to increased mobility (Morelle et al., 2015), improved lean body mass (and thus protein synthesis) is important because it is positively correlated to running speed (Christiansen, 2002; Hirt et al., 2017).

Prioritizing Pd at low AA intakes may thus increase survivability in wild boar, a condition that, while redundant, may persist in the domesticated pig.

Is feeding pregnant sows with a very low AA intake an efficient dietary strategy?

One of the main reported benefits of feeding pregnant sows at very low SID Lys levels, or <50 % of the requirement (compared to low SID Lys levels; ~70% of the requirement) is that sows mobilize more AA during lactation (increased sow BW loss), therefore increasing piglet weaning weight (Shelton et al., 2009; Mallmann et al., 2018; Che et al., 2019; Mallmann et al., 2019). However, increased BW loss during lactation has been associated with many negative metabolic effects such as increased wean-to-estrus intervals, incidence of anestrus, and return to estrus post insemination resulting in reduced farrowing rate (Koketsu et al., 1996; Tantasuparuk et al., 2001; Kemp and Soede, 2004; Thaker and Bilkei, 2005; Hoving et al., 2010; Schenkel et al., 2010; Hoving et al., 2012b; Koketsu et al., 2017; Tokach et al., 2019). Feeding sows with very low SID Lys levels during gestation seems to provide short term benefits with potential negative mid to long-term consequences for the reproductive longevity of the sow.

A potential positive effect of feeding sows very low AA (and low AA) intake levels seems to be the increased efficiency of N utilization (Zhang and Trottier, 2019). However, as previously discussed, increased N efficiency does not appear to equate to increased reproductive efficiency. In fact, improving short-term N utilization efficiency may result in long-term reduced N efficiency and reduced reproductive longevity.

It is critical to recognize that the dietary strategy of providing sows with very low AA intake levels carries significant risks. For example, the incidence of stressful events such as immune challenges could cause sows to divert nutrients to immune response and

other physiological functions that would normally have a lower priority. That is, in disease-challenged sows fed at very low AA intake levels, nutrients diverted for reproduction and metabolic functions other than immune response may be reduced to extremely low levels, causing herd productivity to suffer. Empirical evidence suggests that current AA requirements are insufficient for maintaining the performance of health-challenged pigs (Schweer et al., 2018; Jasper et al., 2020), and that providing levels below the requirement carries an increased risk of reduced sow performance.

Increased aggression is another possible side effect of feeding sows with very low and low AA intakes. Evidence reported for wild boars hasshown that when nutrient supply is low, animals increase aggressive behavior (Esposito et al., 2021). According to Esposito et al. (2021), stress caused by low food resources in wild boar (specifically in lactating females) leads to increased competition for food and the maintenance of herd structures Similarly, in domesticated pigs, when protein supply is low damaging behavior increases (Meer et al., 2017).

In the current study, the SID Lys intake levels deemed as the requirements are proposed as an alternative to low and very low AA intake levels. The improved wean weight of sows fed very low SID Lys levels has also been reported at SID Lys intakes corresponding to the requirement, in addition of other metabolic improvements such as improved colostrum and milk quality and reduced wean to estrus (Table 3-1). Furthermore, if immune response demands increase, health-challenged sows fed to the SID Lys requirement may not experience extremely low AA availability for whole-body metabolic functions. Also, feeding sows to the proposed SID Lys requirements may reduce aggressive behavior in sows, though data on this is limited. As a result, the

metabolic and reproductive benefits of feeding sows at very low SID Lys intake levels are thought to be outweighed by the benefits of feeding sows at the requirement; however, empirical evidence is required to back up this assertion.

Feeding sows at very low SID Lys levels does not appear to be an efficient strategy in general; however, pregnant sows are commonly fed in commercial settings using a visual scoring system, which may be more similar to a low AA intake strategy, as discussed in the following section.

Is feeding pregnant sows based on a body condition score an efficient dietary strategy?

Gestation feeding based on BCS may promote maternal tissue deposition rather than conceptus development. It is predicted that at SID Lys intake levels close to 70% of the requirement maternal weight gain is promoted (Fig. 3-5A), most likely in the form lean body mass deposition. If feeding strategies aim to provide diets to sows up to the point where optimal body condition score (BCS) is achieved, but this BCS is achieved primarily through increased lean tissue deposition, the dietary interventions may provide sows with SID Lys intake levels close to NRmax, or 70% of the requirement. As also shown in Fig. 3-5, at this SID Lys intake reductions in piglet birth weight and litter size are predicted. Therefore, optimal BCS may be achieved primarily through increased lean tissue deposition, which does not optimize reproductive parameters.

This claim is supported by the findings of Maes et al. (2004), who observed that sows with less backfat at the end of gestation had a significantly higher percentage of stillborn piglets. In addition, gilts with less backfat depth at first breeding have reduced total piglets born in the sow's lifetime (Rozeboom, 2015). Accordingly, if body fat measurements are not considered, feeding sows solely on BCS may promote maternal

lean tissue deposition; thus, sows appear to be in good condition, despite their potential suboptimal reproductive performance and metabolic status.

In addition, our findings show that at the SID Lys requirement, the priority for maternal tissue deposition and N retention decreases, suggesting that at SID Lys intakes close to the requirement, body fat is expected to be a greater contributor to the weight gain associated with increments in BCS in comparison to the composition of the weight gain associated with increments in BCS at SID Lys levels above or below the requirement. Therefore, our results suggest that instead of BCS, measures of body fat are preferable. Similarly, Maes et al. (2004) concluded that backfat measures, rather than BCS, are a useful tool to monitor and improve the productivity and efficiency of high producing sow herds. Nevertheless, fat deposition is also related to energy intake levels, so accurate estimates of energy to SID Lys ratios are needed.

Effects of energy on SID Lys requirements

The experimental diets in the studies considered for the validation of the SID Lys requirements (Table 3-1 and Table 3-2) provided 3103±187 kcal metabolizable energy (ME) per kg of feed (mean± standard deviation) and 501±127 kcal ME per g of SID Lys considering our calculated requirements. Hence, the proposed daily SID Lys requirements described by Eq. 3-4 are expected to improve sow reproductive performance in the context of ~500 kcal ME / g SID Lys. Different levels of energy may result in different SID Lys requirements. In this regard, Bikker (1994) data shows that gilts weighing 20 to 45 kg fed 2.5 or 3.0 times their energy requirements for maintenance used that extra energy to increase N retention, which also caused the N requirement to increase (Fig. 3-7). Similarly, Miller et al. (2016) demonstrated that energy is a major

factor influencing Pd and, and therefore AA requirements in pregnant sows. Consequently, our SID Lys requirements are expected to maximize reproductive performance and metabolic status in the context of ~500 kcal ME per g SID Lys intake, however, more research is needed to determine the optimal energy to SID Lys ratio.

Future research

The information presented in this study suggests that current SID Lys requirements are underestimated, and therefore, other essential AA are likely underestimated. Using the N balance technique, Dourmad and Etienne (2002) detected a significant quadratic increase ($P<0.05$) of N retention to graded levels of SID Thr intake; maximized at an intake of 7 g of SID Thr/d during the whole pregnancy period. Shi et al. (2018) also provided graded levels of SID Thr across gestation and observed that an average intake of 9.9 g SID Thr/d maximized litter birth weight, and immunoglobulins (IgG and IgA), prolactin and progesterone concentrations. Thus, considering the results of Dourmad and Etienne (2002) and Shi et al. (2018), it was estimated that NRmax occurred at 7 g SID Thr/d and the SID Thr requirement (NRmin) at 9.9g/d which agree with Eq. 3-3, where, NRmax is expected at ~70% of the SID Thr requirement. Thus, based on Dourmad and Etienne (2002) and Shi et al. (2018) the SID Thr requirement seems to be underestimated in a similar proportion as Lys.

However, with the data available in the literature, it was not possible to estimate NRmax or NRmin for other essential AA. Hence, it is not known to what extent the requirements of other AA are erroneous. Estimates of the requirements for other essential AA become important even for studies evaluating the effects of SID Lys on sow reproductive performance to ensure that they do not become limiting factors.

The linear-logistic model developed for the current study is proposed as an alternative to the traditionally used broken-line regression models. The results of this study suggest that the linear-logistic model allows for the indirect estimation of dietary AA levels that optimize reproduction (and potentially other metabolic functions) using protein retention measurements. Furthermore, using the linear-logistic model in AA requirement research may help to reduce time and costs associated with estimating AA intake that maximize health, specially when minimally invasive methods such as the IAAO technique are used.

Conclusions

The current data-driven approach provides qualitative and quantitative insights into the pregnant sow's dynamic responses to different levels of SID Lys supply. The linear-logistic model is presented as an alternative to the commonly used broken-line regression models, with the added potential benefit of allowing for indirect estimation of dietary AA levels that optimize physiological functions such as reproduction. Predictions made using the linear-logistic model allowed for the calculations of the SID Lys requirements throughout gestation (Eq. 3-4). The calculated SID Lys requirements suggests that current SID Lys requirements are underestimated and likely promote maternal lean tissue deposition rather than conceptus development. Finally, because mathematical models are a result of experimental research rather than a replacement, data must be collected in vivo to validate the conclusions.

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Table 3-1. Experiments evaluating the impact of increased SID lysine intake on sow body weight and piglet birth weight were used to validate the calculated SID Lys requirements.

Source	Litters per treatment, n	Experimental period		Control	Treatment		Increased by treatment		Additional observations	
		days	SID Lys reqmt.[1]	SID Lys, g/d	SID Lys, g/d	% of reqmt.	Sow BW, kg/d	Piglet BW, kg		
Seoane et al. (2020)	31	77 - 107	20.69	18.00	30.00	**145%**	NR	0.03	↑ litter weaned weight	↑ litter size subsequent parity
									↑ Backfat from d 77 to 107	↓ Backfat from d 107 to weaning
Magnabosco et al. (2013)	139	85 - 110	23.55	20.00	27.00	**115%**	-0.03	0.01	↓ Within-litter birth weight variation (CV)	
									↓ Stillborn piglets	↓ Piglets <1.1 kg
Zhang et al. (2011)	50	30 - 110	16.01	10.00	17.10	**107%**	0.14	0.19	↑ Colostrum dry matter	↑ Colostrum protein
									↑ Blood prolactin (d 100)	↓ Blood urea N (d 110)
									↑ Insulin (d 110)	
Heo et al. (2008)	6	80 - 115	23.99	16.00	22.20	**93%**	0.12	0.16	↑ litter weaned weight	↑ piglet growth rate
									↑ Colostrum and milk dry matter, protein, and solid-non-fat	
									↑ Luteinizing hormone post farrowing and at weaning (gilts)	
Mallmann et al. (2019)	244	90 - 115	26.77	11.52	21.12	**79%**	0.47	-0.01	↓ Wean to estrus interval	↑ Piglets <1 kg
									↑ Stillborn piglets	↑ Sow backfat and BW at weaning
									↓ Colostrum yield	↓ Voluntary intake during lactation
Che et al. (2019)	29	90 - 115	26.77	14.70	20.60	**77%**	0.13	0.10	↑ Backfat from d 90 to 110	↑ Lactation BW loss
									↑ Piglet weaned weight	↑ Farrowing duration
Gonçalves et al. (2016)										
gilts	21	90 - 115	26.77	10.70	20.00	**75%**	0.18	0.01	↓ Pre-weaned mortality	↑ Piglet weaned weight
sows	21	90 - 115	26.77	10.70	20.00	**75%**	0.15	-0.01	↑ Stillborn piglets	
Shelton et al. (2009)										
gilts	22	90 - 115	26.77	11.90	17.10	**64%**	0.21	0.09	↓ Wean to estrus interval	↑ piglet growth rate
sows	32	90 - 115	26.77	11.90	17.10	**64%**	0.20	-0.11	↑ Lactation BW loss (not in P3+)	
Mallmann et al. (2018)										
gilts	50	90 - 115	26.77	11.70	14.30	**53%**	0.10	0.01		
sows	145	90 - 115	26.77	11.70	14.30	**53%**	0.14	0.00	↑ Mummified fetuses	↑ Lactation BW loss

BW= body weight; CV = coefficient of variation; Lys = lysine; N = nitrogen; NR = not reported; P3+ = parity 3 or more sows; reqmt. = requirement; SID = standard ileal digestible.

[1] Average SID Lys requirement for the experimental period calculated according to Eq. 3-4.

Table 3-2. Experiments evaluating the impact of increased SID Lys intake on sow body weight and litter size were used to validate the calculated SID Lys requirements.

Source	Litters per treatment, n	Experimental period		Control	Treatment		Increased by treatment	
		days	SID Lys reqmt.[1]	SID Lys, g/d	SID Lys, g/d	% of reqmt.	Sow BW, kg/d	Litter size, n
Virolainen et al. (2004)	6	1 - 35	16.94	12.58	27.05	**160%**	1.21	NR
Virolainen et al. (2005)	6	1 - 35	16.94	12.58	25.16	**149%**	NR	-4.60[2]
Mallmann et al. (2020)	117	6 - 30	16.80	12.24	21.76	**130%**	0.25	-1.10
Hoving et al. (2012)	13	3 - 35	16.63	15.50	20.15	**121%**	0.63	-0.20[2]
Mallmann et al. (2020)	117	6 - 30	16.80	12.24	17.00	**101%**	0.08	0.00
Hoving et al. (2011)	47	3 - 33	16.88	11.75	16.45	**97%**	0.29	2.00
Hoving et al. (2011)	47	3 - 33	16.88	13.20	15.00	**89%**	0.05	0.40
Quesnel et al. (2010)	15	1 - 27	21.15	10.00	12.59	**60%**	NR	-0.50[2]

BW= body weight; Lys = lysine; NR = not reported; reqmt. = requirement; SID = standard ileal digestible.

[1] Average SID lysine requirement for the experimental period calculated according to Eq. 3-4.

[2] Measured as number of viable embryos at slaughter.

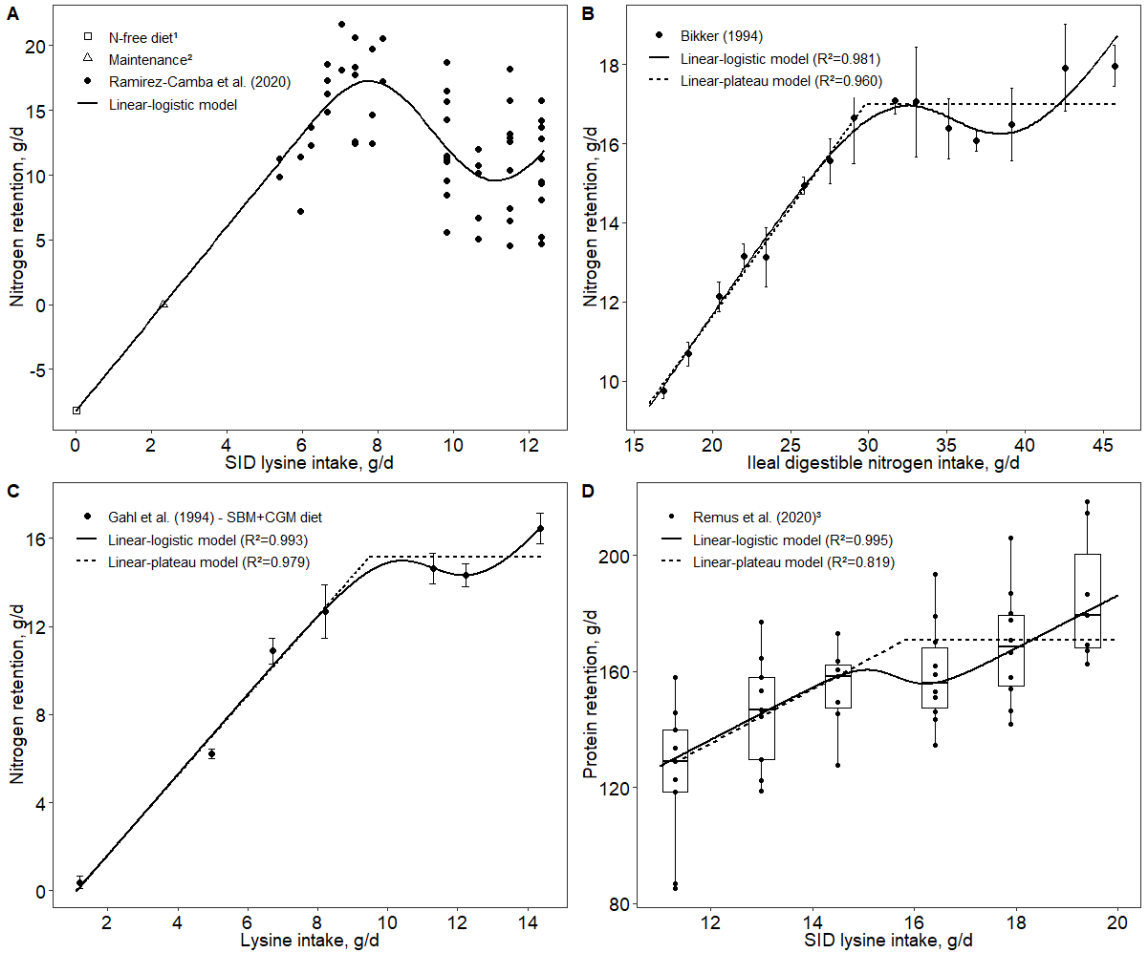

Figure 3-1. The linear-logistic model was developed using nitrogen balance data from gilts at 50 days of pregnancy (A). The linear-plateau broken line regression model and the linear-logistic model's goodness of fit in terms of coefficient of determination (R^2) are shown in data from weaned (B), and growing pigs (C; D).

SMB+CGM diet: Soybean meal and corn gluten meal-based diet

[1] Predicted negative nitrogen retention for a 140 kg pig fed a nitrogen free diet based on Otto et al. (2003).

[2] The SID Lys maintenance requirement for a 140 kg gilt (fed 2.1 kg of a corn-soybean meal diet per day) include the basal endogenous losses (0.522 g/kg dry matter intake; Stein et al., 1999) plus losses due to integuments (4.5 mg/kg $BW^{0.75}$ per day) and basal turnover of protein (23.9 mg/kg $BW^{0.75}$ per day) according to Moughan (1999).

[3] Models were fitted using the median protein retention (50^{th} quantile shown by the boxplot) at each intake level to reduce the effect of influential observations.

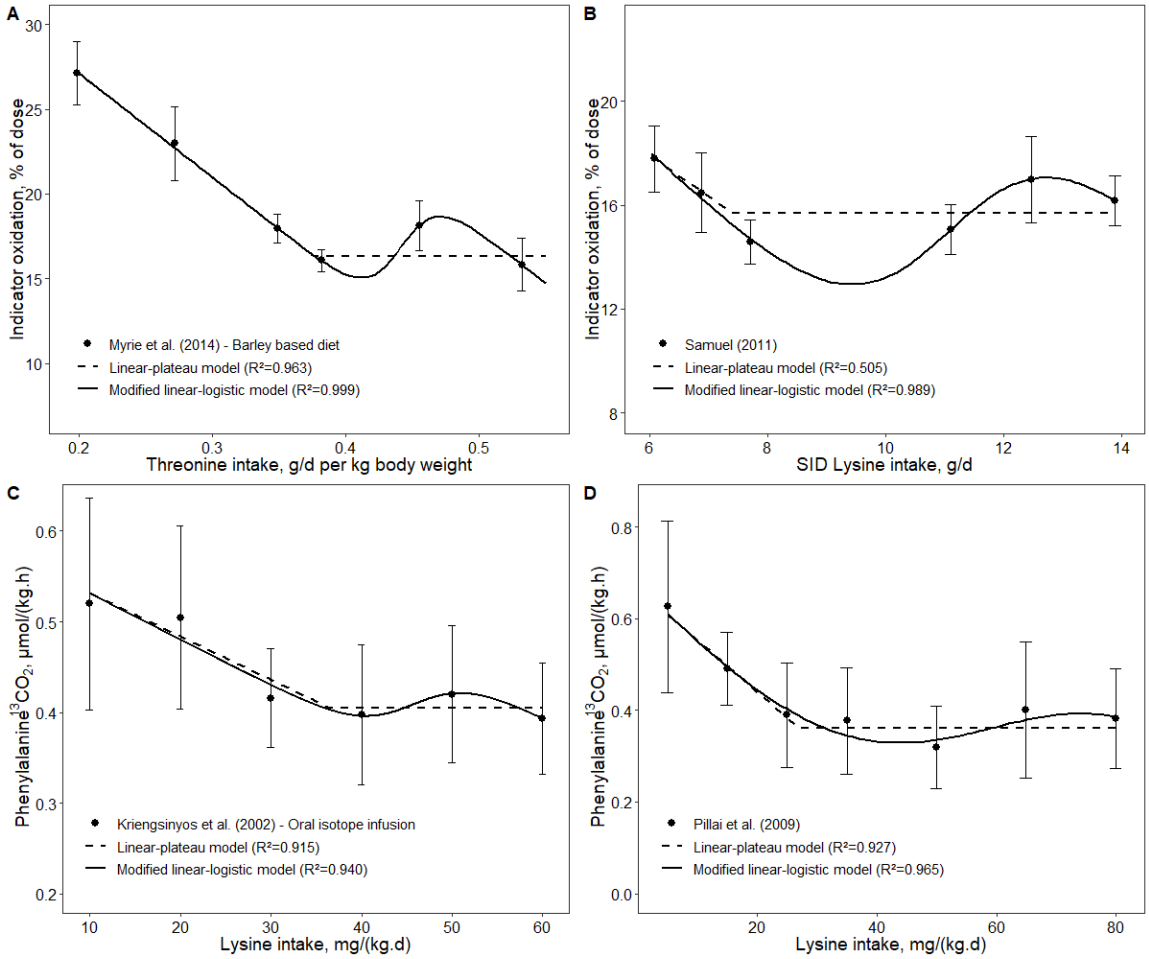

Figure 3-2. Comparison of the goodness of fit in terms of coefficient of determination (R^2) between the linear-plateau regression model and the linear-logistic model in data from growing pigs (A), pregnant sows during early gestation (d 24 – 45; B), adult humans (C), and children (D).

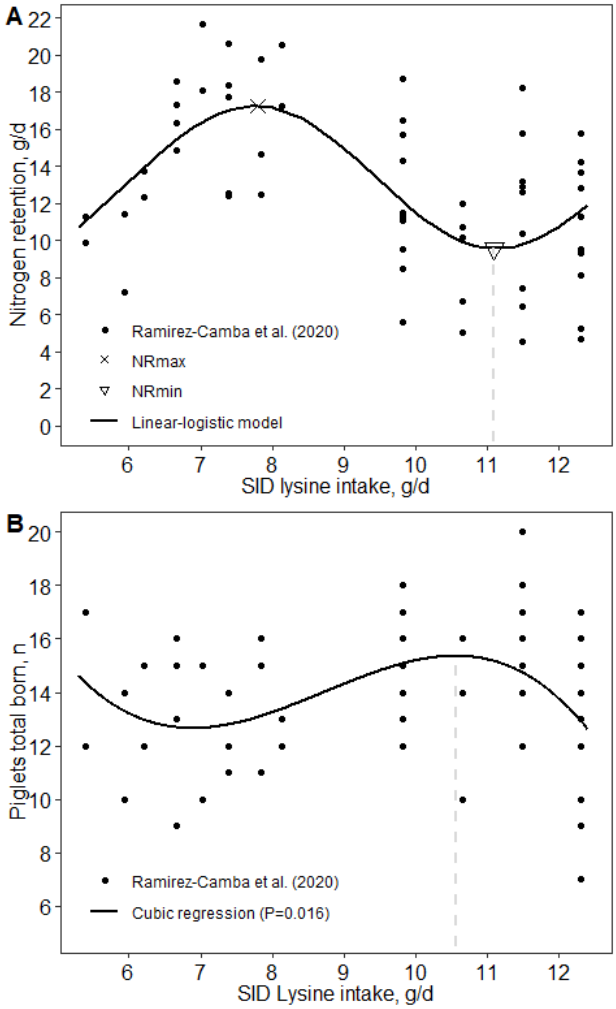

Figure 3-3. The linear-logistic model describes two inflection points defined as maximum N retention inflection point (NRmax) and minimum N retention infection point (NRmin; A).
A reanalysis of Ramirez-Camba et al. (2020) showed that when experimental diets were given to gilts between d 48 to 52 of gestation preceded by 7 d of adaptation, the number of total piglets born was maximized at a SID Lys intake corresponding to NRmin (dotted lines; B).

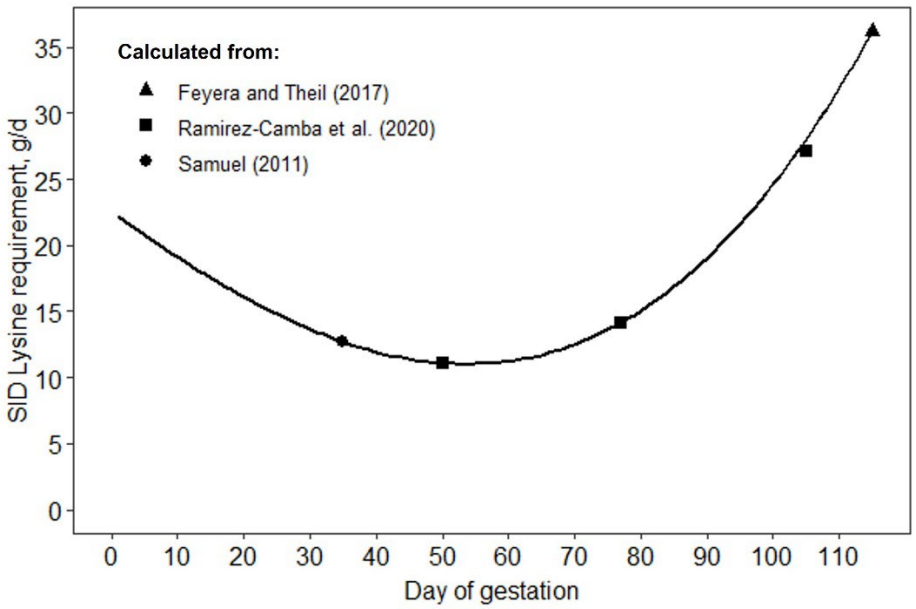

Figure 3-4. Calculated dietary SID Lys levels that maximize sow reproductive performance and metabolic status (i.e. SID Lys requirement) across gestation based on empirical data.

A cubic model (solid line) produced the best fit in terms of R^2 among the linear and nonlinear models evaluated. The cubic model is represented by the following equation: $y = 22.56 – 0.362x + 1.46E\text{-}02x^2 + 2.36E\text{-}05x^3$.

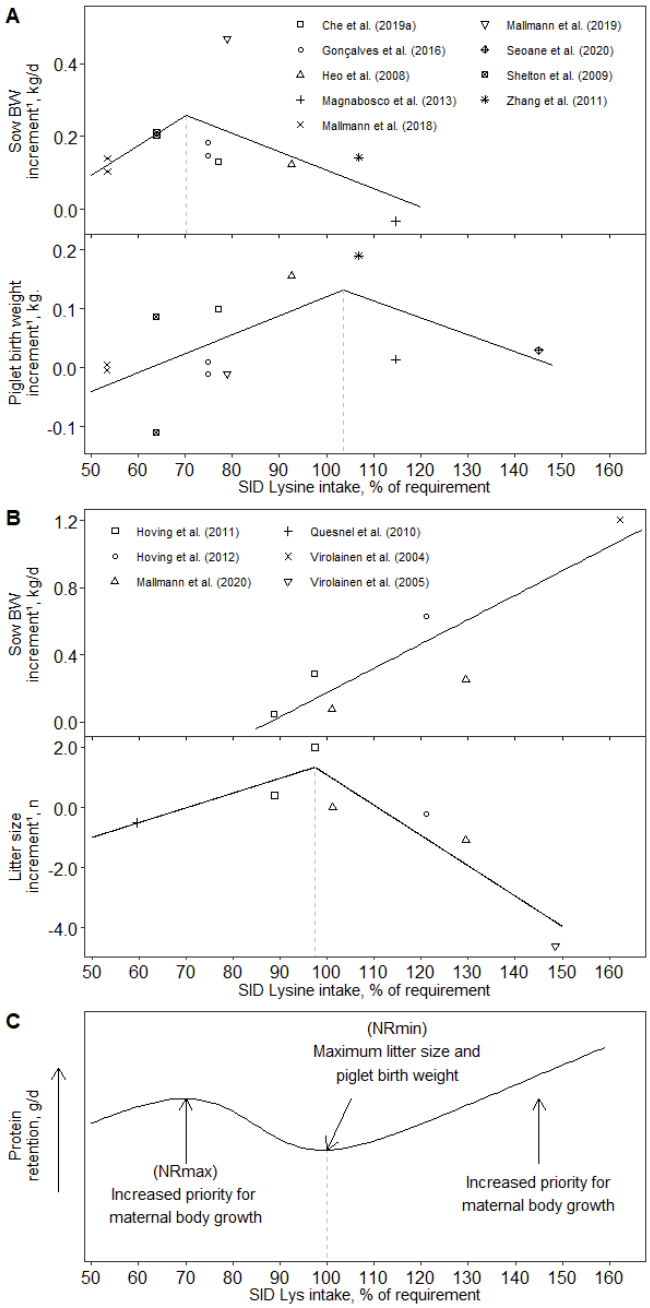

Figure 3-5. Validation analysis of SID Lys requirements of pregnant sows estimated according to Eq. 3-4.
Published studies evaluating the impact of increased SID Lys intake during mid and late gestation (d 30 to 115; Table 3-1) on sow body weight and piglet birth weight (A) together with studies evaluating the impact of increased SID Lys intake during early gestation (d1 to 35; Table 3-2) on sow body weight and litter size (B) were used to validate the calculated SID Lys requirements. Empirical data shows that at intakes corresponding to the NRmin or the SID Lys requirement (dotted lines) an increase in piglet birth weight and litter size occurs (C).
NRmax: inflection point of maximum N retention in a linear-logistic model.; NRmin: inflection point of minimum N retention in a linear-logistic model.

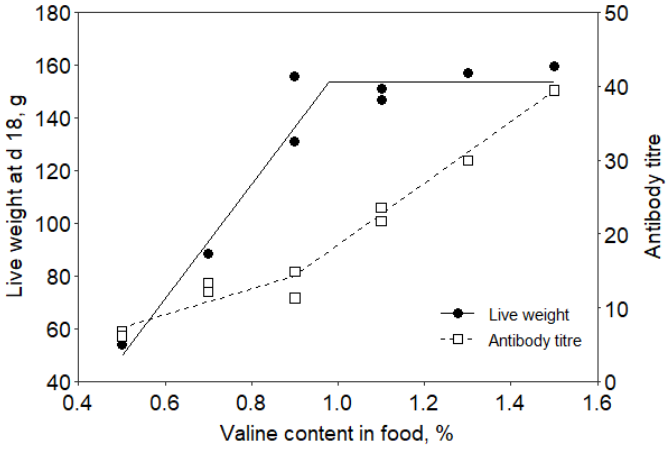

Figure 3-6. The responses in live weight and antibody titres to increasing dietary valine in chicks exposed with a Newcastle virus during an 18-day period; adapted from Bhargava et al. (1970) and France and Kebreab (2008).

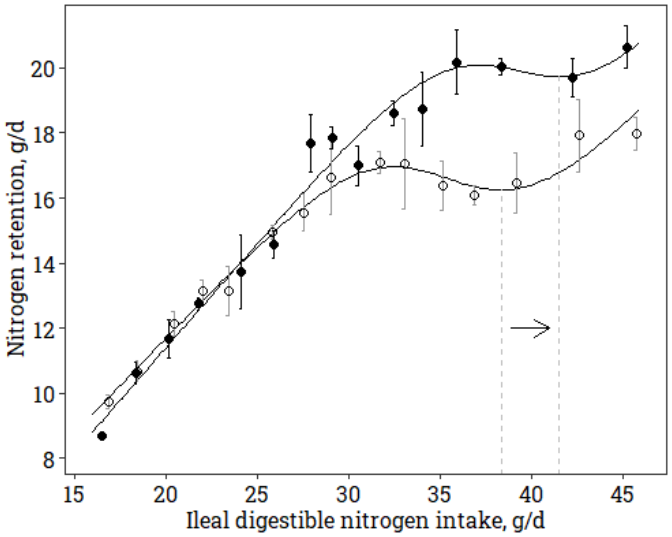

Figure 3-7. Effect of daily ideal digestible nitrogen intake on nitrogen retention in gilts from 20 to 45 kg BW, fed 2.5 × Energy for Maintenance (○), or 3.0 × Energy for Maintenance (●), described with linear-logistic models.
As shown by the arrow, the inflection point of the linear-logistic model where N retention is minimized, which is regarded as the requirement under the current methodology (dotted lines), increases as energy intake increases. Adapted from Bikker (1994).

Supplementary Table 3. Equations and parameter estimates of the comparison between broken line regression and linear-logistic models in three different data sets.

Model	Equation	Parameter estimates					R^2	SE
		a	b	c	d	f		
Bikker (1994)								
Linear-plateau	$a + (bx)(x < c) + (b \cdot c)(x \geq c)$	0.686	0.547	29.807			0.960	0.563
Linear-logistic	$y = a + bx - \frac{c}{1 + de^{-fx}}$	-0.036	0.588	8.377	5.17E+05	0.371	0.981	0.427
Gahl et al. (1994)								
Linear-plateau	$a + (bx)(x < c) + (b \cdot c)(x \geq c)$	-1.981	1.807	9.479			0.979	0.992
Linear-logistic	$y = a + bx - \frac{c}{1 + de^{-fx}}$	-2.036	1.827	7.939	8.89E+05	1.212	0.993	0.797
Remus et al. (2020)								
Linear-plateau	$a + (bx)(x < c) + (b \cdot c)(x \geq c)$	24.980	9.246	15.460			0.819	9.617
Linear-logistic	$y = a + bx - \frac{c}{1 + de^{-fx}}$	26.929	9.120	23.114	5.82E+18	2.762	0.995	2.643

R^2 = Coefficient of determination

CHAPTER 4

METHIONINE REQUIREMENT OF THE GILT AT BREEDING AGE

ABSTRACT

Multiple studies have found that dietary AA levels required to optimize whole-body metabolic status are higher than those required for maximum N or protein retention. The current study aimed to investigate the effects of SID Met intakes greater than current requirements on the metabolic status of gilts at breeding age. Metabolic status of the gilts was evaluated using plasma AA measurements, with a particular emphasis on plasma Tau, a non-proteogenic AA that is biosynthesized from Met and regulates a variety of functions in the body. In addition, the dynamics of N retention at potential SID Met intakes that maximize Tau biosynthesis were investigated. A total of 30 gilts (PIC Camborough x Duroc terminal line; 141.4 kg ± 6.1 kg and 18 mm ± 1.77 backfat at 202.8 ± 1.4 d of age) in a single group were used in the experiment. At 203 d of age gilts were randomly assigned to one of 5 experimental diets balancing as best as possible for BW and backfat. The five experimental diets were designed to provide 4, 4.4, 5.7, 6.9 and 9 g SID Met/d, which corresponds to 133 to 301% of the NRC (2012) SID Met recommendation for pregnant gilts during the first 90 d of gestation. The N-balance study consisted of 7-d diet adaptation and 3-d urine and fecal collections. Prior to and after urinary catheterization, a blood sample was collected from each gilt via jugular venipuncture into heparinized tubes. For all analyses, a $P < 0.05$ was considered significant and $P < 0.10$ was considered a tendency. A significant quadratic relationship was detected between SID Met intake and N retention ($P = 0.039$), quadratic relationship that was minimized at 6.5 g SID Met/d. A significant quadratic relationship was detected

between SID Met intake and plasma Tau ($P = 0.025$), quadratic relationship that was maximized at 6.9 g SID Met/d. These findings indicate that SID Met intakes that maximize Tau biosynthesis and, thus, potentially Tau related metabolic functions, resulted in a decrease in whole-body N retention. It was speculated that at SID Met intakes for optimal Tau biosynthesis, physiological functions such as protein turnover may also increase; because protein is not 100% recycled, increased N excretion would occur, resulting the observed decreased N retention. In addition, significant quadratic relationships were detected between SID Met intake and plasma Arg ($P = 0.044$), plasma Phe ($P = 0.006$) and tendencies for the quadratic relationship between SID Met intake and plasma Ile ($P = 0.072$), plasma Met ($P = 0.061$) and plasma Val ($P = 0.057$). As a result, SID Met intakes that maximized Tau biosynthesis also increased plasma Arg, Phe, Ile, Met, and Val. When metabolic status is assessed, the daily SID Met requirement of gilts at breeding age is 6.9 g corresponding to 230% of the current SID Met recommendations for gilts during early gestation (NRC, 2012). It is recommended that, in addition to N balance, other variables be used to more precisely define AA requirements in gilts.

INTRODUCTION

Amino acid requirements in swine have been defined as the minimum AA intake that maximizes protein synthesis or protein retention (NRC, 2012; van Milgen and Dourmad, 2015). This definition has been deemed adequate for reproductive females based on the assumption that maternal body Pd occurs after dietary AA needs for functions with a higher nutrient utilization hierarchy, such as maintenance and reproduction, has been met (Lewis and Southern, 2000). As a result, it is assumed that at

the AA intake that maximizes protein retention (requirement), dietary AA required for metabolic functions are already met. However, different physiologic functions seem to be maximized at AA intakes above those required for maximum protein retention (Marchini et al., 1993; Robinson et al., 2016; Chalvon-Demersay, 2021). Therefore, the current study aimed to investigate the effects of SID Met (Met) intakes greater than current requirements on the metabolic status of gilts at breeding age together with measures of protein retention.

With respect to assessing metabolic status related to dietary Met supply, concentrations of plasma Tau may be used (Yamori et al., 1996; Schuller-Levis and Park, 2006; De Boo and Harding, 2007; Yamori et al., 2010; Hasanzadeh et al., 2018). Tau is a non-proteogenic AA that is biosynthesized from Met and regulates a variety of functions in the reproductive system, central nervous system, renal system, among other important physiological functions (Norberg et al., 1998; Ripps and Shen, 2012; Tang et al., 2018; Mu et al., 2019; Wen et al., 2019). Tau is also an essential AA in utero and for early embryo development (Holm et al., 2018; Mu et al., 2019). Dietary SID Met levels that maximize Tau biosynthesis are hypothesized to maximize the metabolic status of gilts at breeding age. An optimal metabolic status at breeding may result in improved reproductive performance both pre- and post- implantation.

MATERIALS AND METHODS

The experiment protocols were approved by the South Dakota State University Animal Care and Use Committee (2009-041A) and followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Third Ed., 2010). The experiment was conducted from December 2020 to January 2021.

Animals and General Management

The experiment was conducted at South Dakota State University Swine Education and Research Facility, Brookings, SD. A total of 30 gilts (PIC Camborough x Duroc terminal line; 141.4 kg ± 6.1 kg and 18 mm ± 1.77 backfat at 202.8 ± 1.4 d of age) in a single group were used in the experiment. Females were kept in gestation stalls (61 cm x 1.98 m) from 164 to 213 d of age. From 164 to 180 d of age, gilts were offered a common corn-soybean meal finishing diet (3350 kcal/kg ME and 0.65% SID Lys) at a feed allocation of 2.1 kg/d to maintain a target body condition score of 3 on a 5-point scale. From d 181 to d 202 gilts were provided 2.1 kg/d of a high Met (HM) diet (Table 4-1). Young and Marchini (1990) reported that under low AA intake, positive N balance is achieved by reducing metabolic functions in a stage termed accommodation. The purpose of the HM diet was to provide increased Met levels for three weeks so that gilts could potentially increase metabolic functions that may have been limited by previous Met intakes, thereby reducing the chances of an accommodation state during the experiment.

From d 203 to d 212 experimental diets were provided for 12 days to conduct a N-balance study. All diets were provided in meal form and water was provided ad libitum via a single water nipple drinker. Pigs and facilities were checked twice daily by trained personnel during the N balance periods. Daily feed disappearance was monitored for feed spillage and feed refusal. Sow illness, lameness, and clinical signs of infection over the course of catheterization were noted.

Dietary Treatments

At 203 d of age gilts were randomly assigned to one of 5 experimental diets balancing as best as possible for BW and backfat. The five experimental diets were

formulated to achieve 95 – 150% of the NRC (2012) SID Met for pregnant gilts during the first 90 d of gestation (3 g/d). Non-pregnant gilts were used to estimate SID Met intakes that could potentially maximize Tau biosynthesis pre-implantation; the estimated SID Met intakes are intended to serve as a baseline for future research aiming to estimate dietary SID Met levels that maximize reproductive performance during the first third of gestation. All sows were fed once a day at 0630h at a rate of 2.1 kg/d. The desired levels of SID Met were accomplished by supplementing a base diet (0.19% dietary SID Met, Met-1) with 0.02 (Met-2), 0.08 (Met-3), 0.14 (Met-4) and 0.24 % (Met-5) crystalline Met. Dietary SID Lys was set at 12 g/d (0.57% dietary SID Lys). The ratios of other essential AA to Lys were above the NRC (2012) requirements. Titanium dioxide was included at 0.40% as an indigestible marker to calculate total tract N digestibility.

Nitrogen Balance

The N-balance study consisted of 7-d diet adaptation and 3 to 5-d urine and fecal collections. Nitrogen balance observations were based on total urine collection using urinary catheters and determination of fecal N-digestibility using indigestible marker (Short et al., 1996). Urine was collected as described by Miller et al. (2016). Prior to collection, urinary catheters (Lubricath, 2-way, 30 mL balloon, 18 French; Bard Medical Division, Covington, GA, USA) were lubricated and inserted flaccidly through the urethra and the balloon was inflated with 30 mL saline solution to retain the catheter in the bladder. Catheters were connected to closed containers using polyvinyl tubing and urine collected. Sulfuric acid was added to the containers to maintain pH <3. A representative subsample (1% of the successful daily collection) was obtained, pooled within each 5-d collection period and stored at 4 °C until further analysis. Urine

collection was considered successful when at least 3 complete 24-h collections were accomplished. Urinary catheters were removed at the end of the N-balance period. Fecal samples were obtained by rectal palpation and daily collections were pooled per gilt and stored at -20 °C until further analysis.

Blood Sampling

A blood sample was collected from each gilt prior to urinary catheterization and before feeding (i.e. 0600h) on d 210 of age via jugular venipuncture into heparinized tubes; a second blood sample was collected before feeding (i.e. 0600h) on d 213 of age. Samples were centrifuged at 2,000 × g; 15 min at 4 ℃. Plasma was transferred to 1.5 microcentrifuge tubes and stored at −80 ℃ until analysis.

Chemical Analysis

After diet mixing, a subsample of feed was collected, pooled and homogenized. Prior to analyses, aliquots from urine samples were placed in 120 mL specimen cups and approximately 200 g of each experimental diet and freeze-dried feces were ground using rotor mill (Centrifugal Mill ZM 200; Retsch GmbH, Haan, Germany) with 0.50 mm sieve. Urine, freeze-dried feces and experimental diets were analyzed for N content using combustion method (Rapid N III, Elementar Analysensysteme, GmbH, Hanau, Germany); crude protein was calculated as N x 6.25. Dry matter and titanium dioxide content in feces and feeds were quantified according to Short et al. (1996). Absorbance of standard and samples were read using Spectra MAX 190 plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at 408 nm wavelength. Amino acid and proximate compositions of experimental diets were completed by a commercial laboratory (Agricultural Experiment Station Chemical Laboratories, University of Missouri,

Columbia, MO). Plasma AA concentrations were determined via the HPLC analysis of the phenylisothiocyanate derivatives, as previously described by Urschel et al. (2011).

Calculations

N retention (g/d) was calculated from daily feed allowance and analyzed diet N content, minus N content of wasted feeds and daily N excretion in feces and urine. Fecal N excretion (g/d) was calculated from N intake and total tract N digestibility. Daily gilt whole body protein retention (g/d) was calculated as daily N retention x 6.25. Daily SID Met intake was calculated as the product of daily feed intake (kg/d), Met level of diet (g/kg) and SID coefficient (%). SID coefficients were calculated considering the inclusion of corn, soybean meal, and synthetic AA for each diet and their respective digestibility coefficients according to NRC (2012).

Statistical Analyses

Statistical analyses were performed using R (version 3.6.3) and R Studio (version 1.3.1073). The relationship between N retention and SID Met intake was analyzed with simple linear regression (linear and quadratic) using the R stats package. The relationship between plasma AA and SID Met intake was analyzed with mixed-effects models (linear, quadratic, and cubic) with gilt and day of collection as random effect and SID Met intake as fixed effect using the lme4 package. The relationship between urinary N excretion and plasma AA was analyzed with a linear mixed-effect model with gilt and day of collection as random effect using the lme4 package. For all analyses, statistical assumptions (homogeneity of variances, normality of residuals) were confirmed apriori using the R stats package. For all analyses, a $P < 0.05$ was considered significant and $P<0.10$ was considered a tendency.

RESULTS

The nutrient loadings of DDGS used in diet formulation were low relative to analyzed values. When the mixed diet nutrient values were recalculated using the analyzed DDGS nutrient loadings, the diets provided 4, 4.4, 5.7, 6.9 and 9 g SID Met/d, which corresponds to 133 to 301% of the NRC (2012) SID Met recommendation for pregnant gilts during the first 90 d of gestation. These revised formulation values match well with analyzed values for the complete mixed diets. The total number of successful observations for the N balance was 28 due to two failed catheterizations. Significant positive quadratic relationships were detected between SID Met intake and N retention (P = 0.039; Fig. 4-1A), a negative quadratic relationship between SID Met intake and fecal N excretion (P= 0.005; Fig. 4-1B), and a tendency for a negative quadratic relationship between SID Met intake and urinary N excretion (P = 0.092; Fig. 4-1C).

Summary statistics of the concentration of AA in the plasma of all 30 gilts at 210 d of age are shown in Table 4-2. Significant positive linear relationships were detected between SID Met intake and plasma Met (P=0.007). In addition, a significant negative linear relationship between SID Met intake and plasma Lys was detected (P=0.004; Fig. 4-2). Significant negative quadratic relationships were detected between SID Met intake and plasma Arg (P=0.044; Fig. 4-3A), plasma Phe (P= 0.006; Fig. 4-3B), and plasma Tau (P= 0.025; Fig. 4-3C), and tendencies for the negative quadratic relationship between SID Met intake and plasma Ile (P= 0.072; Fig. 4-3D), plasma Met (P= 0.061; Fig. 4-3E) and plasma Val (P= 0.057; Fig. 4-3F). Besides, significant cubic relationships were detected between SID Met intake and plasma asparagine (P=0.015; Fig. 4-4A), plasma glutamine (P=0.033; Fig. 4-4B), plasma Ile (P=0.012; Fig. 4-4C), plasma Leu

(*P*=0.038; Fig. 4-4D) and plasma proline (*P*=0.031; Fig. 4-4E). In addition, a significant positive linear relationship was detected between urinary N excretion and plasma Arg (*P*= 0.031; Fig. 4-5A) and a tendency for the negative relationship between urinary N excretion and plasma Ornithine (*P*= 0.057; Fig. 4-5B).

DISCUSSION

Plasma Tau as a marker of metabolic function

Met is an essential AA for protein synthesis and for important metabolic functions such as the synthesis of Tau. Tau is a non-proteogenic AA with antioxidant, anti-inflammatory and osmoregulator properties; it is also involved in energy metabolism in muscles, adipose tissue and the liver (Wen et al., 2019). Taurine also regulates a number of functions in the reproductive system, central nervous system, renal system, and digestive system (Norberg et al., 1998; Riedijk et al., 2007; Ripps and Shen, 2012; Tang et al., 2018; Mu et al., 2019; Wen et al., 2019). Taurine is synthesized from Met via transmethylation reactions, which are inhibited at low Met intakes. At low dietary Met intakes the conservation of Met (or reduced rate of Met oxidation) is achieved via a reduction in the rate at which Met enters the transmethylation reactions relative to the rate at which Met enters pathways of protein synthesis (Young and Marchini, 1990; Robinson et al., 2016). As a result, at low Met intakes, protein synthesis is prioritized over Tau biosynthesis, while at intakes above maximum protein synthesis, Tau biosynthesis rates increase. In this study plasma Tau was maximized at SID Met intakes of 6.9 g/d, which is 230% of the current SID Met requirement for gilts during early gestation (NRC, 2012). Additional empirical evidence supports the notion that sows require higher SID Met intakes than are currently recommended in order to achieve optimal metabolic status. For

example, Xia et al. (2019) showed that dietary SID Met levels that maximized placental angiogenesis and reproductive performance in sows (early: 7.28, mid: 5.95, and late gestation: 7.54 g SID Met/d) are higher than current recommendations for pregnant sows. Similarly, Bin et al. (2018) reported an increase in average birth weight when feeding 0.48% Met (12.2 g SID Met/d) diets to sows and an increase in piglet survival ratio when feeding 0.60% Met (15.7 g SID Met/d) diets to sows from d 90 to farrowing. Robinson et al. (2016) demonstrated that protein synthesis is prioritized over metabolic functions involving transmethylation reactions in neonatal piglets and it is unsurprising that the same occurs in gilts at breeding age. Targeting Met intake levels to maximize transmethylation reactions may be especially important for early embryo development because during early gestation porcine embryos are primarily fed by uterine fluids, in which Tau is abundant (Li et al., 2007).

The results of the current study suggest that AA requirements besides measures of Pd should consider metabolic criteria for which free plasma AA measures may be used. However, the traditional assumption is that increased free plasma AA is associated with excess dietary AA intake and, therefore, considered inadequate for optimal performance. As discussed in the next section, this assumption may be inaccurate.

Free plasma AA levels for estimating AA requirements

Under the plasma AA method, the AA requirement has been defined as the point where additional dietary AA supply causes an increase in plasma AA levels, based on the assumption that the body lacks a storage compartment for free AA (France and Kebreab, 2008). In addition, increased plasma AA levels have been regarded as waste and potentially harmful, based on the observation that excess amounts of AA accumulate in

the body in rare disorders such as phenylketonuria (France and Kebreab, 2008). However, the assumption that increased plasma AA have negative effects is contradicted by multiple studies that show that increased plasma AA is associated with increased conceptus development (Li et al., 2014; Wang et al., 2018; Che et al., 2019; Xia et al., 2019) and increased AA availability for immune response (Le Floc'h et al., 2018). Additionally, De Boo and Harding (2007) reported that, in sheep, increased maternal plasma Tau levels resulted in increased fetal AA concentrations and reduced fetal hypoxia-induced inflammation.

In Chapter 3, the linear-logistic model was proposed as an alternative to the broken-line regression for the estimation of AA requirements. The N retention response to AA supply described by a linear-logistic model reveals that at AA intakes that potentially maximize metabolic functions, protein or N retention decreases. In the current study a similar pattern was observed; SID met intakes that maximized Tau synthesis (and potentially transmethylation reactions) also resulted in a reduction in N retention. It is thus possible that SID Met intake levels that maximize protein synthesis also minimized plasma Tau or Met, whereas SID Met intake levels that maximize metabolic functions maximize plasma Tau. These observations suggest that plasma free AA measures may be used for the estimation of dietary AA levels that maximize metabolic functions, which, for pregnant animals, may be considered as the requirement. Amino acid requirements that optimize metabolic functions that promote fetal development may be more desirable during gestation than AA dietary levels that promote maternal tissue deposition.

In the current study, 6.9 g SID Met/d maximized both plasma Tau and fecal N excretion. These findings suggest that SID Met intakes of 6.9 g SID Met/d are optimal for

maximizing both transmethylation reactions and protein turnover in gilts at breeding age. Adequate levels of protein turnover have been associated with long term health (Marchini et al., 1993) and optimal newborn size in humans (Duggleby and Jackson, 2001). Therefore, feeding sows at SID Met intakes optimal for metabolic status may increase sow longevity and improve reproductive performance.

Dietary SID Met levels that maximized plasma Tau also resulted in increased plasma Arg levels which has been linked to improved gilt reproductive performance (Palencia et al., 2018). Furthermore, plasma concentrations of branched chain AA and Phe increased at SID Met intakes close to the level that maximized plasma Tau (proposed requirement). Increased branched chain AA and Phe may imply increase AA availability for physiological functions. This claim, however, needs to be investigated further. In addition, at low SID Met intake levels (<60% of the SID Met level that maximized Tau biosynthesis) and at SID Met intake levels that approach NRC (2012) recommendations for early pregnancy (3 g SID Met/d) plasma asparagine, glutamine, Ile, Leu, and proline concentrations increased (predicted using cubic regression). This increase in plasma AA at dietary SID Met close to recommended levels for optimal protein retention (i.e. current requirements) could be linked to the previously discussed reductions in metabolic functions at low AA intake, which could increase the availability of AA for protein synthesis. Because a reduction in plasma Tau was observed at SID Met intake levels close to NRC (2012) recommendation, it is assumed that current recommendations may increase protein synthesis, and thus lean tissue deposition, but not Tau biosynthesis and its associated metabolic functions.

The findings of the current study show that as urine N excretion decreased, so did the plasma Arg/ornithine ratio. Reductions in the plasma Arg/ornithine ratio have been linked to metabolic disorders in humans (Galkina et al., 2019). These findings imply that SID Met levels that maximize the efficiency of N utilization may result in a decreased metabolic status, which may influence sow longevity. Furthermore, as SID Met intake increased, plasma Lys decreased, and some evidence suggests that higher plasma Lys concentrations are associated with lower chances of disease recovery in humans (Mauhin et al., 2017). Thus, a decrease in plasma Lys due to SID Met intake may be associated with improved metabolic status. Another possibility for the negative relationship between SID Met intake and plasma Lys is that a mutual inhibition between these two AA exists where Met transport in the intestine and kidneys has been shown to be partially inhibited by Lys, and vice versa. (Broer, 2008). Our findings suggest a more holistic approach to determining AA requirements in gilts, taking into account both protein synthesis and metabolic criteria.

In conclusion, dietary SID Met levels that optimize Tau biosynthesis, and potentially related metabolic functions, are higher than those needed for maximum N retention (i.e. current AA requirements). When metabolic status is assessed, the daily SID Met requirement of gilts at breeding age is 6.9 g corresponding to 230% of the current SID Met recommendations for gilts during early gestation (NRC, 2012). It is recommended that, in addition to N balance, other variables be used to more precisely define AA requirements in gilts.

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Table 4-1. Ingredient composition and nutrient content of the low and high master diets.

Item	HM	Met-1	Met-2	Met-3	Met-4	Met-5
Ingredients, %						
Corn, yellow dent	68.18	66.83	66.81	66.77	66.73	66.67
Soybean meal	9.58	8.90	8.90	8.89	8.89	8.88
Corn DDGS	9.00	9.96	9.96	9.95	9.95	9.94
Soybean hulls	7.57	7.54	7.54	7.54	7.53	7.52
Soybean oil	1.15	1.99	1.99	1.99	1.99	1.99
DL-Met	0.18	0.00	0.02	0.08	0.14	0.24
L-Arg	0.00	0.02	0.02	0.02	0.02	0.02
L-Lys HCl	0.20	0.22	0.22	0.22	0.22	0.22
L-Thr	0.15	0.16	0.16	0.16	0.16	0.16
L-Trp	0.00	0.00	0.00	0.00	0.00	0.00
Monocalcium phosphate	1.89	1.89	1.89	1.89	1.89	1.89
Limestone	1.33	1.33	1.33	1.33	1.33	1.33
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral Premix[1]	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Titanium dioxide	0.00	0.40	0.40	0.40	0.40	0.40
Calculated nutrient content[3]						
ME, kcal/kg	3226.6	3253.3	3253.8	3255.0	3256.3	3258.4
NE, kcal/kg	2400.1	2430.3	2430.6	2431.6	2432.6	2434.4
Crude protein, %	13.88	13.64	13.65	13.67	13.70	13.75
SID Lys, %	0.57	0.57	0.57	0.57	0.57	0.57
SID Met, %	0.38	0.19	0.21	0.27	0.33	0.43
SID Met+Cys, %	0.57	0.39	0.40	0.46	0.52	0.62
SID Thr, %	0.50	0.51	0.51	0.51	0.51	0.51
SID Trp, %	0.10	0.10	0.10	0.10	0.10	0.10
CF, %	5.51	5.53	5.53	5.52	5.52	5.51
Ca, %	0.88	0.88	0.88	0.88	0.88	0.88
P, %	0.65	0.65	0.65	0.65	0.65	0.65

Arg: arginine; CF: crude fiber; Cys: cysteine; DDGS: distiller's dried grains with solubles; HM: high methionine diet Lys: lysine; ME: metabolizable energy; Met: methionine; NE: net energy; Thr: threonine; Trp: tryptophan.

[1] Mineral premix provided the following per kilogram of diet: 33000 IU vitamin A, 33000 IU vitamin D3, 284 IU vitamin E, 0.132 mg vitamin B12, 13 mg menadione, 30 mg riboflavin, 99 mg D-panthothenic acid, 165 mg niacin, 13 mg folic acid, 45 mg pyridoxine, 10 mg thiamine, 1 mg biotin.

[2] Vitamin premix provided the following per kilogram of diet: 12 mg Zn as $ZnSO_4$, 12 mg Fe as $FeSO_4$; 1 mg Cu as $CuSO_4$, and 3 mg Mn as $MnSO_4$.

[3] Calculated chemical concentrations using values of analyzed feed ingredients used at South Dakota State University's Feed Mill.

Table 4-2. Summary statistics (mean ± standard deviation) of the concentration of the AA in the plasma of gilts at 210 d of age per SID Methionine intake level.

Plasma AA, μmol/L	SID Methionine intake, g/d					P-value[1]		
	4.09	4.51	5.77	7.03	9.13	Linear	Quadratic	Cubic
Alanine	623.4 ± 151.2	556.1 ± 121.8	631.2 ± 128.1	709.8 ± 285.0	666.3 ± 108.8	0.273	0.441	0.217
Arginine	100.8 ± 23.8	90.0 ± 40.0	119.9 ± 45.5	112.3 ± 68.5	86.8 ± 28.5	0.661	**0.044**	0.867
Asparagine	97.1 ± 17.0	82.6 ± 19.7	88.8 ± 14.0	98.5 ± 18.9	86.5 ± 14.3	0.348	0.518	**0.015**
Aspartate	94.3 ± 20.8	90.1 ± 26.6	92.8 ± 22.5	109.3 ± 26.2	94.4 ± 22.4	0.750	0.248	0.108
Citrulline	118.8 ± 26.9	127.8 ± 25.6	125.7 ± 23.2	118.3 ± 22.7	120.9 ± 32.0	0.626	0.915	0.262
Glutamine	496.8 ± 84.2	434.7 ± 113.1	445.9 ± 55.5	504.3 ± 123.5	457.4 ± 91.3	0.376	0.977	**0.034**
Glutamate	406.4 ± 136.0	444.1 ± 168.9	449.2 ± 149.4	520.3 ± 166.8	482.5 ± 81.1	0.275	0.297	0.709
Glycine	1258.3 ± 216.5	1184.2 ± 300.0	1203.8 ± 206.6	1232.3 ± 274.1	1231.8 ± 244.5	0.928	0.774	0.537
Histidine	91.8 ± 19.3	89.4 ± 20.8	94.0 ± 15.4	99.2 ± 25.1	92.6 ± 18.4	0.743	0.297	0.453
Isoleucine	94.9 ± 24.4	79.0 ± 16.7	89.9 ± 14.1	97.3 ± 22.6	78.6 ± 16.7	0.102	**0.072**	**0.012**
Leucine	247.9 ± 52.6	215.0 ± 36.6	236.2 ± 36.6	253.1 ± 47.5	236.1 ± 27.2	0.903	0.566	**0.039**
Lysine	321.4 ± 77.5	300.8 ± 104.3	306.0 ± 73.5	294.6 ± 91.8	256.6 ± 87.3	**0.004**	0.686	0.880
Methionine	46.5 ± 8.8	48.1 ± 9.0	51.1 ± 9.9	60.1 ± 15.4	55.6 ± 6.8	**0.007**	**0.061**	0.192
Ornithine	145.2 ± 98.3	144.2 ± 69.8	117.6 ± 24.0	158.0 ± 70.1	143.0 ± 42.0	0.903	0.726	0.243
Phenylalanine	72.0 ± 15.5	69.8 ± 20.1	78.0 ± 15.7	90.1 ± 34.0	68.3 ± 11.6	0.683	**0.006**	0.147
Proline	280.8 ± 45.1	254.4 ± 56.8	272.8 ± 40.2	302.6 ± 41.3	277.4 ± 42.8	0.462	0.313	**0.031**
Serine	167.8 ± 31.2	157.9 ± 38.7	168.4 ± 34.4	166.0 ± 28.5	160.8 ± 21.7	0.568	0.686	0.830
Taurine	279.5 ± 41.1	278.1 ± 62.5	304.6 ± 61.9	342.2 ± 99.7	289.9 ± 62.8	0.495	**0.025**	0.253
Threonine	195.5 ± 18.4	192.8 ± 52.6	184.2 ± 23.8	205.9 ± 50.2	179.2 ± 38.4	0.496	0.432	0.136
Tryptophan	2.6 ± 1.1	2.8 ± 1.3	2.6 ± 1.1	3.1 ± 2.7	2.6 ± 1.0	0.978	0.495	0.500
Tyrosine	125.8 ± 18.9	115.4 ± 25.6	123.2 ± 23.1	131.1 ± 30.6	126.3 ± 18.4	0.520	0.711	0.247
Valine	280.4 ± 70.6	256.9 ± 65.1	283.2 ± 47.6	298.0 ± 74.8	247.0 ± 46.4	0.162	**0.058**	0.216

[1] The p-values of the linear, quadratic, and cubic relationships between SID Methionine intake and plasma AA are shown. Mixed-effects models with animal and day of collection as random effect and SID Methionine intake as fixed effect.

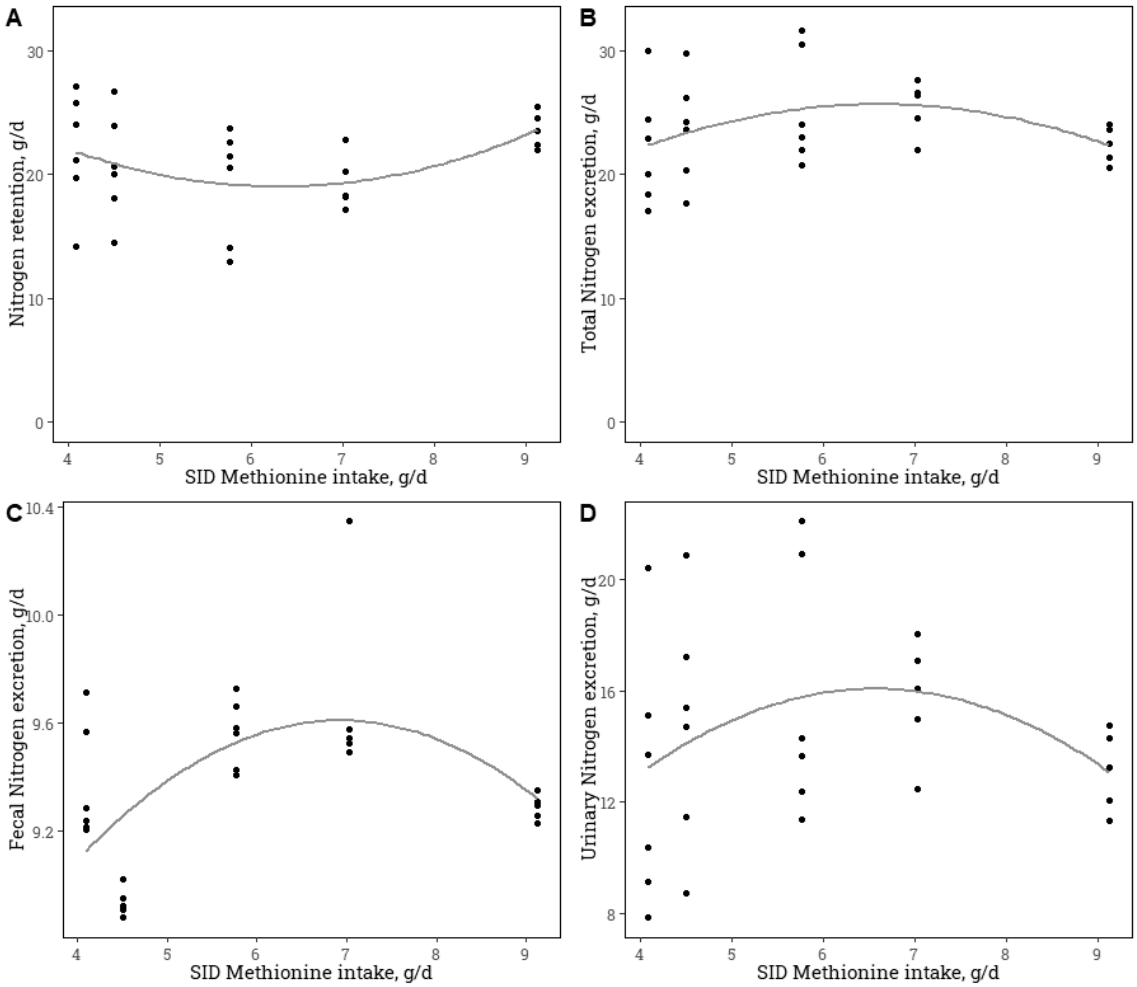

Figure 4-1. Significant quadratic relationships were detected between SID methionine intake and nitrogen retention (P= 0.039; A) and fecal nitrogen excretion (P= 0.005; B), and tendencies for the relationship between SID methionine intake and total nitrogen excretion (P=0.059; B) and urinary nitrogen excretion (P= 0.092; C).

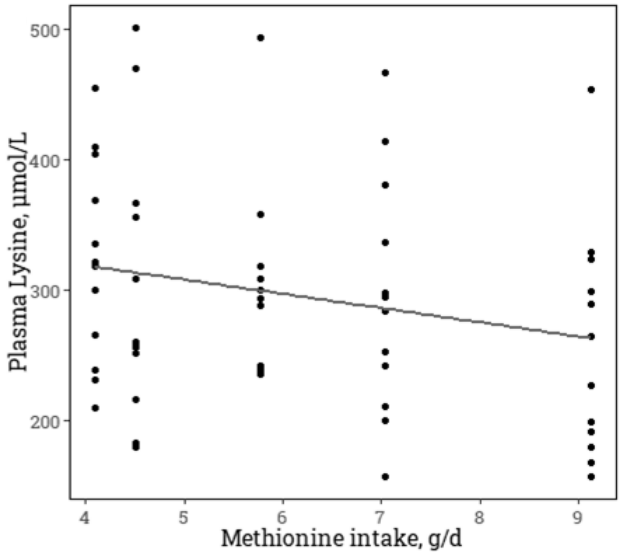

Figure 4-2. A significant linear relationship was detected between SID Methionine intake versus plasma Lysine (P=0.004).

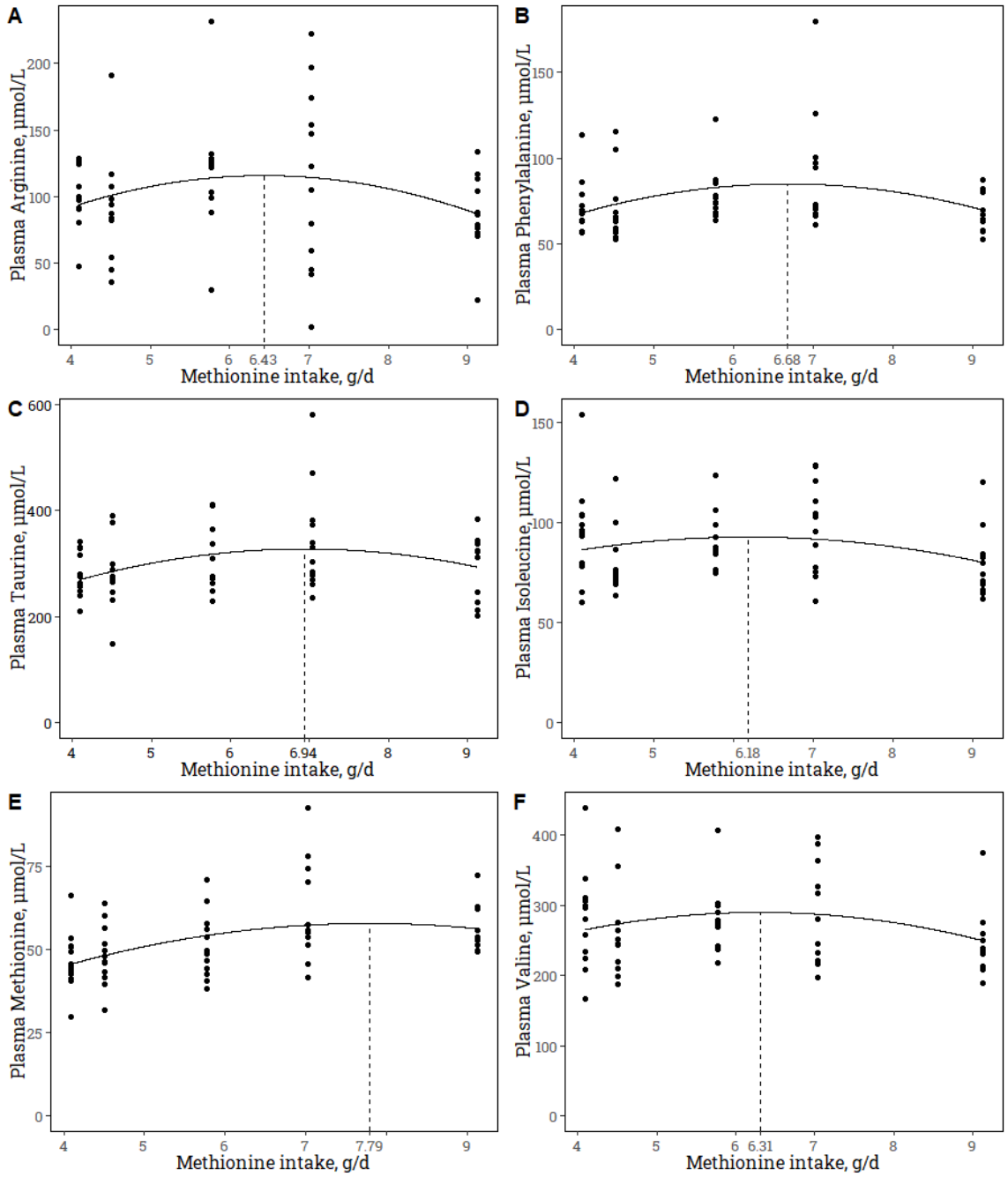

Figure 4-3. Significant quadratic relationships were detected between SID Methionine intake versus plasma Arginine (P=0.044; A), Phenylalanine (P= 0.006; B), and Taurine (P= 0.025; C), and tendencies for the quadratic relationship between SID Methionine intake versus plasma Isoleucine (P= 0.072; D), Methionine (P= 0.061; E) and Valine (P= 0.058; F). Dotted lines represent SID methionine intakes that were projected to maximize plasma AA levels.

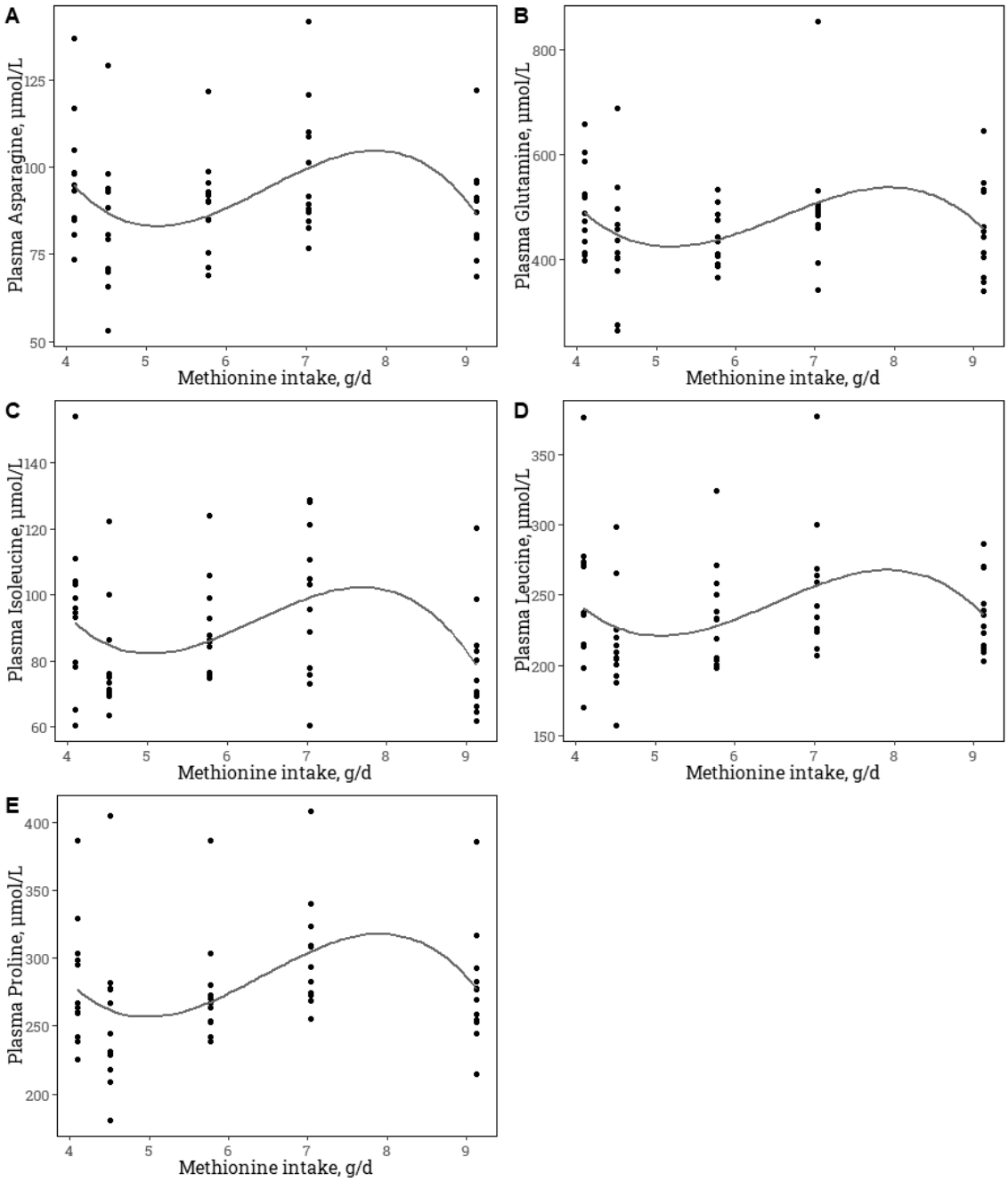

Figure 4-4. Significant cubic relationships were detected between SID Methionine intake versus plasma Asparagine (P=0.015; A), Glutamine (P=0.033; B), Isoleucine (P=0.012; C), Leucine (P=0.038; D) and Proline (P=0.031; E).

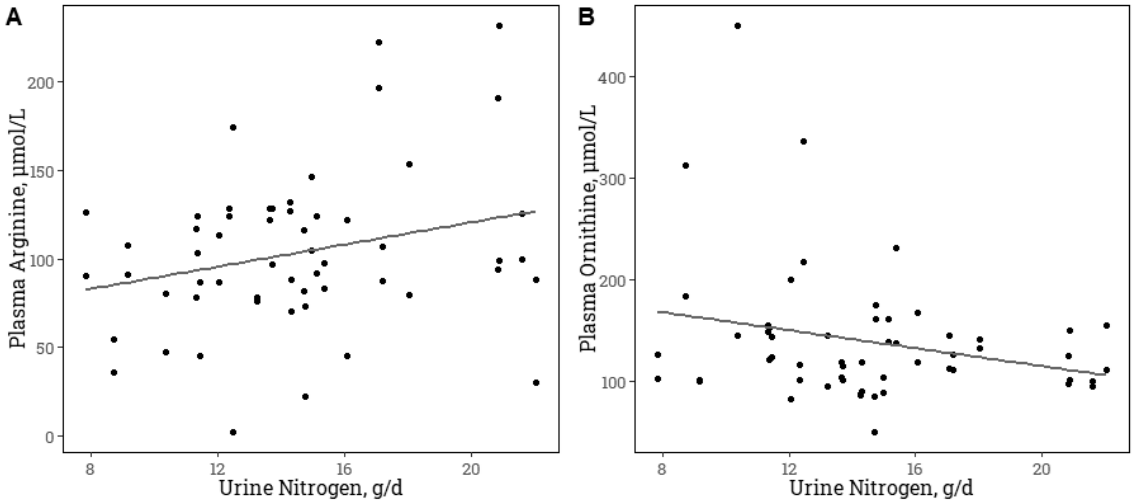

Figure 4-5. A significant linear relationship was detected between urinary nitrogen excretion versus plasma Arginine (P= 0.031; A) and a tendency for the relationship between urinary nitrogen excretion and plasma Ornithine (P= 0.057; B).

CHAPTER 5

EFFECTS OF METHIONINE SUPPLEMENTATION DURING EARLY GESTATION ON SOW BODY WEIGHT GAIN: A PILOT STUDY

ABSTRACT

A previous study predicted that at optimal levels of AA intake, there would be a decrease in sow maternal lean tissue deposition and an increase in reproductive efficiency (i.e. piglet birth weight and litter size). In the current study, pregnant sows were provided SID Met level expected to influence metabolic status (measured as plasma Tau levels) during early gestation to compare the effects of these SID Met doses on sow maternal tissue deposition. Because during early gestation the weight gained by the embryos is negligible in comparison to the weight gained by the maternal body, changes in total body weight during this period are assumed to represent primarily changes in maternal tissue. The hypothesis of the current study is that AA requirements for optimal metabolic status result in a reduction in maternal lean tissue deposition and improvements in the reproductive function. However, reproductive variables were not considered because this proof-of-concept study lacked the statistical power to detect differences in these variables. A total of 39 females (PIC 1050; 22 gilts and 13 Parity 1 sows) were used in the experiment. At day 1 after the last female of each group was breed (d 2.8 ± 1.6), sows were randomly assigned to either a control or Met top-dress, balancing as best as possible for parity and body condition score (BCS) within each block. All sows were fed a traditional corn-soybean meal diet in daily allocations of 1.8, 2.3, and 2.8 kg/d, respectively, for sows with fat, ideal, and thin BCS. Regardless of BCS, the dietary SID Met to SID Lys ratio remained constant in both the control and Met top-dress groups, at

0.39 and 0.54, respectively. Individual BW were measured daily from day 3 after breeding until pregnancy confirmation. Sow BW gain varied by day of gestation in a positive linear ($P < 0.001$) and quadratic ($P = 0.002$) relationship. There was a tendency for a difference in weight gain between the control and Met top-dress groups ($P = 0.070$) with sows in the control group gaining 1.67 kg more than those in the Met group. Because of the lower weight gain, it is possible that the Met supplemented group reduced their AA utilization priority for lean tissue deposition while increasing their priority for using AA for reproduction. In addition to the low statistical power for detecting differences in reproductive performance, any impact on litter characteristics could not be evaluated because of a Porcine Reproductive and Respiratory Syndrome (PRRS) outbreak that occurred during the experiment. In conclusion, the current pilot study shows that SID Met supplementation had an effect on sow growth during early gestation.

INTRODUCTION

In practical commercial livestock production, the AA requirement can be defined as the dose required to provide a high enough level of AA to allow maximal economic return for the production unit (France and Kebreab, 2008). In sows, the AA requirement is influenced by economic factors such as increasing reproductive efficiency, as measured, for example, by the number of pigs produced per sow per year (Lewis and Southern, 2000). There is evidence, however, that current AA requirements for gestating sows promote maternal lean tissue deposition rather than promoting variables related to the sow's reproductive efficiency (Chapter 3). As discussed in Chapters 1, 3 and 4, the AA doses needed to optimize metabolic functions, including reproduction, appear to be higher than those needed to maximize lean tissue deposition. A previous study in gilts at

breeding age (d 210) revealed that the dietary SID Met level that maximized the biosynthesis of Tau, an essential non-proteinogenic AA for embryo development and fetal growth (Holm et al., 2018), was 230% of the NRC (2012) SID Met recommendation for gilts during early gestation (Chapter 4).

The primary focus of the current study is to investigate the dynamics of weight gain during early gestation at SID Met levels that maximized metabolic status (measured as SID Met levels that maximized plasma Tau) in gilts at breeding age. Because AA intakes for optimal metabolic status are predicted to result in a decrease in the priority of AA use for lean tissue deposition and an increase in AA use for physiological functions such as reproduction (Chapter 3 and 4), the SID Met supplementation levels used in this study are hypothesized to result in a decrease in maternal lean tissue deposition.

MATERIALS AND METHODS

Animals and general management

The experiment was conducted at South Dakota State University Swine Education and Research Facility, Brookings, SD. A total of 39 females (PIC 1050; 22 gilts and 13 Parity 1 sows) in 3 subsequent breeding groups (10, 18, and 11 sows each) were used in the experiment. Females were kept in gestation stalls (61 cm × 1.98 m) from breeding to pregnancy confirmation and then moved to gestation pens. From pregnancy confirmation to 110 d of gestation females were offered a common gestation diet (3,300 kcal/kg ME and 0.46% SID Lys) at a feed allocation per day (i.e. 2.27 kg/d) to maintain a target body condition score of 3 on a 5-point scale.

From day 110 of gestation until weaning at day 21 of lactation, females were housed in farrowing crates (1.83 m × 2.44 m) and offered a common lactation diet (3,300

kcal/kg ME and 0.64% SID Lys), according to standard feed curve protocol of the swine unit. Lactation feed was administered by an electronic feeding system (Gestal 3G; Jyga Technologies, Greeley, KS) allowing daily intake up to 20% above the set curve, thus providing for ad libitum intake. Gestation and lactation diets were provided in meal form and water was provided ad libitum via a single water nipple drinker.

Dietary treatments

At day 1 after the last sow of each group was bred (d 2.8 ± 1.6), females were randomly assigned to either a control or a Met top-dress diet, balancing as best as possible for parity and body condition score (BCS) within each block. All sows were fed a traditional corn-soybean meal diet (Table 5-1) in a single meal at (6:30 AM) and in daily allocations of 1.8, 2.3, and 2.8 kg/d, respectively, for sows with fat, ideal, and thin BCS. The control group received 50 g of corn top-dressed daily, whereas the Met top-dress group received a 50 g top-dressed mix of corn and crystalline Met (DL-Met, Feed grade 99%; Evonik Antwerpen N.V.), which varied depending on BCS. Fat, ideal, and thin sows were given 1.57, 2 and 2.44 g/d crystalline Met, respectively, along with additional corn for a total top-dressing dose of 50 g per sow. Regardless of BCS, the dietary SID Met to SID Lys ratio remained constant in both the control and Met top-dress groups, at 0.39 and 0.54, respectively.

Body weight, litter size, and general observations

Individual BWs were measured every other day at 6:00 AM before sows received their meal from day 3 after breeding until pregnancy confirmation. At farrowing, litter size at birth (born alive, still birth, and mummified) was recorded. Daily feed

disappearance was monitored for feed spillage and feed refusal. Sow illness, lameness, reproductive, and clinical signs of disease over the course of the experiment were noted.

Statistical analyses

Changes in BW due to SID Met supplementation were analyzed using a linear mixed model with day and treatment as fixed effects and parity as a random effect. Changes in BW as influenced by day of gestation were analyzed under simple linear regression (linear and quadratic) for each parity. Differences in litter size at birth (born alive, still birth, and mummified) due to SID Met supplementation were analyzed using Two-way ANOVA (main factors: treatment and parity). All statistical analyses were carried out in R version 4.0.5 and R studio version 2021.09.0, with the lme4 package used to fit the linear mixed model and the Stats package used to fit simple linear regressions and Two-way ANOVAs. For all analyses, $P < 0.05$ was considered significant and $P < 0.10$ was considered a tendency.

RESULTS AND DISCUSSION

Five females were removed from the study: four sows were not pregnant (negative pregnancy confirmation) and one sow displayed signs of lameness that prevented it from walking through the scale. Confirmation of pregnancy occurred at d 26.7 ± 1.8. A total of 34 sows were considered in the experiment from which 21 where gilts (control: 10; Met top-dress: 11) and 13 were P1 sows (control: 6; Met top-dress: 7). One female was considered in a fat BCS (control group), 26 were considered in ideal BCS (control: 12; Met top-dress: 14) and 7 were considered in a thin BCS (control: 4; Met top-dress: 3). In the Met group, fat, ideal, and thin sows were set to receive daily allocations of 5.3, 6.8, and 8.3 g SID Met/d, respectively, which is 177, 227 and 277 % of

the SID Met requirement for a gilt and 204, 262 and 319 % of the SID Met requirement for a P1 sow, according to NRC (2012).

During the experiment, a Porcine Reproductive and Respiratory Syndrome (PRRS) outbreak occurred affecting primarily groups 2 and 3 which were bred in August and September of 2021, respectively. Six abortions occurred in group 2, with four in the control group and two in the Met top-dress group. For group 1 (bred July 2021), average litter size at birth was 13.56 (13 liveborn; 0.56 stillborn and 0.56 mummies), for group 2 it was 10.4 (7.2 liveborn; 3.2 stillborn and 4.4 mummies), and for group 3 it was 12.1 (9.8 liveborn; 2.4 stillborn and 3.5 mummies). No differences between the treatment groups or between parities were detected for any reproductive performance variables. The current study's sample size (approximately 17 sows per treatment) would allow for the detection of differences in reproductive performance between treatments if these differences are greater than ~20% (Chapter 1), which is unlikely. Furthermore, the numeric range of the observed litter size variables increased because of the PRRS outbreak, resulting in increased variance and thus even lower statistical power than originally expected.

Using linear mixed models, sow BW gain varied by day of gestation in a linear ($P < 0.001$) and quadratic ($P = 0.002$; Fig. 5-1 A) relationship, and there was a tendency for a difference in weight gain between the control and Met groups ($P = 0.070$). Sows in the control group gained on average 1.67 kg more than those in the Met group (Fig. 5-1A). As discussed in Chapter 3, it appears that at AA doses for optimal metabolic status, animals reduce their AA utilization priority for lean tissue deposition while increasing their priority for using AA for reproduction. The reduced BW gain in the SID Met supplemented group would be explained by a reduction in the priority for lean tissue

deposition caused by adequate SID Met intake levels; however, because the effects of dietary SID Met on litter size could not be determined, this claim is not fully supported. However, the current findings suggest that the SID Met levels given to sows in the current study influence the dynamics of N utilization, which may have implications for improving sow reproductive performance.

For P1 sows, simple linear regression revealed a linear (P = 0.005) and quadratic (P = 0.002; Fig. 5-1B) relationship between day of gestation and BW gain. For gilts, simple linear regression revealed a linear (P = 0.029) relationship but not significant quadratic relationship (P = 0.108; Fig. 5-1C). Thus, the quadratic relationship between day of gestation and sow BW gain detected using the linear mixed model seems to be primarily driven by P1 sows (Fig. 5-1). The quadratic relationship between day of gestation and sow BW gain shown in Fig. 5-1B predicts that P1 sows lose on average 1.9% of BW at breeding by d 10 of gestation. Similar results were observed by Buis (2016) who reported negative growth during the first two weeks of gestation in gilts. The maximum negative growth observed by Buis (2016) occurred at d 12 of gestation with a 2.3% reduction in BW relative to initial BW (Fig. 5-2A). The author attributed this weight loss to sows learning to eat from electronic feeders; however, because the sows in the current study were kept in stalls and a similar trend in sow BW gain was observed for P1 sows, the weight loss observed by Buis (2016) could be due to a physiological response rather than management.

The data reported by Thomas et al. (2018) and Thomas (2019) indicate a similar pattern which suggests that maternal tissue mobilization during the first days of gestation may be a common phenomenon under current feeding strategies. Thomas et al. (2018)

reported 671 gilt BWs considered as accurate across gestation but weights of the first week of gestation were not reported. However, Thomas (2019), report the average initial BW (161.9 kg) from the dataset reported in Thomas et al. (2018). When the initial BW was considered, the data reported by Thomas et al. (2018) showed a similar pattern of reduced BW in the first days of gestation as that reported in Buis (2016). The analysis of Thomas et al. (2018) using splines regression showed that the maximum negative growth occurred at d 12 of gestation 3% reduction in BW relative to initial BW (Fig. 5-2B). In the Buis (2016) and Thomas et al. (2018) studies, gilts returned to positive growth after d 12.

This observed negative growth during the first two weeks of gestation may be related to insufficient energy demands. Sturmey and Leese (2003) reported that in pigs, blastocoel expansion is a highly energy demanding process that triples ATP consumption between morula and early blastocyst stages during pregnancy [day 6; Perry and Rowlands (1962)], most likely to supply the Na+/K+ ATPase pump (Donnay and Leese, 1999; Booth et al., 2005). It is possible that sows supply this increase in energy demands by mobilizing maternal tissue. Leal et al. (2019) in a recent systematic review reported that increased energy intake during early gestation was detrimental for embryo survival in 4 out of the 15 research papers. Included in the analysis, 3 papers were published more than 25 years ago, which suggest potential benefits of increased energy intake on embryo survival. Furthermore, Leal et al. (2019) reported an increase in sow BCS, when sows consumed greater energy levels than current recommendations during the first weeks of pregnancy, especially in P1 sows. Leal et al. (2019) attributed benefits in reproductive performance and sow BCS to increased energy levels; however, the studies included in

their systematic review provided increased feed intake, which included increased AA intake levels.

In addition, although a reduction in sow BW gain during the first two weeks of gestation may be perceived as a negative response, it is possible that during this period the priority for reproduction increases relative to the priority for lean tissue deposition. Thus, sows with a greater weight loss during the first two weeks of gestation may have a greater availability of dietary and mobilized AA (or other nutrients) for reproduction. If this is the case, in the current experiment the diets provided to P1 sows may be more adequate for improving reproductive performance than for gilts, as P1 sows showed a reduced priority for growth compared to gilts. However, these hypotheses must be empirically tested using an adequate number of experimental units that allow to detect expected differences in farrowing performance variables which would also be translated into growth curves that allow a better understanding of the dynamics of sow BW gain during early gestation.

In conclusion, the current pilot study provides preliminary evidence that the supplemented SID Met levels had an effect on sow growth during early gestation. The tendency for the reduced sow BW gain suggest that the provided SID Met levels may have reduced the priority for lean tissue deposition and potentially increased the priority for reproduction, however, this hypothesis must be tested in a study with an adequate experimental design and statistical power.

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Table 5-1. Ingredient composition and nutrient content of the common gestation diet.

Ingredients, %	Gestation diet
Corn	81.61
Soybean meal, 46.5%	14.54
Monocalcium phosphate	1.84
Limestone	1.31
Salt	0.50
Nursery Vitamin premix	0.05
Trace Mineral premix	0.15
Calculated nutrient content[1]	
ME, kcal/kg	3285
NE, kcal/kg	2452
Crude protein, %	13.50
Total Lys, %	0.63
SID Lys, %	0.53
Ratio to SID Lys	
SID Met+Cys	0.78
SID Met	0.39
SID Thr	0.76
SID Trp	0.24
SID Val	1.00
SID Ile	0.87
Total Ca, %	0.89
Digestible P, %	0.41

[1] Calculated based on ingredient nutrient content and digestibility coefficients according to NRC (2012).

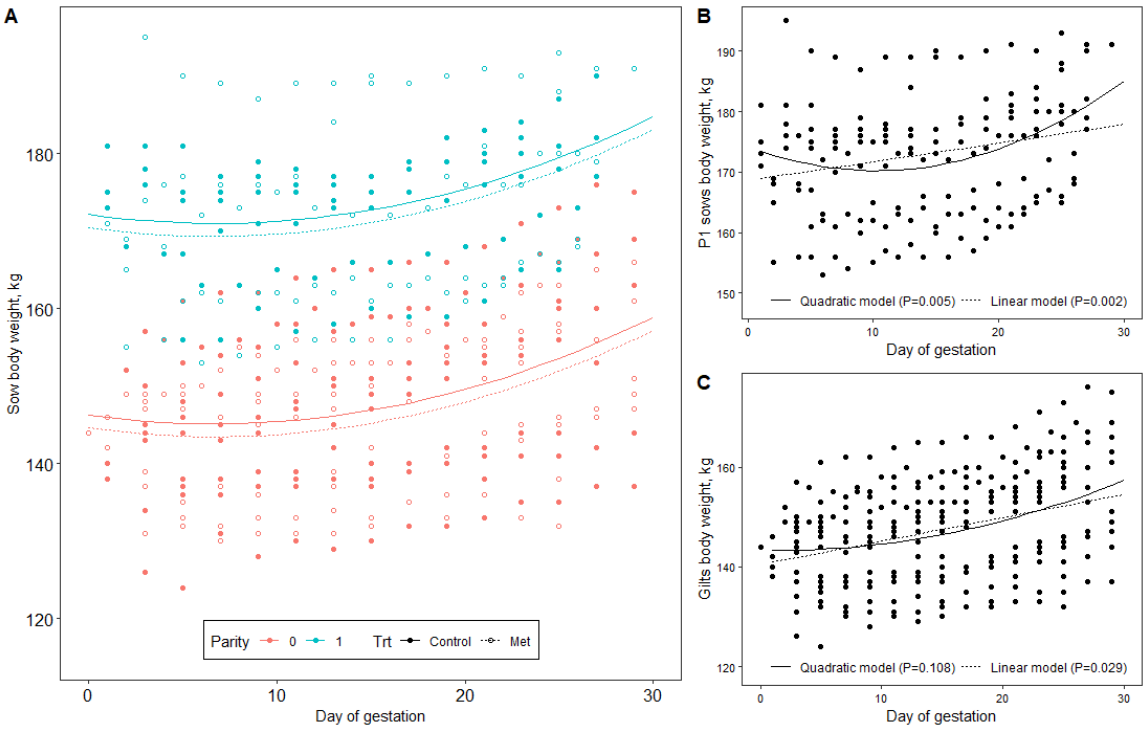

Figure 5-1. Predicted sow body weight gain by parity and treatment using a quadratic mixed model (P = 0.002); under the same model a tendency for the difference between the control group and the methionine supplemented group were detected (P = 0.070). The quadratic response between sow body weight and day of gestation (A) seems to be influenced mainly by P1 sows (B) rather than gilts (C).

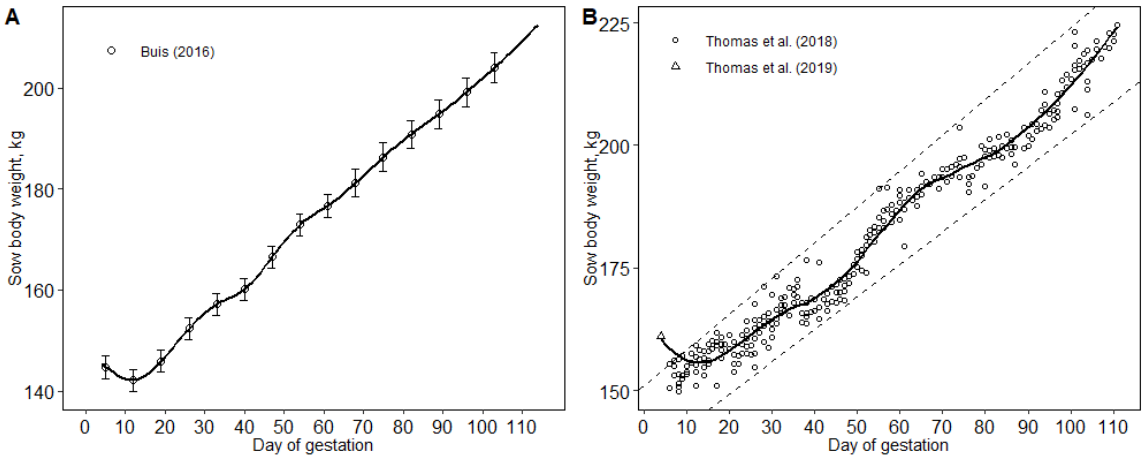

Figure 5-2. The data reported by Buis (2016; A), and Thomas et al. (2018) and Thomas (2019; B), show a negative growth during the first 12 days of gestation.
Thomas et al. (2018) reported 671 gilt body weights considered as accurate across gestation. Weights obtained 5% above or below predicted weights were deemed inaccurate and removed from the dataset (dotted lines). Thomas (2019), which reported data from the same commercial barn in the same time period of time as Thomas et al. (2018), reported an average initial BW of 161.9 kg. Solid line represents the data fitted using splines regression.

CHAPTER 6

PHOSPHORUS BALANCE AS A MEANS TO DETERMINE AMINO ACID REQUIREMENTS IN SOWS: A PILOT STUDY

INTRODUCTION

Feeding pigs diets with an AA balance that corresponds to the animal's requirements is critical for the efficiency of the swine industry. In practical commercial livestock production, AA supply should be adequate for optimizing reproductive efficiency, as measured, for example, by the number of pigs produced per sow per year. On the other hand, AA requirements have traditionally been based on crude protein synthesis measurements (often N balance). Nonetheless, it is unknown whether the protein synthesized at the AA requirement is fetal or maternal tissue or what the ratio of deposition between these two tissues is. As a result, empirically determined AA recommendations may promote maternal rather than fetal tissue growth. A potential alternative to measures of crude protein to estimate AA requirements is to consider phosphorus (P) deposition. The concentration of P in fetal tissue is 10 fold greater than the concentration of P in the maternal tissue gained during gestation (NRC, 2012). Therefore, AA intakes that maximize P retention are more likely to maximize fetal growth than maternal tissue, and thus may be considered as the requirement; a hypothesis that the current study seeks to test.

METHODS

Animals and General Management

The experiment was scheduled to be conducted at the South Dakota State University Swine Education and Research Facility in Brookings, SD, from July 2021 to

January 2022. A total of 51 sows (PIC 1050) in three consecutive groups (breed in July, August, and September 2021, respectively) were used in the experiment (parity 1: 12 sows; parity 2: 18 sows; parity 3: 7 sows; parity 4: 10 sows; parity 5: 3 sows and parity 6: 1 sow). Females were kept in gestation stalls (61 cm × 1.98 m) from breeding to 110 d of gestation and were offered a common gestation diet (3,300 kcal/kg ME and 0.46% SID Lys) at a feed allocation per day (i.e. 2.27 kg/d) to maintain a target body condition score of 3 on a 5-point scale. From day 110 of gestation until weaning at day 21 of lactation, females were housed in farrowing crates (1.83 m × 2.44 m) and offered a common lactation diet (3,300 kcal/kg ME and 0.64% SID Lys), according to standard feed curve protocol of the swine unit. Lactation feed was administered by an electronic feeding system (Gestal 3G; Jyga Technologies, Greeley, KS) allowing daily intake up to 20% above the set curve, thus providing for ad libitum intake. Gestation and lactation diets were provided in meal form and water was provided ad libitum via a single water nipple drinker. Pigs and facilities were checked twice daily by trained personnel.

Dietary Treatments

When confirmed pregnant, animals were randomly assigned to 1 of 6 experimental diets balancing as best as possible for parity and BW within block. The SID Met levels in the six experimental diets (Table 6-1) were set to provide from 95 to 525% of the model predicted daily SID Met for protein retention (NRC, 2012) for a parity 3 sow during mid gestation (days 68 to 79, 2.2 g/d), and from 76 to 416% of the model predicted daily SID Met for protein retention for a parity 3 sow during late gestation (days 96 to 107, 3.7 g/d). The ratios of other essential AA to Lys were above the NRC (2012) requirements. Experimental diets were provided once a day at 0630h at a rate of

2.21 kg/d during mid-gestation and at 2.61 kg/d during late gestation to meet NRC (2012) energy requirements and ensure energy was not limiting the response to test AA level in late gestation. Titanium dioxide was included at 0.20% as an indigestible marker to calculate total tract N and P digestibility.

Nitrogen Balance

The N and P-balances were planned for 7-day diet adaptation and 3 to 5-day urine and fecal collections. Both N and P balance were based on total urine collection using urinary catheters and determination of fecal N and P digestibility using indigestible marker (Short et al., 1996). Urine collection procedure was based on Miller et al. (2016). Prior to collection, urinary catheters (Lubricath, 2-way, 30 mL balloon, 18 French; Bard Medical Division, Covington, GA, USA) are lubricated and inserted flaccidly through the urethra and the balloon was inflated with 30 mL saline solution to retain the catheter in the bladder. Catheters are connected to closed containers using polyvinyl tubing and urine collected. Sulfuric acid is added to the containers to maintain pH <3. A representative subsample (1% of the successful daily collection) was obtained, pooled within each 5-d collection period and stored at 4 °C until further analysis. Urine collection is considered successful when at least 3 complete 24-h collections were accomplished. Urinary catheters are removed at the end of the N and P balance period. Fecal samples were obtained by rectal palpation and daily collections were pooled per gilt and stored at -20 °C until further analysis.

Chemical Analysis

After diet mixing a subsample of feed was collected, pooled and homogenized. Prior to analyses, aliquots from urine samples are placed in 120 mL specimen cups and

approximately 200 g of each experimental diet and freeze-dried feces are ground using rotor mill (Centrifugal Mill ZM 200; Retsch GmbH, Haan, Germany) with 0.50 mm sieve. Urine, freeze-dried feces and experimental diets were analyzed for N content using combustion method (Rapid N III, Elementar Analysensysteme, GmbH, Hanau, Germany); crude protein was calculated as N x 6.25. Dry matter and titanium dioxide content in feces and feeds are quantified according to Short et al. (1996). Feed and urine samples are digested with a nitric-perchloric acid mixture (3:1 v/v) using procedures previously described by Darriet et al. (2017). Absorbance of standard and samples for titanium dioxide content and concentrations of P in feed and urine are read using Spectra MAX 190 plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at 408 nm wavelength. The molybdovanadate method was used to determine P concentrations in feed and urine digests. Amino acid and proximate compositions of experimental diets wew completed by a commercial laboratory (Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO).

Calculations

Nitrogen and P retention (g/d) were calculated from daily feed allowance and analyzed diet N and P content, minus N and P content of wasted feeds and daily N and P excretion in feces and urine. Fecal N and P excretion (g/d) were calculated from intake and total tract digestibility. Daily gilt whole body protein retention (g/d) was calculated as daily N retention x 6.25. Daily SID Met intake is calculated as the product of daily feed intake (kg/d), Met level of diet (g/kg) and SID coefficient (%). SID coefficients are calculated considering the inclusion of corn, soybean meal, and synthetic AA for each diet and their respective digestibility coefficients according to NRC (2012).

Unanticipated study cessation: The first group of animals was successfully collected during mid gestation; however, abortions occurred throughout the herd following this collection period due to an outbreak of PRRS, forcing the experiment to be canceled.

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Table 6-1. Ingredient composition and nutrient content of the experimental diets.

	Experimental diets					
Ingredients, %	Met-1	Met-2	Met-3	Met-4	Met-5	Met-6
Corn Starch	17.06	17.06	17.06	17.06	17.06	17.06
Corn, Yellow Dent	30.16	30.16	30.16	30.16	30.16	30.16
Rye	25.00	25.00	25.00	25.00	25.00	25.00
Soybean Hulls	18.81	18.81	18.81	18.81	18.81	18.81
Soybean oil	3.66	3.66	3.66	3.66	3.66	3.66
L-Lysine HCl	0.31	0.31	0.31	0.31	0.31	0.31
L-Threonine	0.24	0.24	0.24	0.24	0.24	0.24
L-Tryptophan	0.04	0.04	0.04	0.04	0.04	0.04
L-Isoleucine	0.14	0.14	0.14	0.14	0.14	0.14
L-Valine	0.18	0.18	0.18	0.18	0.18	0.18
L-Methionine	**-**	**0.05**	**0.12**	**0.21**	**0.32**	**0.45**
L-Glutamic Acid	**1.50**	**1.45**	**1.38**	**1.29**	**1.18**	**1.05**
Monocalcium phosphate 21%	1.30	1.30	1.30	1.30	1.30	1.30
Limestone	0.66	0.66	0.66	0.66	0.66	0.66
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Sow Vitamin Premix	0.10	0.10	0.10	0.10	0.10	0.10
Trace Mineral Premix	0.15	0.15	0.15	0.15	0.15	0.15
Titanium Oxide	0.20	0.20	0.20	0.20	0.20	0.20
Calculated nutrient content[1]						
ME, kcal/kg	3300	3300.0	3300	3300	3300	3300
NE, kcal/kg	1980	1980	1980	1980	1980	1980
Crude protein, %	9.00	9.00	9.00	9.00	9.00	9.00
Total Lys, %	0.55	0.55	0.55	0.55	0.55	0.55
SID Lys, %	0.45	0.45	0.45	0.45	0.45	0.45
Ratio to SID Lys						
SID Met+Cys	0.46	0.79	0.94	1.14	1.39	1.68
SID Met	0.22	0.33	0.49	0.69	0.93	1.22
SID Thr	0.93	0.93	0.93	0.93	0.93	0.93
SID Trp	0.18	0.18	0.18	0.18	0.18	0.18
SID Val	0.93	0.93	0.93	0.93	0.93	0.93
SID Ile	0.71	0.71	0.71	0.71	0.71	0.71
Total Ca, %	0.60	0.60	0.60	0.60	0.60	0.60
Digestible P, %	0.29	0.29	0.29	0.29	0.29	0.29

[1] Calculated based on ingredient nutrient content and digestibility coefficients according to NRC (2012).

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Current AA requirements have been estimated using the ideal protein concept model, which is based on Lys requirements empirically estimated using N retention measures and an ideal AA profile based on whole animal AA tissue composition (Chapter 1). However, N retention measures do not seem to be adequate for the estimation of Lys doses that maximize reproductive performance (Chapter 1, 3 and 4) and AA profiles of ideal protein based on tissue composition do not consider important physiological functions such as protein turnover (Chapter 1). Therefore, current AA recommendations do not seem adequate for maximizing reproductive performance. As a result, currently considered adequate doses of essential AA may be limiting sow performance, which could explain the transient increase in maternal retention around d 30 of gestation, referred to as time-dependent Pd (Chapter 2).

The transient increase in maternal retention around d 30 (i.e. time dependent Pd) could also be explained by a reduction in N retention during the first 30 days of gestation and a reduction around d 50 when compared to N retention pre breeding (d 0) and at d 68 (Chapter 2). Observations made in Chapter 5 suggest that the transient increase in N retention around day 30, or the time-dependent Pd, may be due to animals regaining weight lost during the first two weeks of pregnancy. As a result, the idea of reduced N retention during early and possibly mid gestation appears plausible. The findings of Chapter 3 show that SID Met intake that maximizes Tau biosynthesis in gilts at breeding age is 230% of current AA requirements for gilts during early gestation, indicating that current AA requirements are underestimated when important metabolic functions for

optimal reproductive performance are considered. The meta-analysis performed in Chapter 3 on the effects of SID Lys intake during early gestation on litter size predicted that SID Lys requirements based on increased Lys intake during the first days of gestation relative to the needs during mid gestation (d 50) resulted in increased litter size and reduced maternal body growth. It is possible that the increase in litter size is related to an increase in the supply of other AA, such as sulfur AA, rather than an increase in Lys supply; the studies included in the meta-analysis increased daily feed intake and not only dietary Lys.

Overall, the findings of the current dissertation suggest that AA requirements based on traditional measures of N retention and the ideal protein based on animal tissue composition do not accurately describe the AA needed for optimal reproductive performance because important dynamic processes such as protein turnover are not well characterized or considered. The linear-logistic model is proposed as a method for using measures of N retention to estimate AA requirements that maximize metabolic functions such as reproduction while potentially taking into account factors such as protein turnover (Chapters 3 and 4). The meta-analyses conducted in Chapter 3 demonstrate that SID Lys requirements estimated using the linear-logistic model, as well as their assumptions, have the potential to maximize sow and piglet performance.

Because of its potential to describe AA doses that maximize lean tissue and metabolic functions, the linear-logistic model could be used to estimate AA requirements for different stages of production, each with its own set of goals. AA doses that maximize lean tissue deposition may be considered optimal for growing pigs, whereas AA doses that maximize metabolic status may be considered optimal for gestating sows and weaned

pigs, as these AA doses may result in increased reproductive performance as well as piglet survivability and robustness. As discussed in Chapter 4, plasma AA has the potential to be used as a method for estimating AA requirements that maximize metabolic status, but not as the traditional point at which additional AA increased plasma AA, but rather as the AA intake that maximizes plasma AA.

In conclusion, the current dissertation characterizes the essential AA deposition that occurs in the main tissues that comprise the pregnant sow throughout gestation. However, having estimates of the composition of the tissue gained throughout gestation is insufficient for estimating AA requirements in pregnant sows, especially because tissues that make up a relatively small proportion of the animal's body appear to require large amounts of AA for maintenance (higher turnover rates). Because dynamics of N utilization, such as protein turnover, are not considered in AA deposition models or the ideal protein concept model based on tissue composition, these methods are not recommended for estimating AA requirements during gestation; more research is needed to make these methods viable for estimating AA doses that maximize pregnant sow reproductive performance. In addition, it is suggested that AA requirements be estimated using dose-response studies that directly measure reproductive performance or indirectly measure N retention using the proposed dose-response curve defined as the linear-logistic model. The linear-logistic model demonstrated to be superior to the previously used broken-line regression (e.g., linear-plateau or quadratic plateau broken line regression models) for the estimation of AA requirements. The linear-logistic model captures not only AA doses that maximize lean tissue deposition but AA doses that potentially maximize reproductive performance and the biosynthesis of essential metabolites for

optimal reproductive performance such as Tau. The use of the methods and considerations presented in this dissertation have the potential to improve pig production efficiency beginning with gestation.