The Effect of Supplementing Low Crude Protein Diets with Crystalline Amino Acids on Growth Performance and Skin Collagen Abundance of Nursery Pigs

by

Kayla E. Silva

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Animal Biosciences

Guelph, Ontario, Canada

ABSTRACT

THE EFFECT OF SUPPLEMENTING LOW CRUDE PROTEIN DIETS WITH CRYSTALLINE AMINO ACIDS ON GROWTH PERFORMANCE AND SKIN COLLAGEN ABUNDANCE OF NURSERY PIGS

Kayla E. Silva
University of Guelph, 2018

Advisor:
Dr. Lee-Anne Huber

Low-crude protein (**CP**) diets supplemented with crystalline amino acids (**CAA**) in swine production reduces nitrogen (**N**) excretion into the environment, and reduces the concentration of non-essential amino acids (**NEAA**) and N, potentially limiting growth and important metabolic processes. Therefore, this thesis investigates whether supplementing NEAA glycine (**Gly**) and serine (**Ser**) to a low-CP diet will improve growth performance, and skin collagen abundance, and to determine if additional threonine (**Thr**) supplementation will spare Gly and Ser. Glycine and Ser supplemented diets had similar skin collagen abundance to CON while glutamate (**Glu**) diets resulted in lower collagen abundance and growth performance. Pigs fed low-CP diets supplemented with Thr had lower performance, but supplementation with 2.8 x Thr rescued collagen abundance in experiment two. Skin collagen abundance and processes beyond protein retention, have unknown implications for long-term productivity of pigs, therefore when feeding low CP diets supplementation with specific NEAA may be warranted.

In memory of Dr. Kees de Lange

I am deeply honoured and humbled to be your last graduate student

ACKNOWLEDGMENTS

First, I consider myself fortunate to work under Dr. Kees de Lange. Thank you for taking me in and giving me this opportunity to pursue graduate school, and showing me a whole new world with pigs. I am deeply honoured to complete a project that you came up with, and I hope I made you proud. Dr. Anna-Kate Shoveller, thank you for directing me to Kees, your constant support and guidance and valued input towards my project. To Dr. John Cant, thank you for your willingness to become my advisor after Kees, and your knowledge and direction throughout my master's degree. I cannot express enough gratitude to Dr. Wilfredo Mansilla. You have been the backbone to this research, and I appreciate all of the questions you helped me answer, and all the laughs we had in between. Last but definitely not least, a huge thank you to my "third times a charm" advisor Dr. Lee-Anne Huber for your guidance, support, encouragement, and overall enthusiastic and exceptional personality. It was nothing but an honour and privilege to work with you.

A special shout out to lab mom and dad (Julia Zhu, and Doug Wey) I cannot put into words how thankful I am for you two. We have had many laughs, jokes, great conversations, as well as your constant support and technical assistance throughout my project. To the de Lange family: Wilfredo, Lee-Anne, Adam Totafurno, Emily Miller, Quincy Buis, Youngji Rho, and Marko Rudar, just to name a few, thank you for being the hardest working individuals I know, always so willing to help, and being an all-around great group of friends. Thank you to my officemates, Youngji, Adam, Wilfredo, Alison Lee, Angie Wilson, Natalie Ferriman, and Josh De Vos and my good friends Leann Denich and Aleks Kobylinski for the good conversations and friendships. I would also like to thank the many undergraduate students who have volunteered their time to help me with my project.

To my friends and family, thank you for believing and supporting me, I know you enjoyed all of the pig pictures you have received throughout the years. A special shout out to my aunt and godmother Suset Silva, thank you for being such an influential role model, for your unconditional love and support and guiding me through my university career. I will be forever in your debt. Thank you to the rest of my family, Flo, my grandparents, the Ross clan (Bridget, Dave, Tara, Ian, Mathew, Shawna) for becoming such a huge part of my life, and last but not least, thank you to my partner Dean, for helping me at the barn, your unconditional love and your witty and charming personality, I couldn't have made it out sane without you!

Finally, I would like to thank the staff at the Arkell Swine Research Station for their hard work. A special thank you to Dr. John Htoo for his constant support and guidance throughout my research project. This thesis would not have been completed without the generous support of Evonik Nutrition & Care, Ontario Pork, OMAFRA and Swine Innovation Porc.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibility
BW	Body weight
CAA	Crystalline amino acid
CP	Crude protein
DM	Dry matter
E:TN	Essential to total nitrogen ratio
EAA	Essential amino acid
G:F	Gain to Feed
Gly	Glycine
His	Histidine
Hyp	Hydroxyproline
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
ME	Metabolizable Energy
Met	Methionine
mTOR	Mammalian target of rapamycin
N	Nitrogen
NEAA	Non-essential amino acid

NRC	National Research Council
Phe	Phenylalanine
Pro	Proline
PSR	Picrosirius red
Ser	Serine
SHMT	Serine hydroxymethyl transferase
SID	Standardized ileal digestibility
TA	Threonine aldolase
TDG	Threonine dehydrogenase
TDH	Threonine dehydratase
Thr	Threonine
Trp	Tryptophan
Val	Valine

1. LITERATURE REVIEW

1.1. Introduction

The National Research Council (**NRC**) recently summarized the body of literature that estimated the essential amino acid (**EAA**) requirements for swine (NRC, 2012). Essential amino acids are well characterized since they are required by mammals as necessary building blocks for protein synthesis, and cannot be endogenously synthesized by the animal in sufficient quantities to meet requirements for protein synthesis (Kim et al., 1983). Consequently, it is critically important to assess these EAA in feedstuffs to determine and predict bioavailability and digestibility values that are additive among mixtures of ingredients, to produce diets that optimize pig performance. On the other hand, research determining requirements for nitrogen (**N**) and non-essential amino acids (**NEAA**) are scarce due to the impression that mammals have the ability to endogenously synthesize sufficient quantities of these compounds (Wu et al., 2013). The latter is in part due to feeding common diets (i.e. corn- and soybean meal- based) that are not limiting in N or NEAA (Kerr et al., 1995). Moreover, the desire to reduce livestock competition with humans for plant-based protein sources has increased interest in research toward determining the effects of low crude protein (**CP**) diets on swine production (Kerr et al., 1995).

Alternative ingredients such as crystalline amino acids (**CAA**) allow for the formulation of low CP diets that are not limiting in EAA, and improve N utilization efficiency for net protein gain, but in turn, reduce NEAA supply (Gloaguen et al., 2014). Research has shown that reducing the dietary CP concentration by more than 4 percent (i.e. to less than 13 % CP in nursery pig diets, and 12.8 % CP in finishing pig diets) had a negative effect on growth

performance (Tuitoek et al., 1997; Powell et al., 2011; Ospina-Rojas et al., 2013). In low CP diets, endogenous synthesis of specific NEAA or total N may limit protein synthesis and optimum growth, especially in young pigs (Ospina-Rojas et al., 2013; Wang et al., 2014b). Consequently, Wu (2014), extrapolated research that was conducted by Baker (1997) and NRC (1988) along with estimated amino acid synthesis rates, accretion, and dietary amino acid uptake by the portal vein in pigs, and suggested that pigs have a rather high dietary requirement for NEAA, which was observed by an increase in body weight gain (**BWG**) when glycine (**Gly**) was included in low CP diets fed to young pigs (Powell et al., 2011; Wang et al., 2014b). However, the increase in BWG with Gly supplementation could in part be due to a threonine (**Thr**) sparing effect, implying that Thr was limiting growth, as shown in research conducted in pigs by Le Floc'h et al. (1997). When dietary Gly supply is low, Thr catabolism may increase to produce extra Gly through the Thr dehydrogenase pathway (**TDG**; Le Floc'h et al., 1995). Le Floc'h et al. (1995) conducted an experiment that showed 80 % of Thr oxidation produced Gly as the end product in swine. Therefore, Thr could generate a sparing effect for Gly, and provide some of the Gly required by the pig when feeding a low CP diet (Le Floc'h et al., 1995).

Considering the holistic needs of the pig (growth and maintenance), Gly may be required for specific metabolic functions, such as neurotransmission, purine and DNA synthesis, and particularly collagen synthesis (Wu, 2014). Collagen represents more than 25 % of total protein in the body (Waterlow, 1984) and of that, Gly accounts for roughly 33 % of amino acid residues in collagen (Gregg and Rogers, 1986). Therefore, it is imperative to re-evaluate the essentiality of Gly, and other NEAA, when feeding low CP diets, as it may be necessary to maintain a healthy collagen turnover rate as well as other non-protein metabolic processes (Gibson et al., 2002).

The present literature review focuses on the importance of amino acid bioavailability and digestibility, optimal EAA to total nitrogen (**E:TN**) ratio in low CP diets, the important interactions between Thr, Gly, and Ser, as well as the metabolic pathway of TDG. Emphasis will be on the NEAA Gly and its functions and influence on collagen synthesis.

1.2. Amino acid bioavailability and digestibility

Formulating balanced diets that meet amino acid requirements is important for optimum growth of swine, and a reduction in environmental pollutants via N excretion (Hobbs et al.,1996). It is critical to determine amino acid bioavailability and digestibility in feed ingredients, since it is highly correlated with production expenses, in order to formulate diets low in CP that meet estimated EAA requirements. The terms bioavailability and digestibility, however, are not interchangeable. Amino acid bioavailability is defined as dietary amino acids that are ingested and absorbed in a form that can be used for protein synthesis (Batterham, 1992). Amino acid digestibility is often used as a measure of amino acid bioavailability, and is defined as the disappearance of amino acid from the intestinal lumen (Sauer and Ozimek, 1986; Stein et al., 2007a).

Ileal digesta collection has been the standard and practical method for determining amino acid bioavailability (Stein et al., 2007b). The least invasive method to sample digesta is through a surgical procedure with a simple T-cannula at the terminal ileum (Stein et al., 2007b). An indigestible marker such as titanium dioxide or chromic oxide is used to calculate the digestibility of amino acids. The samples collected from the distal ileum are used to calculate apparent ileal digestibility (**AID**) coefficients of dietary protein sources (Fuller, 2012; Eq. 1)

$$\text{[Eq. 1] AID (\%)} = 100 - \left[\left(\frac{C_{input} \times AA_{output}}{C_{output} \times AA_{input}}\ \text{g/kg DM}\right) \times 100\right]$$

where C_{input} and C_{output} are the concentrations of index compounds in feed and ileal digesta, respectively and AA_{input} and AA_{output} are the concentrations of amino acid in feed and ileal digesta, respectively (Kong and Adeola, 2014). It should be noted, however, that the AID coefficients include endogenous amino acid losses in the form of endogenous enzymes, sloughed cells, and mucous (Stein et al., 2007a). Therefore, AID underestimates the true digestibility of proteins.

Research conducted by Stein et al. (2005), showed that varying the concentration of dietary CP can negatively affect AID values. In this study, three ingredients (corn, soybean meal and canola meal) were mixed together in different concentrations to create seven diets with CP levels ranging from 9 % to 20 %. Since the corn diet had a low CP level compared to every other diet (9 % vs 20 %) and when fed, pigs had greater endogenous amino acid relative to the total flow of CP and amino acid at the terminal ileum (Stein et al., 2005). Therefore, the AID values calculated for low CP ingredients underestimate the AID of the complete diet (Stein et al., 2005). This leads to controversy about the validity of AID as a method of measuring amino acid digestibility since the digestibility across feed ingredients are not additive and estimated digestibility is dependent on the amino acid and CP concentration in the diet (Stein et al., 2007b).

Correcting for estimates of basal endogenous amino acid losses in AID values is termed standardized ileal digestibility (**SID**; Mosenthin et al., 2000; Eq. 2).

$$\text{[Eq. 2] SID (\%)} = \text{AID} + \left[\left(\frac{\text{Basal endogenous loss}}{AA_{input}}\right) \times 100\right]$$

Formulating optimum non-ruminant diets has predominantly focused on using SID because it is additive among feed ingredients and independent of amino acid concentration in the diet (Mosenthin et al., 2000). In the same study for determining AID values in feed ingredients, Stein et al. (2005) also calculated SID values. The results showed that in 81 out of 88 observations for the four complete diets (corn-soybean meal, corn-canola meal, soybean meal-canola meal, and corn soybean meal-canola meal diets), SID values were smaller than AID values. This leads to the conclusion that SID values in complete diets predict digestibility better than AID (Furuya and Kaji, 1991; Stein et al., 2005) and are additive among a wide range of nutrient concentrations and feed ingredients. Therefore, SID coefficients have been adopted as the most common and accurate digestibility values to formulate practical diets worldwide (NRC, 2012).

1.3. Low crude protein diets in swine nutrition

Due to the increase in demand for high quality protein sources for human consumption, an alternative source of protein is required for livestock species. An ideal source will reduce N excretion and losses into the environment. In swine, 54 % of N ingested in a high CP diet on average will be excreted via the urine and feces (Millet et al., 2018). In order to lessen the burden on the environment, commercially available CAA allow for the formulation of low CP diets that still meet the estimated requirements of EAA. It has been reported that for every 1 % reduction in CP in diets supplemented with CAA, there is an 8-10 % reduction of N losses (Obrock, 1997; Le Bellego et al., 2001). Furthermore, the formulation of low CP diets will lessen the demand for quality protein sources for swine diets, and the impact on the environment.

Over the last 20 years, prominent researchers have analyzed the use of low CP diets in swine (Russell et al., 1987; Kephart and Sherritt, 1990; Hansen et al., 1993; Kerr et al., 1995; Powell et al., 2011; Gloaguen et al., 2014). Experiments conducted by Kephart and Sherritt (1990) in growing pigs showed that a diet containing 11 % CP supplemented with crystalline EAA did not restore growth performance compared to pigs fed a typical corn-soybean meal diet with 17 % CP. These results were supported by Hansen et al. (1993) who found that supplementation of EAA to a 12 % CP sorghum-soybean meal diet fed to growing pigs did not improve growth performance versus pigs fed diets containing 16 % CP. Conversely, pigs fed a diet containing 14 % CP with 0.07 % additional Thr, above estimated requirements, achieved growth performance not different from those fed the 16 % CP diet (Hansen et al., 1993).

On the contrary, Kerr et al. (1995) conducted an experiment with weaning to finishing pigs on growth performance and carcass characteristics as major outcomes. It was determined that pigs fed a diet containing 15, 12, or 11 % CP (starter, grower, and finisher, respectively) corn-soybean meal diets supplemented with Lysine (**Lys**), Tryptophan (**Trp**), and Thr in each phase, exhibited similar growth performance compared to those fed a high CP diet. More recently, research conducted by Gloaguen et al. (2014) is supportive of the aforementioned work, as it was found that decreasing the dietary CP level from 17.6 % to 13.5 % did not affect growth performance of 10 to 20 kg pigs when also supplementing with the EAA Leucine (**Leu**), Isoleucine (**Ile**), Histidine (**His**), Valine (**Val**), and Phenylalanine (**Phe**). Powell et al. (2011) also found that growing pigs fed a low CP, corn-soybean meal diet supplemented with EAA (Lys, Methionine (**Met**), Thr, Trp, Val, and Ile) in the form of CAA in addition to the NEAA Gly, exhibited growth performance not different from pigs fed an 18 % CP diet. In many of these studies, it is generally agreed upon that a reduction in CP by 4 percentage units is

acceptable to maintain maximum growth performance (Russell et al., 1983; Russell et al., 1987; Kerr and Easter, 1995; Figueroa et al., 2003; Gloaguen et al., 2014).

1.4. Non-essential amino acid and Nitrogen

Feeding low CP diets supplemented with crystalline EAA results in reduced N and NEAA supply (Gloaguen et al., 2014). In practical swine diets, the endogenous synthesis of NEAA are sufficient to meet metabolic demands. However, in low CP diets supplemented with CAA, total N can limit NEAA production (Powell et al., 2011). Non-essential amino acids are defined as amino acids that can be produced endogenously in adequate quantities to meet the needs for growth and maintenance (Wu et al., 2013). Since NEAA are accepted as compounds that are always sufficiently produced within the body, dietary NEAA are considered irrelevant for optimal growth, even though they supply over 50 % of total N ingested (Heger, 2003). Numerous studies have shown that NEAA should be provided in a certain ratio to EAA to allow optimum protein utilization and maximum growth in monogastric animals (Frost and Sandy, 1951; Heger, 2003). It should be noted, however, that NEAA play a vital role in metabolism including; DNA, RNA, and glutathione synthesis, and they become conditionally essential in times of stress, disease, lactation, pregnancy, and neonatal development (Ball et al., 1986). Although requirements have not been established for NEAA, a recent study conducted by Hou et al. (2015) suggested that NEAA should be examined carefully since they are necessary in order for pigs to achieve their maximum genetic potential.

There are several metabolic processes that require NEAA in order to maintain homeostasis within the body. These metabolic pathways include but are not limited to: regulation of gene expression and energy metabolism, cell signalling, wound healing, and

neurological functions (Hou et al., 2015). Non-essential amino acids are critical for these intermediate steps, which ultimately play a bigger role in maintaining the integrity of physiological systems including the immune and central nervous system (Reeds, 2000). Wu et al. (2000) suggested a new term "functional amino acids", which includes EAA and NEAA required for all metabolic functions, beyond synthesis of protein and peptides, to improve growth, health, and reproduction. Since the concept of NEAA importance is relatively new, further research is needed to determine the long-term implications of feeding pigs low CP diets without supplementation of NEAA.

1.5. Essential amino acid nitrogen to total nitrogen ratio (E:TN)

In order to obtain optimal growth and protein deposition in pigs, a balance of EAA and NEAA is required, according to the ideal protein concept (Heger, 2003). Diets that solely supply EAA as the dietary source of N failed to promote growth in rats and chicks and the same was true with only supplementing NEAA as dietary N (Kinsey and Grant, 1944; Luckey et al., 1947). Therefore, to maximize growth it is necessary to find an appropriate balance between EAA and NEAA which is often referred to as the EAA to total N ratio(**E:TN**; Mitchell et al., 1968).

There has been a considerable amount of research directed towards determining a balanced ratio of E:TN (Frost and Sandy, 1951; Stucki and Harper, 1961; Heger et al., 1998; Lenis et al., 1999). Previous research to establish the optimum E:TN was conducted on rats and chicks and the estimated ratio was between 0.50 and 0.65 (Frost and Sandy, 1951; Stucki and Harper, 1961). More recently, Lenis et al. (1999) conducted an experiment on growing pigs focusing on the effect of three E:TN ratios (0.35, 0.45, 0.56) at three dietary protein levels (11.8,

14.3, and 18.8 % CP). The results indicated that a ratio slightly below 0.50 promoted optimal N retention and utilization, and the ratio becomes increasingly important with low dietary CP levels (Lenis et al., 1999).

Additionally, Heger et al. (1998) studied the effects of six isonitrogenous diets (15.3 % CP) fed to growing pigs with six ratios of E:TN (between 0.25 and 0.86). At the highest E:TN value, feed intake, N intake, and N retention were reduced (Heger et al., 1998); an N retention breakpoint occurred at a ratio of 0.48 (Heger et al., 1998; Lenis et al., 1999). Although an optimum E:TN ratio has been established for growth and protein utilization using the N balance technique, there is a need to define an optimum E:TN ratio to account for physiological functions beyond protein metabolism.

1.6. Glycine metabolism and functions

Over the last century, there has been a general consensus to disregard the importance of NEAA in diets (NRC, 2012). Glycine is considered the simplest NEAA, and can be produced endogenously in order to satisfy requirements for maintenance and growth, and therefore is not supplemented as CAA in the diet (Darling et al., 2000; NRC, 2012). Currently, controversy continues to surround Gly and whether it should be deemed essential, conditionally essential, or non-essential. Research conducted by Stucki and Harper (1961) in poultry included Gly as a component of EAA. In contrast, Alleman et al. (2000) considered Gly as a NEAA. On the contrary, Graber and Baker, (1973) deemed Gly a conditionally essential amino acid. More recently, Wang et al. (2014a) concluded that in milk-fed young pigs, endogenous synthesis of Gly cannot meet the requirements for whole body growth and protein synthesis. All of these inconsistent views prove that more research is necessary to determine the importance of NEAA

in mammals and demonstrates that functions beyond protein synthesis are not considered in requirement studies.

The NRC (2012) provides fundamental information on EAA, but, it neglects the importance of NEAA in metabolic functions beyond protein synthesis, especially in young animals. Deng et al. (2009) conducted an experiment to determine whether low CP diets (12.7, or 16.7 %) with equal amounts of EAA as a high CP diet (20.7 %) would restore growth performance such as final body weight (**FBW**), average daily feed intake (**ADFI**), average daily gain (**ADG**), and gain:feed (**G:F**) in 4-6-week-old piglets. The effect on final BW, and ADFI were not significant, however, BWG and G:F declined when piglets were fed the low CP diet, and this was due to inadequate synthesis rates of NEAA, which in part inhibited the mammalian target of rapamycin (**mTOR**) signalling pathway and limited protein synthesis (Deng et al., 2009). More recently, Wu and colleagues have published a substantial amount of research suggesting that there is a dietary need for Gly, and other NEAA, as functional AA that benefit health, reproduction, and survival (Wu, 2009; Wu, 2010; Wu et al., 2010; Wu et al., 2013; Wu, 2014).

Glycine has significant involvement in key metabolic pathways that are often overlooked (Table 1; Akinde, 2014; Wang et al., 2014b; Hou et al., 2015). Glycine directly influences purine and serine synthesis, gene expression via nucleic acid production, constituents of collagen and elastin, and creatine and glutathione synthesis (Wu, 2009). Melendez-Hevia et al. (2009) analyzed pathways of Gly production and consumption within the human adult to determine if Gly should be considered a conditional AA (Table 2). The results from this study suggest that the utilization of Gly through metabolic processes such as the synthesis of collagen and metabolites is greater than dietary supply. In order to satisfy all reactions that include Gly

as a precursor, an extra 10 g/day is needed in the diet to meet requirements in an adult (Melendez-Hevia et al., 2009). This research is imperative as it can be directly related to Gly consumption in non-ruminant animals, specifically swine.

Milk has been historically considered the most balanced protein, which supplies all amino acids needed for offspring growth and development (Wang et al., 2014a). However, analysis of sow's milk showed that a 7-day old piglet will only receive around 20 % of the daily Gly needed for protein synthesis, meaning that the piglet will need to endogenously synthesize 80 % of the Gly necessary to satisfy protein synthesis within the body (Wu, 2010). Similarly, in swine and poultry, only 50 % of the Gly 'requirement' is present in a practical diet, meaning that endogenous synthesis must be sufficient enough to provide the remainder of Gly 'requirements' (Hou et al., 2016). However, a study conducted by Ospina-Rojas and colleagues (2012) in male chicks showed that a reduction in dietary CP by 3 to 4 % with supplementation of Gly and Ser restored growth performance to reach levels comparable to those fed the control (high CP) diet. This study agrees with work conducted by Corzo et al. (2004) and shows that endogenous synthesis of Gly is insufficient to support optimal growth.

To establish the importance of dietary Gly in piglets, Wang et al. (2014a) conducted an experiment where piglets received a milk replacer diet with four levels of supplemental Gly (0, 0.5, 0.1, 0.20 %). With addition of Gly, ADG increased, villus height in the small intestine was enhanced, and jejunal mRNA levels for Gly transporter, GLYT1, increased (Wang et al., 2014a). This research provides compelling evidence that supplemental Gly in diets fed to milk-fed piglets is needed since endogenous synthesis of NEAA is inadequate to sustain optimal performance.

1.7. Role of glycine in collagen synthesis

Collagen is an extracellular triple-helix protein that maintains structural integrity in all body tissues and organs (Shoulders and Raines, 2009; Devlin, 2011). Collagen is recognized as the most abundant protein in humans, with skin having the highest collagen content by weight at 74 %, followed by the cornea at 64 % (Devlin, 2011). Of the AA that comprise collagen, 33 % is Gly and 13 % is Proline (**Pro**; Gregg and Rogers, 1986); the tripeptide chain sequence is either Gly-Pro-X or Gly-X-Hydroxyproline (**Hyp**), or X-X-Gly where X represents any amino acid (Devlin, 2011). It has been shown by Midas et al. (2005) that the amino acids Ala and Ser are able to replace Gly residues in collagen and stabilize the tripeptide chain, however it is in contradiction with research conducted by Horng et al (2007).

Human and pig skin are known to have similar properties such as the relative thickness of the epidermis and dermis, making the pig a great model for studying wound healing and other analyses, but the collagen content of fast growing genotypes is currently unknown (Tzeng et al., 2018). Out of the six collagen types present in tissues, Type I and III are found in skin, while Type III has the highest content of Gly and Hyp (Devlin, 2011). Based on this knowledge, a staining technique that targets Type III collagen, such as Picrosirius Red, is beneficial for the determination of skin collagen abundance.

1.7.1. Glycine and Serine

Glycine can be interconverted to Ser by the addition of a 1-carbon unit via the enzyme Ser hydroxymethyl transferase (**SHMT**) in the cytosol of cells (Wixom et al., 1955; Wu, 2003). For this reaction, SHMT catalyzes the regeneration of tetrahydrofolate from N^5, N^{10}-methylene tetrahydrofolate and as a result, Gly is the methyl acceptor (Wu, 2003). In poultry, because Gly

and Ser are biosynthetically linked, the nutritional composition of diets will frequently combine these two amino acids (Ospina-Rojas et al., 2013) and a similar diet formulation adaptation should be considered for swine.

1.8. Threonine degradation

Threonine is an EAA that is commercially available as a dietary supplement and is the third limiting after Lys and Met for growing-finishing pigs fed commercial corn and soybean meal diets (Kerr et al., 1995). Indeed, it is crucial to meet Thr requirements for optimal growth. However, there is evidence that supplying Thr above requirements can also create a sparing effect for the NEAA Gly. Therefore, dietary Thr supplementation can be used instead of Gly, as it is commercially available for inclusion in swine diets (Seve et al., 1993; Le Floc'h et al., 1994; Ospina-Rojas et al., 2013).

There are three known irreversible degradative enzymes for Thr in mammals: Thr aldolase (EC 4.1.2.5; **TA**; Karasek and Greenberg, 1957), Thr dehydratase (EC 4.2.1.16; **TDH**; Sayre et al., 1956) and Thr dehydrogenase (EC 1.1.1.103; **TDG**; Green and Elliot 1964). Schirch and Gross (1968) revealed that TA and serine-glycine hydroxymethyltransferase were in fact the same protein involved in the interconversion of Gly and Ser and the activity of TA did not contribute to Thr degradation. Less attention has been given to TA and TDH, as they have low, or even undetectable activity in liver mitochondria, in rats and pigs and therefore TDG is the main enzyme responsible for Thr degradation (Ballevre et al., 1990; Le Floc'h et al., 1995).

1.8.1. Threonine dehydrogenase enzyme

Threonine degradation begins with TDG, a mitochondrial enzyme that converts Thr to 2-amino-3-oxobutyrate. With the help of 2-amino-3-oxobutryate ligase, 2-amino-3oxobutyrate is then broken down to form acetyl CoA and Gly, while aminoacetone is formed spontaneously and non-enzymatically (Figure 1; Bird et al., 1984; Le Floc'h et al., 1995; Davis and Austic, 1997). Studies have shown that 80 % and 87 % of Thr catabolism in the pig and rat, respectively, produces Gly and acetyl CoA as an end product, suggesting that TDG is the major pathway for Thr degradation in mammals (Bird and Nunn, 1983; Ballevre et al., 1990; Kao and Davis, 1994). Similarly, David and Austic (1997) found that TDG was the only enzyme present in chicken liver for Thr degradation.

Lee et al. (2011) conducted two experiments with rats and chickens to determine the effect of dietary Thr and protein level on TDG activity. Rats were supplied with a low or high CP diet (12 and 18 %, respectively) with three dietary levels of Thr (0.28, 0.42, 0.72 % for low CP and 0.42, 0.52, 0.72 % for high CP). Chickens were supplemented with either a low or high CP diet (18.5 or 22.5 %, respectively) with two dietary Thr levels (0.45 and 0.60 %). As dietary Thr supply increased in the rat, TDG activity in the liver increased, but, there were no differences in activity with an increase in CP content, which is also in agreement with Le Floc'h et al. (1994). Conversely, increasing dietary Thr supply in the chicken did not increase TDG activity, although an increase in CP upregulated TDG activity, which is reported by Davis and Austic (1997).

Furthermore, it has been shown that the activity of TDG is upregulated by Thr imbalance (Davis and Austic 1994). Davis and Austic (1994) formulated four Thr imbalanced diets by

supplementing these diets with branched chain amino acids (**BCAA**), with the addition of 3 % serine, 6 % BCAA, or 5.6 % EAA to a basal diet, versus a control diet. When chicks were fed the 6 % BCAA and 5.6 % EAA diets, feed intake, and weight gain were reduced, while TDG activity increased by 50 % compared to chicks fed the control diet (Davis and Austic, 1994). The accumulation of Gly relative to the amount of aminoacetone found in TDG enzyme activity in chicks fed any of the Thr imbalanced diets increased by four times compared to the control diet (Davis and Austic, 1994). The TDG enzyme activity in chicks fed the diets supplemented with BCAA or EAA increased by about seven times versus chicks fed the control diet (Davis and Austic, 1994). Moreover, Bird and Nunn (1983) showed that at low concentrations of dietary Thr, Gly production exceeds aminoacetone production. Davis and Austic (1993) report that in the case of Thr imbalance, that there is possibly a metabolic need or 'requirement' for Gly (Davis and Austic, 1993). More research is necessary to determine the activity of TDG during different feeding regimens, and if Gly is needed as a precursor for adequate growth and maintenance of pigs.

1.8.2. Threonine and Glycine interaction

As previously stated, Gly is produced from Thr via the TDG pathway (Bird and Nunn, 1983). Earlier studies on the interaction between Thr and Gly provided conflicting results (Baker et al., 1972; D'Mello, 1973; Corzo et al., 2009; Ospina-Rojas et al., 2013). Ospina-Rojas et al. (2013) fed broilers eight diets with four levels of Gly+Ser (1.84, 1.98, 2.12, and 2.26 %) and two levels of Thr (0.93 and 1.07 %) to meet 100 and 115 % of estimated Thr requirements, respectively, to determine the amino acid level for optimum growth in broilers fed a low CP diet. At the highest supplementation of Gly+Ser and Thr (2.26, and 1.07%, respectively), BWG, and feed efficiency were lower than birds fed the low CP diet, but

increased with 1.84 % Gly+Ser, and 1.07 % Thr. These results suggest that over-supplementation of Thr can reduce the need for Gly+Ser when they are supplied at lower dietary levels, likely through the TDG pathway, and high Thr concentrations are not required if the diet has sufficient Gly+Ser concentrations (Ospina-Rojas et al., 2013).

On the contrary, D'Mello (1973) added 0.3, 0.5 or 1.0 % Thr and 0 or 1.2 % Gly to a basal diet for chicks. Two assays were analyzed for the conversion of Thr to Gly and acetaldehyde, and the results indicated that Thr degradation to Gly was negligible in the chick (D'Mello, 1973). Studies including the interaction between Thr and Gly have been prominent in poultry where Gly+Ser is required in the diet for optimal growth performance in chicks, but, there is extrapolated evidence that Gly+Ser may be needed in the diet of other species as well, including swine (Le Floc'h et al., 1995; Corzo et al., 2009; Wu, 2009; Wu, 2014).

1.9. Conclusion

In recent years, NEAA in swine diets have received more attention due to their importance when feeding diets low in CP. The importance of NEAA have been inconsistent throughout the literature, although NEAA play a vital role in processes beyond protein metabolism including DNA, RNA, and glutathione synthesis. Similarly, researchers have proven that Gly is required for optimum growth, specifically in milk-fed piglets. Alternatively, supplementing low-Gly diets with Thr, is an option for producers, instead of directly supplying Gly. It is important to maintain a balance of E:TN so that N is not limiting in low CP diets which will ultimately have a detrimental effect on animal performance. Therefore, research is required to determine the fates of NEAA beyond protein metabolism so that a well-balanced

feed ration is provided to satisfy all protein and metabolic requirements of swine species and to improve the sustainability of animal production.

Table 1-1. *Glycine as a precursor or substrate to a myriad of functions in human and animal metabolism.*

Glycine functions	Description
Synthesis of Lecithin	Consumes adenosyl-methionine (source of all methylation reactions). The methyl groups are taken from tetrahydrofolate-C, with the enzyme glycine hydroxymethyltrasnferase (GHMT) which plays a role in Gly synthesis [a]
Biosynthesis of purine bases	Purine synthesis consumes one Gly molecule, and yields two C_1 and two Gly molecules, therefore producing one molecule of Gly [a]
Biosynthesis of Thymine	Thymine is directly involved in DNA synthesis and consumes C_1 units for methyl groups. The consumption of one methyl group signifies a production of one Gly molecule [a]
Synthesis of Glutathione	Important as an antioxidant, free radical scavenger and regulation of gene expression. Glutathione synthase adds Gly which in turn creates glutathione [a]
Sarcosine Metabolism	Often referred to as methylglycine, produces glycine through oxidative transfer of the methyl group to tetrahydrofolate (THF) [b]
Glyoxylate Metabolism	Produces Gly with enzyme alanine-glyoxylate transaminase (AGT). Hydroxyproline degradation produces glyoxylate, being a source of Gly [cd]
Biosynthesis of Carnitine	The pathway for carnitine produces Gly as a by-product through 3-hydroxy-trimethyl-lysine with help from hydroxytrimethyl-lysine aldolase [e]
Biosynthesis of Porphyrins	Glycine is crucial for porphyrins as eight Gly molecules are needed for each haem group, which is important in haemoglobin, myoglobin, and cytochromes [f]
Synthesis of Collagen and Elastin	Collagen and elastin both have a Gly content of over 30 % which consumes a great portion of Gly [g]
Calcium regulation	Modulates intracellular calcium levels and cytokines [h]
Synthesis of hormones	Participates in steroid synthesis including estrogen and androgen [i]
Neurotransmitter	Regulates feed intake, homeostasis and behaviour through the central nervous system [j]
Bile Conjugation	Glycine contributes to the conjugation of bile acids thereby influencing digestion and absorption of lipids and other nutrients [k]

[a] Adapted from Melendez-Hevia et al. (2009)
[b] Mudd et al. (1980)
[c] Ruiz-Torres and Kurten (1976)
[d] Thompson and Richardson (1967)

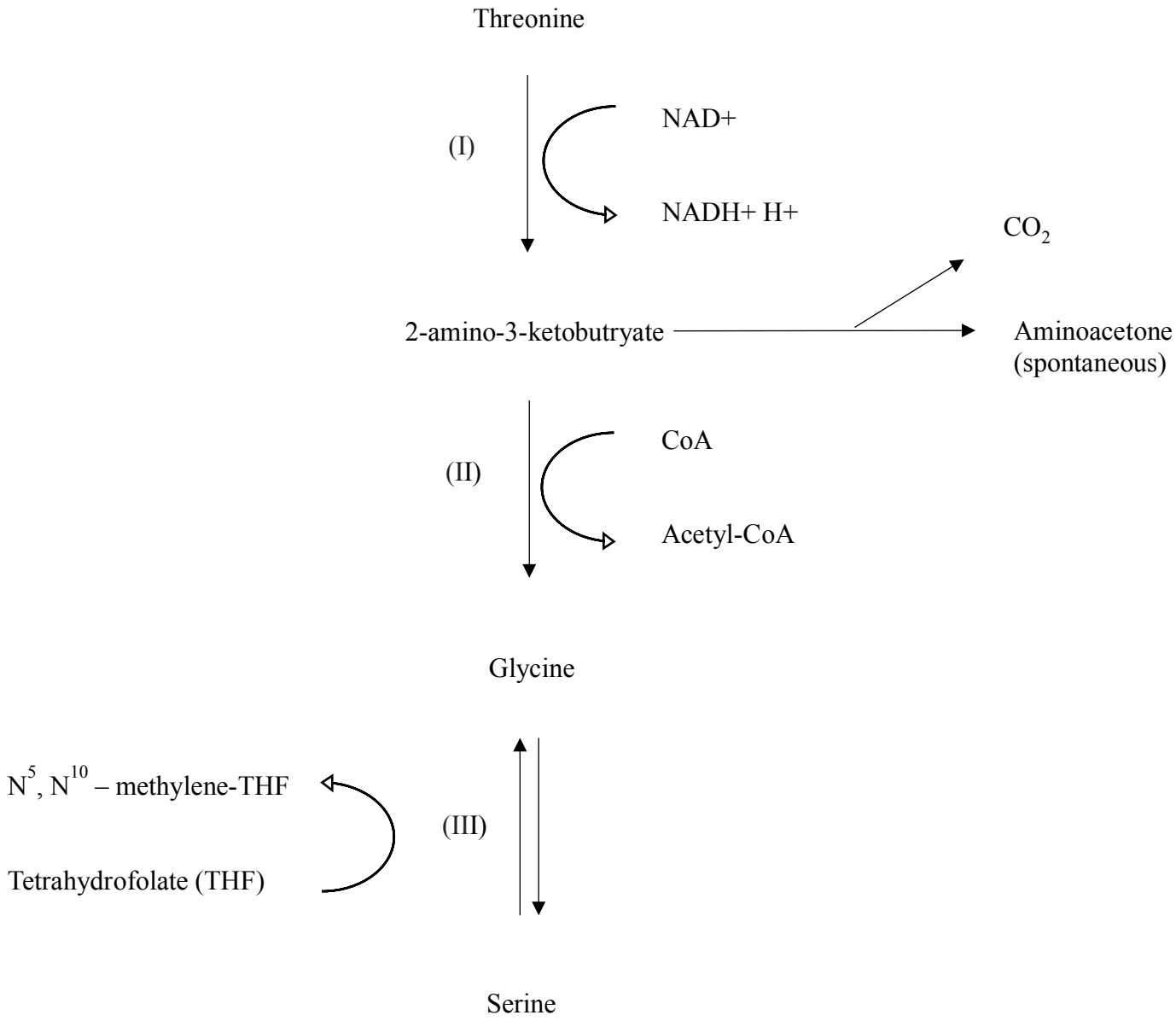
[e] Vaz and Wanders (2002)
[f] London et al. (1950)
[g] Boudier et al. (1981)
[h] Wu (2009)
[i] Finkelstein (1998)
[j] Rajendra et al. (1997)
[k] Hafkencheid and Hectors (1975)

Table 1-2. *Glycine consumption (g/day) in an average human*[a]

Source	Glycine Flux g/day
Protein hydrolysis / Glycine synthesis	+2.3[b]
Metabolism synthesis	+2.9[c]
Metabolite synthesis	-1.5[b]
Non-collagen protein synthesis	-1.0[b]
Collagen synthesis	-12[d]
Gly Balance	-9.3

[a] Data adapted from Melendez-Hevia et al., 2009.
[b] Reference by Gibson et al., 2002.
[c] Remethylation by methionine synthase or homocysteine methyltransferase.
[d] Melendez-Hevia et al., 2009; Collagen synthesis assuming that 95 % Gly is recycled. Values are dependent on this assumption and will change accordingly with amount of Gly recycled.

Figure 1-1. *Threonine dehydrogenase and Gly to Ser interconversion pathway in mammals. (I): Threonine dehydrogenase; (II): Amino ketobutyrate lyase; (III): Serine hydroxymethyl transferase. Adapted from Ballevre et al., 1990.*



2. RESEARCH RATIONALE AND OBJECTIVES

Interest is on the rise for low CP diets in livestock production because they will decrease harmful environmental pollutants and lessen the competition between humans and livestock for dietary protein sources. However, lowering the CP content in the diet has been shown to negatively impact growth performance of livestock (Kephart and Sherritt, 1990; Hansen et al., 1993). The introduction of CAA to diet formulations enables a reduction in CP, while increasing efficiency of EAA utilization for net protein gain. Little attention has been focused on NEAA since animals can synthesize these components endogenously (Darling et al., 1999). The endogenous synthesis of NEAA, however, may limit maximal growth when feeding low CP diets, if insufficient NEAA are provided in the diet. Current research has shown that among the NEAA, Gly may play an important role to maximize growth performance when animals are fed a low CP diet (Powell et al., 2011). Additionally, Wu et al. (2009) consider Gly, and other NEAA crucial for metabolic functions such as gene expression, DNA methylation, and collagen and glutathione synthesis. Furthermore, Thr catabolism can alleviate a low Gly supply by producing Gly via the TDG pathway (Baker et al., 1972; Le Floc'h et al., 1995; Corzo et al., 2009). To further explore the potential 'requirement' for Gly, collagen, a protein, which is Gly rich (33 % of collagen), needs to be evaluated. To initiate a healthy collagen turnover rate, enough Gly should be produced endogenously, or supplied in the diet (Li and Wu, 2018). It has been reported that the synthesis of collagen is sensitive to low dietary supply of Gly (Gibson et al., 2002).

Therefore, the objectives of the studies presented in this thesis are:

1. To determine the effect of supplementing Gly and Ser in a low CP diet on protein retention, growth performance, and skin collagen abundance of nursery pigs (Chapter 3)
2. To determine the effectiveness of supplementing Thr above estimated requirements to compensate for low dietary Gly in a low CP diet on growth performance, protein deposition, and skin collagen abundance in nursery pigs (Chapter 4)

3. THE EFFECT OF SUPPLEMENTING GLYCINE AND SERINE TO A LOW CRUDE PROTEIN DIET ON GROWTH PERFORMANCE AND SKIN COLLAGEN ABUNDANCE OF NURSERY PIGS

3.1. Abstract

A total of 104 newly weaned barrows, 21 days post weaning (initial body weight (**BW**) = 6.41 ± 0.61 kg ± SD) were used to determine the effect of a low crude protein (**CP**) diet supplemented with glycine (**Gly**) and serine (**Ser**) on pig growth and skin collagen abundance in comparison to a high CP corn- and soybean meal-based commercial diet during the nursery phase. Barrows were assigned to one of three diets in a 3-phase feeding program (Phase 1, 2, 3; **P1, P2, P3,** respectively) according to BW: 1) conventional corn-soybean meal diet; Control (**CON**; 20.3 - 23.1 % CP; as-fed, analyzed contents); 2) low CP diet (14.8 – 21.4 % CP) supplemented with G+S to the same dietary concentration as the CON; 3) low CP diet supplemented with glutamate (**Glu**) to maintain the same CP level and essential to total nitrogen (**E:TN**) as the G+S diet (**GLU**; 15.0 – 22.1 % CP). All diets were formulated to meet estimated essential amino acid requirements (NRC, 2012). Pigs were weighed individually each week for six consecutive weeks to determine average daily gain (**ADG**) and feed weigh backs were collected every week per pen to determine average daily feed intake (**ADFI**). On day 21 (n = 8) and 35 (n = 8) pigs were euthanized for the determination of physical and chemical body composition and the collection of skin samples, that were taken from the ham, stained with Picrosirius red (**PSR**), and analyzed for skin collagen abundance. Pigs fed the CON diet had a greater final BW (26.6 kg) and overall ADG (0.50 kg/d) compared to GLU (24.1 kg, and 0.43 kg/d; P = 0.01), while intermediate values were observed for G+S (24.8 ± 0.6 kg, and 0.46 ± 0.01 kg/d, respectively). Overall ADFI, and gain to feed (**G:F**) were not influenced by dietary

treatment. Carcass weights on day 21 and 35 were greater for pigs fed CON (14.3, 22.4 kg, respectively) than G+S (12.3, 19.0 kg) or GLU (12.5, 20.4 kg; $P < 0.05$). Viscera weights on days 21 and 35 were greater for CON (2185, 3373 g, for days 21 and 35, respectively) than G+S (1888, 2912 g; $P < 0.05$); intermediate values were observed for GLU (1961, 3186 g). Overall, whole-body N retention and N intake were greater for CON (11.98, 38.3 g/d, respectively) than G+S (9.02, 27.5 g/d) or GLU (9.52, 29.1g/d; $P < 0.05$). Pigs fed G+S and CON diets (P2: 72.8, 72.0 %; P3: 72.0, 71.8 % for G+S and CON, respectively) had greater skin collagen abundance than pigs fed GLU (P2: 67.2 and P3: 69.3, ± 0.63 %; $P < 0.01$). Supplementing low CP diets with GLU did not maintain pig growth performance compared pigs fed a conventional diet, while pigs fed G+S had intermediate values. Only the G+S diet maintained pig skin collagen abundance not different from pigs fed CON. Supplementing specific NEAA as well as measures beyond growth performance should be considered when formulating low-CP diets.

Key words: glycine, serine, diet, low-crude protein, collagen, nursery pigs

3.2. Introduction

Previous studies have demonstrated that nursery pigs fed a low crude protein (**CP**) diet supplemented with lysine, methionine, threonine and tryptophan had similar growth performance compared to pigs fed a conventional high CP corn and soybean meal diet (Kerr et al., 1995; Tuitoek et al., 1997). Feeding reduced CP diets that will closely match amino acid requirements will benefit the environment by reducing nitrogen (**N**) excretion through closely matching amino acid requirements, and will also lessen the competition between humans and livestock for high quality protein sources. However, the reduction of protein in the diet by

supplementing essential amino acids (**EAA**) will also result in the reduction of dietary N and non-essential amino acids (**NEAA**). Therefore, endogenous synthesis of NEAA that are found in reduced quantities in low CP diets, could limit optimum growth in swine. A recent study conducted by Powell et al. (2011) examined a low CP diet supplemented with Gly compared to a high CP corn and soybean meal diet, and found that pigs fed diets supplemented with Gly had similar average daily gain (**ADG**) and gain to feed (**G:F**) as pigs fed the high CP diet, suggesting that the NEAA Gly can restore growth performance when pigs are fed a low CP diet (Powell et al., 2011). Since Gly and Ser are interconvertible, and to ensure that the response seen is not due to a limitation in Ser specifically, both of these amino acids will be included in the current study.

The hypothesis of this chapter is that supplementing Gly and Ser to nursery pigs fed a low CP diet will result in growth performance and skin collagen abundance not different from those of pigs fed a high CP corn- and soybean meal-based commercial diet. The objective is to determine the effect of Gly and Ser supplementation in a low CP diet on protein deposition, growth, and skin collagen abundance of pigs during the nursery phase.

3.3. Methodology

3.3.1. Animals and Diets

The experimental protocol was approved by the University of Guelph Animal Care Committee and followed the Canadian Council of Animal Care guidelines (CCAC, 2009; AUP e3786). A total of 104 newly weaned barrows (initial BW = 6.41 ± 0.61 kg ± SD) were used and housed at the Arkell Swine Research Station (Ontario Ministry of Agriculture, Food and

Rural Affairs, Guelph, ON: University of Guelph, Ontario, Canada). Barrows were weighed and separated by BW to either a light or heavy category (5.97 ± 0.43 kg, 6.81 ± 0.49 kg, respectively). Eight initial barrows were sent to the University of Guelph and immediately euthanized via exsanguination to determine initial body protein mass. The remaining 96 barrows were randomly assigned to 24 pens (190 cm x 157 cm) with 4 pigs per pen according to weight class.

Three experimental diets were created at the Arkell Feed Mill (Guelph, Ontario, Canada) and were provided in a 3-phase feeding program according to BW (NRC, 2012). Diet samples were collected immediately after they were created, and were sent to Agri-Food Laboratories (Guelph, Ontario, Canada) for chemical analysis. Diet samples were also sent to Evonik Nutrition & Care GmbH Analytical Services (Frankfurt, Germany) and analyzed for total amino acid content. All diets were formulated 10 % above EAA requirements according to the NRC (2012). Phase 1, Phase 2, and Phase 3 (P1, P2, P3, respectively) continued for 7, 14, and 18 days, respectively. Newly weaned pigs received easily digestible protein sources such as whey, blood plasma, and blood meal for all P1 diets and the control (CON) diet for P2 to ensure adequate protein levels. The CON diet for P1, P2, and P3 (23.1, 22.7, 20.3 % CP as-fed; analyzed contents respectively) was formulated as a conventional corn- and soybean meal-based diet (commercial diet). The low CP, semi-purified Gly and Ser diet (G+S) for P1, P2 and P3 (21.4, 17.2, 14.8 % CP, respectively) was formulated by adding crystalline Gly and Ser, so that the dietary level matched the level of G+S found in the CON diet. The low CP glutamate (GLU) diet for P1, P2 and P3 (22.1, 17.8, 15.0 % CP, respectively) was formulated by replacing all crystalline Gly and Ser with Glu to maintain the same CP level and E:TN as the G+S diet.

Pigs were fed ad libitum in feed troughs and had free access to water throughout the entire study.

3.3.2. Observations and analysis

On days 21 and 35, 1 pig per pen (8 pigs per treatment) was taken to the University of Guelph to be euthanized for the determination of physical body composition and body protein mass. Viscera were thoroughly rinsed with water, ensuring blood and intestinal contents were removed, and weighed. Carcasses and viscera were stored in a - 20°C freezer until further analysis. The rest of the pigs were kept on trial for measurement of ADG and ADFI until day 39. Pigs were weighed individually each week for 6 consecutive weeks for the determination of ADG. Feed refusals were weighed every week per pen to determine ADFI. Gain-to-feed was calculated per pen as the ratio of total ADG to total ADFI.

3.3.3. Whole-body protein analysis

Frozen, whole carcasses and viscera were ground 3 times using a meat grinder (Model B-801, Autio Company, Astoria OR, USA) to ensure proper mixing. Two-hundred gram ground subsamples of carcass and viscera were weighed before and after freeze drying to determine dry matter (**DM**) content (Mansilla 2017). The DM contents of freeze-dried carcass, viscera and diets were determined via forced air oven drying for 2-h at 135 ˚C according to AOAC (1997; Method 930.15; Model 737F, Fisher Scientific, Hampton, New Hampshire, United States). Nitrogen content was determined by combustion analysis according to AOAC (1997; Method 990.03; Model; 600, LECO Corporation, Saint Joseph, MI, USA). Representative diet sub-samples were sent to a commercial laboratory (SGS Agri-Food Laboratories, Guelph, Ontario, Canada) and analyzed for DM, moisture, CP, calcium, phosphorus, potassium,

magnesium, and sodium contents using chemical and near infra-red spectroscopic analysis (AOAC, 1997).

3.3.4. Skin collagen analysis

Skin samples were collected on days 21 (n = 24) and 35 (n = 24) from each freshly slaughtered pig from the ham, superficial to the coxal joint (3 × 3 cm^2). Samples were rinsed thoroughly with saline, blotted dry and placed immediately in a container of 10 % buffered formalin solution for preservation until analysis of skin collagen abundance (Carleton et al., 1980). One skin sample was collected per pig at the time of slaughter. Skin samples were free of pig hair, and were separated with a scalpel between the dermis layer (i.e. connective tissue, hair follicles), and the hypodermis (i.e. subcutaneous fat, blood vessels; Kolarsick et al., 2011). Sub-samples were then collected from the dermis layer, where collagen resides (Sullivan et al., 2001). Out of the 3 x 3 cm^2 skin sample, two sub-samples (replicates) were sliced into 2 cm x 0.2 cm thick pieces per pig to ensure samples fit into the cassette. Four skin samples (two pigs from the same treatment) were enclosed in each cassette and sent to the Animal Health Histology Lab (University of Guelph, Guelph, Ontario, Canada) for further processing. In total, 480 samples (two replicates per pig, n = 8 per treatment) were embedded in paraffin and were stained using Picrosirius red (**PSR**) staining for the visualization of collagen I and III fibers (Junquiera et al., 1979). Picrosirius red is an anionic dye with sulphonic acid groups that react and bind with the basic groups of collagen fibers (Junqueira et al., 1979).

Skin collagen stained with PSR is generally analyzed under polarized light which increases the sensitivity and resolution of the collagen fibers (Junquiera et al., 1979; Debessa et al., 2001) but, circular light was not available for the present study, so Bright Field

microscopy (Leica Microsystems Model: DMR, Concord, Ontario, Canada) was used. Under Bright Field microscopy, collagen fibers appear red on a pale-yellow background. Images were obtained with a 10x objective lens and were subsequently projected on a monitor (Apple iMac, 27-inch desktop, USA) using the Open Lab Improvision software (University of Warwick Science Park, Coventry, England, United Kingdom). For each skin sample, five pictures were taken to ensure an accurate representation of collagen distribution within a sample. Processed collagen fiber images were then analyzed by ImageJ software developed for microscopy (Version 1.50 i; National Institute of Health, United States). To quantify collagen using ImageJ, collagen images were converted to a 32-bit grayscale and an automatic threshold was applied to each individual sample according to the instructions provided by ImageJ. The automatic threshold separated the image into the foreground and background and computed the average of the pixels according to:

$$\text{threshold} = (\text{average background} + \text{average foreground})/2$$

ImageJ calculated skin collagen abundance as a percentage area of each image, which was expressed in pixels (Rich and Whittaker, 2005).

3.3.5. Calculations

Nitrogen retention was calculated as the difference between N mass in each pig, above the average of the initial group (n = 8), divided by the period length. Nitrogen intake was calculated as feed intake of the whole trial (per pen) multiplied by the N content of each diet.

3.3.6. Statistical analysis

All statistical analyses for physical composition, whole body N retention and utilization, skin collagen abundance and growth performance were determined using Proc glimmix of SAS 9.4 (SAS Inst. Inc., Car, NC, USA). Dietary treatment was the fixed effect, pig within pen was the random effect, and initial BW was used as a covariate. The repeated measures option was considered for growth performance, but was not significant ($P > 0.05$) and was taken out of the model. When a significant treatment effect was detected, differences among individual means were assessed using the Tukey-Kramer post-hoc test. Statistical analyses were considered significant when $P < 0.05$ and a trend when $0.05 \leq P < 0.10$.

3.4. Results

All pigs used in this experiment remained healthy throughout the study except for one pig that became lame and had to be euthanized on day 7. All pigs readily consumed the experimental diets (Table 3-1). The analyzed values for dietary N and amino acids are given in Table 3-2 and agree with the calculated values.

3.4.1. Growth Performance and Physical Composition

Final BW for pigs fed the CON diet was greater than for pigs fed GLU ($P = 0.010$), while intermediate values were observed for G+S (Table 3-3). Average daily gain between day 7 and 21 was greater for pigs fed CON versus pigs fed G+S or GLU ($P < 0.001$), while overall ADG between day 0 and 39 was greater for pigs fed CON versus GLU ($P = 0.010$) with intermediate values observed for G+S. Average daily feed intake for pigs fed the CON diet between day 7 and day 21 was greater than for pigs fed G+S or GLU ($P = 0.001$). Gain-to-feed

between day 7 and day 21 tended to be greater for pigs fed CON versus pigs fed G+S (P = 0.079), while intermediate values were observed for GLU.

Carcass weights on day 21 (P2) and 35 (P3) for pigs fed the CON diet were greater than for pigs fed G+S or GLU (P = 0.004, P = 0.001, respectively). Blood weight for pigs on day 21 and 35 fed the CON diet was greater than pigs fed G+S (P = 0.042, P = 0.003) while intermediate values were observed for GLU. Liver weights on day 21 were greater for pigs fed CON than pigs fed GLU (P = 0.037); intermediate values were observed for G+S. On day 35, liver weights were greater for pigs fed the CON diet than pigs fed G+S (P = 0.029); intermediate values were observed for GLU. Kidney weights on day 35 were greater for pigs fed the CON versus pigs fed G+S (P = 0.039); intermediate values were observed for GLU. Pancreas weights on day 21 and 35 were greater for pigs fed the CON diet compared to pigs fed G+S (P = 0.046, P = 0.013, respectively), while intermediate values were observed for GLU. Intestinal weights on day 21 were greater for pigs fed the CON diet compared to pigs fed G+S (P = 0.019); intermediate values were observed for GLU. On day 35, intestinal weight was not influenced by dietary treatment. Visceral weight on day 35 was greater for pigs fed the CON diet than pigs fed G+S (P = 0.015), while intermediate values were observed for GLU.

3.4.2. Whole body N retention

Nitrogen intakes between day 7 and 21, between day 21 and 39 and over the entire experimental period (between day 0 and 39) were greater for pigs fed the CON diet versus pigs fed G+S or GLU (P = 0.001, P = 0.001, P = 0.009, respectively; Table 3-4). Carcass N retention on day 21 and day 35 were greater for pigs fed the CON diet versus pigs fed G+S or GLU (P = 0.006, P = 0.003, respectively). On day 35 pigs fed CON had greater carcass N retention than

pigs fed G+S (P = 0.009), while intermediate values were observed for GLU. Viscera N retention on day 35 tended to be greater when pigs were fed the CON diet compared to G+S (P = 0.081), while intermediate values were observed for GLU. Over the entire experimental period (day 0 to day 39), viscera N retention was greater for pigs fed the CON diet versus pigs fed G+S or GLU (P = 0.021). Whole body N retention between day 7 and 21 and day 0 and 35 were greater for pigs fed the CON diet compared to pigs fed G+S or GLU (P = 0.007, P = 0.003, respectively), and between day 21 and 35, whole body N retention was greater for pigs fed the CON diet compared to pigs fed G+S (P = 0.007), while intermediate values were observed for GLU. Apparent N utilization efficiency for protein retention above maintenance in each phase was not influenced by dietary treatment.

3.4.3. Skin collagen abundance

In total, 432 out of 480 sample snap shots were used for statistical analysis while the rest were discarded due to uneven staining technique. On d21 (P2) and 35 (P3), pigs fed CON and G+S diets had greater skin collagen abundance versus pigs fed GLU (P < 0.01; Figure 3-1).

3.5. Discussion

The objectives of the present study were to explore the effect of a low CP diet supplemented with G+S or GLU on growth performance, protein retention, and skin collagen abundance of nursery pigs. Pigs fed a low CP diet supplemented with GLU did not maintain body weight gain compared to pigs fed a conventional high CP corn and soybean meal diet, while a low CP diet supplemented with G+S supported a final BW not different from CON or

GLU. Being that final BW was not different between CON and G+S-fed pigs, and whole body N retention was less for the pigs fed G+S, these outcomes could be attributed to increased fat content for G+S pigs. Alternatively, being that carcass and viscera weights were less for G+S- versus CON-fed pigs, the final BW may also have been influenced by gut fill, despite no differences in ADFI during P3. Furthermore, pigs fed the GLU diet were unable to maintain skin collagen abundance compared to the CON and G+S, indicating that the addition of G+S in the low CP diet allowed for the partitioning of amino acids toward skin collagen retention. The long-term implications of this observation remain to be elucidated.

High CP diets that are reduced by 4 percentage units in CP (e.g., 20 % versus 16 % CP) have been shown to have negative effects on growth performance including a reduction in ADG and feed efficiency (Kephart and Sherritt, 1990; Hansen et al., 1993). The low CP GLU diet used in nursery phases 2 and 3 (P2; 17.78 and P3; 15.01 % CP, as-fed; analyzed contents), had an average reduction of 5.1 percentage units compared to the CON (P2; 22.72 and P3; 20.32 % CP) and also resulted in lower growth performance, which is in agreement with previous studies. The addition of G+S to a low CP diet, supported final BW and overall ADG intermediate to CON and GLU. Perhaps, dietary supplementation of G+S greater than the levels used in this study may be beneficial for growth performance when CP is reduced by more than 4 %. Powell and colleagues (2011) showed that supplementation of Gly and Ser (1.7 % as-fed, sum of G+S) to a low CP diet (13.4 % CP; as-fed; calculated contents) fed to 20 – 50 kg BW pigs improved growth performance, while another experimental diet with the inclusion of Glu (2.3 %) did not improve performance, proving that the addition of Gly and Ser was not solely supplying N, and ultimately rescued growth performance.

When analyzing the diets in phase 1, the low CP diets G+S and GLU had a higher analyzed value for CP (9 and 12 % difference compared to calculated values, respectively), which could be attributed to an error in sampling or mixing of the diets with the whole ingredients (e.g., whey, blood plasma, blood meal). The higher value for CP is supported by the slight increase of each analyzed amino acid relative to the calculated value in these diets, suggesting that one of the whole ingredients may have been added in excess or had greater CP concentration than predicted. One particular amino acid, Glu, showed the biggest difference between calculated and analyzed values. This is due to the inclusion of glutamine when analyzing the values of Glu since during acid hydrolysis the amide N in glutamine is removed; therefore, converting it to Glu.

Nitrogen intake was greater for pigs fed the CON diet during each phase and over the entire experimental period compared to G+S and GLU. This is attributed to the decreased protein content in these latter diets. Whole body N retention also followed the same pattern where pigs fed the CON diet had greater N retained than G+S and GLU, while there were no differences in total N retention efficiency. The ADFI and ADG between day 7 and 21 (P2) and the carcass and viscera weights on day 21 were greater for pigs fed the CON diet than pigs fed either G+S or GLU, which corresponds to reduced feed intake during this phase and smaller stomach and intestinal weights at the end of this phase for G+S and GLU. By the end of the study, however, there were no differences in physical body composition.

In addition to protein retention, NEAA are important for a myriad of underlying metabolic pathways (Yu et al., 1985). Recent research has demonstrated that NEAA, specifically Gly, play many important roles in animal metabolism including but not limited to; synthesis of DNA, RNA, purines, glutathione, hemoglobin, creatine, and collagen protein

(Wang et al., 2013, Li and Wu, 2018); Gly contributes to about 30 % of the amino acid profile of collagen. Pigs fed a diet supplemented with Gly and Ser maintained skin collagen abundance not different to that of pigs fed CON, while pigs fed a low CP diet without additional Gly and Ser supplementation had significantly lower skin collagen abundance. Mansilla (2017) found that pigs fed a low CP (10.8 % CP as-fed, analyzed contents), NEAA-supplemented diet (according to Mahan and Shields, 1998), Gly was the only NEAA that had a positive value for the endogenous synthesis rate, suggesting that de novo synthesis may not have been sufficient for the numerous roles Gly plays in metabolic processes, relaying its necessity for various metabolic pathways, as stated above.

In the current experiment, the supplementation of Glu in P2 and P3 (3.6 and 3.1 % as-fed; analyzed contents; respectively) did not maintain growth performance, which is in agreement to research conducted by Powell et al. (2011). This implies that the GLU diet may have been limiting in an amino acid, total N, or a combination of both. Since the G+S and the GLU diet were isonitrogenous, it is evident that Gly did not act as a N source in the G+S diets to support N retention. Instead of supplementing pig diets with NEAA, which may not be economically feasible due to the lack of CAA availability, alternative non-protein N sources (e.g. urea and ammonia) have been proposed as an efficient way to meet the needs for N when feeding low CP diets (Rose et al., 1949; Mansilla et al., 2015). Mansilla et al., (2015) concluded that the addition of ammonia to a low CP diet was as efficient at supplying N as a NEAA mix of Ala, Asp, Gly, Glu, Pro, Ser for BW gain, and N retention in growing pigs. However, the addition of ammonium salts resulted in a trend to decrease ADFI, perhaps indicating that ammonium salts are not as palatable as other feed ingredients. Similar observations were made

by Rose et al. (1949). Using ammonium salts can supply the N required when feeding a low CP diet, however, these studies did not examine any metabolic functions beyond protein retention.

In contrast, Shemin and Rittenberg (1946) conducted an experiment in rats to determine if Gly, Glu, Pro, Leu, or ammonia were effective at providing N as a precursor for NEAA in the synthesis of hemoglobin. The results of this study indicate that Gly was the most effective precursor for hemoglobin synthesis compared to ammonia or any other NEAA. When looking at the supply and demand for NEAA such as Gly, the daily production of hemoglobin results in a net utilization of 20 umol/kg/h of Gly (Jackson, 1991; Melendez-Hevia et al., 2009), which is over and above the requirements for protein synthesis. Taking this into consideration, specific NEAA are needed for a variety of functions beyond protein synthesis that should be considered when making nutrient recommendations, especially for nursery animals.

Currently, the NRC (2012) does not consider requirements for NEAA in any stage of production for swine. Research conducted by Wang et al. (2014) has shown that for maximum protein accretion in milk-fed piglets, and for cell growth in intestinal epithelial cells, dietary Gly, along with other NEAA, become nutritionally essential amino acids. With the new development in swine genetics for leaner and faster growing genotypes, and the potential use of low CP diets to alleviate competition between humans and livestock for high quality protein sources, the term "non-essential" should be re-evaluated.

3.6. Conclusions and Implications

The NRC (2012) estimated amino acid requirements based solely on production for any life stage of swine. Currently, there is an option to decrease protein content in swine diets,

which in turn reduces the competition between humans and livestock for high quality protein sources and reduces environmental N excretion. However, the reduction in protein content requires careful assessment of the NEAA and N needs, since the endogenous supply of these compounds are reduced when replacing whole protein sources with CAA. The reduction in dietary NEAA may have consequences for biological processes (e.g. hemoglobin production) and the distribution of protein retention between muscle and support molecules such as collagen, which have unknown long-term consequences for the pig. In this study, the dietary and endogenous supply of NEAA in low CP diets supplemented with Glu were insufficient to meet demands for skin collagen production. Further research on metabolic processes and demands for NEAA when feeding low CP diets are warranted as well as the consequences of not meeting these demands with dietary or endogenous NEAA supply.

Table 3-1. *Ingredient composition and nutrient content of the experimental low CP diets pigs with supplemental G+S or GLU and a conventional high CP corn and soybean meal diet fed to nursery pigs (as-fed basis).*

	Phase 1[1]			Phase 2			Phase 3		
				Treatment[2]					
Item	CON	G+S	GLU	CON	G+S	GLU	CON	G+S	GLU
Ingredient composition, % (as-fed basis)									
Soybean Meal	22.00	19.00	19.00	25.00	15.00	15.00	31.57	11.00	11.00
Dry Corn	11.00	21.00	21.00	32.00	60.00	60.00	60.00	74.00	74.00
Cornstarch	3.40	21.45	20.73	9.26	13.21	12.53	2.24	4.91	4.45
Barley	24.00	-	-	10.00	-	-	-	-	-
Whey, dried	25.00	25.00	25.00	10.20	-	-	-	-	-
Blood Plasma, AP920	4.80	2.00	2.00	5.00	-	-	-	-	-
Blood meal, spray dried	3.00	2.00	2.00	2.00	-	-	-	-	-
Monocalcium phosphate	1.69	1.70	1.70	1.33	1.40	1.40	0.90	0.90	0.90
Sodium bicarbonate	0.40	0.40	0.40	0.10	0.30	0.30	-	0.40	0.40
Limestone	0.72	0.80	0.80	0.98	1.23	1.23	1.18	1.18	1.18
Salt	0.60	0.60	0.60	0.70	0.60	0.60	0.38	0.42	0.42
Swine Premix[3]	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Animal-Vegetable Fat	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lysine-HCl	0.14	0.63	0.63	0.17	1.17	1.17	0.38	1.04	1.04
DL-Methionine	0.15	0.22	0.22	0.13	0.24	0.24	0.14	0.18	0.18
Cysteine-HCl	-	0.18	0.18	-	0.28	0.28	-	0.22	0.22
Threonine	0.01	0.24	0.24	0.03	0.48	0.48	0.11	0.41	0.41
Tryptophan	-	0.03	0.03	-	0.11	0.11	-	0.10	0.10
Isoleucine	-	0.16	0.16	-	0.31	0.31	-	0.26	0.26
Valine	-	0.16	0.16	-	0.43	0.43	-	0.36	0.36
Leucine	-	0.14	0.14	-	0.46	0.46	-	0.28	0.28
Histidine	-	0.07	0.07	-	0.21	0.21	-	0.16	0.16
Arginine	-	-	-	-	-	-	-	-	-
Phenylalanine	-	0.16	0.16	-	0.33	0.33	-	0.26	0.26
Tyrosine	-	0.04	0.04	-	0.14	0.14	-	0.11	0.11
Glutamate[4]	-	-	1.65	-	-	1.68	-	-	1.16
Glycine[5]	-	0.65	-	-	0.52	-	-	0.31	-
Serine[6]	-	0.27	-	-	0.48	-	-	0.39	-
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutrient content[7]									
CP, %	23.64	19.67	19.67	22.84	16.47	16.47	20.54	14.77	14.77
ME, kcal/kg	3338	3458	3463	3397	3440	3445	3391	3451	3440
NE, kcal/kg	2458	2580	2581	2494	2605	2607	2514	2646	2637
Crude Fat, %	3.92	3.65	3.65	4.27	4.67	4.67	4.92	5.09	5.09

SID[8] Lysine, %	1.53	1.53	1.53	1.43	1.43	1.43	1.24	1.24	1.24
SID Arginine, %	1.21	0.90	0.90	1.25	0.68	0.68	1.22	0.60	0.60
SID Histidine, %	0.64	0.53	0.53	0.61	0.50	0.50	0.48	0.43	0.43
SID Isoleucine, %	0.81	0.78	0.78	0.78	0.73	0.73	0.74	0.64	0.64
SID Leucine, %	1.83	1.53	1.53	1.77	1.43	1.43	1.51	1.25	1.25
SID Methionine, %	0.44	0.44	0.44	0.41	0.41	0.41	0.41	0.36	0.36
SID Met + Cys, %	0.84	0.84	0.84	0.79	0.79	0.79	0.69	0.69	0.69
SID Cysteine, %	0.40	0.40	0.40	0.37	0.37	0.37	0.28	0.33	0.33
SID Phenylalanine, %	1.02	0.89	0.89	1.00	0.84	0.84	0.87	0.74	0.74
SID Phe + Tyr, %	1.68	1.41	1.41	1.67	1.32	1.32	1.43	1.16	1.16
SID Threonine, %	0.89	0.89	0.89	0.84	0.84	0.84	0.74	0.74	0.74
SID Tryptophan, %	0.31	0.25	0.25	0.29	0.23	0.23	0.22	0.20	0.20
SID Valine, %	1.13	0.97	0.97	1.04	0.91	0.91	0.80	0.80	0.80
SID Glutamate, %	1.81	1.71	3.34	2.30	1.89	3.55	3.15	1.76	2.90
SID Glycine, %	1.18	1.18	0.53	0.92	0.92	0.41	0.68	0.68	0.38
SID Serine, %	1.00	1.00	0.73	0.97	0.97	0.50	0.85	0.85	0.46
SID Glycine + Serine, %	2.17	2.17	1.27	1.89	1.89	0.91	1.53	1.53	0.84

[1] Phase 1 (P1; 7-11 kg), Phase 2 (P2; 11-20 kg), and Phase 3 (P3; 25- 30 kg) were fed for 7, 14, and 18 days, respectively.

[2] Dietary treatments were as followed: CON diet for P1, P2, and P3 (23.1, 22.7, 20.3 % CP, respectively). G+S diet for P1, P2 and P3 (21.4, 17.2, 14.8 % CP, respectively). GLU diet for P1, P2 and P3 (22.1, 17.8, 15.0 % CP, respectively). P1, P2, and P3 were fed for 7, 14 and 18 days, respectively.

[3] Supplied per kg of complete diet: vitamin A, 10,000 IU as retinyl acetate (2.5 mg) and retinylpalmitate (1.7 mg); vitamin D3, 1,000 IU as cholecalciferol; vitamin E, 56 IU as dl-α- tocopherol acetate (44 mg); vitamin K, 2.5 mg as menadione; choline, 500 mg; pantothenic acid, 15 mg; riboflavin, 5 mg; folic acid, 2 mg; niacin, 25 mg; thiamine, 1.5 mg; vitamin B6, 1.5 mg; biotin, 0.2 mg; vitamin B12, 0.025 mg; Se, 0.3 mg from Na2SeO3; Cu, 15 mg from CuSO4.5H2O; Zn, 104 mg from ZnO; Fe, 100 mg from FeSO4; Mn, 19 mg from MnO2; and I, 0.3 mg from KI (DSM Nutritional Products Canada Inc., Ayr, ON).

[4-6] Amino acids that changed in concentration depending on the experimental diet.

[7] Calculated using ingredient values according to NRC (2012).

[8] Standardized ileal digestible.

Table 3-2. *Analyzed crude protein, Ca, P, K, Mg, Na (%) and total amino acid (%) content of each experimental low CP diet with supplemental G+S or GLU and a conventional high CP corn and soybean meal diet fed to nursery pigs (as-fed basis).*

Analyzed, %[1]									
	Phase 1[2]			Phase 2			Phase 3		
	Treatments[3]								
Item	CON	G+S	GLU	CON	G+S	GLU	CON	G+S	GLU
CP, %	23.05 (23.65)[9]	21.43 (19.67)	22.14 (19.67)	22.72 (22.84)	17.23 (16.47)	17.78 (16.47)	20.32 (20.54)	14.77 (14.77)	15.01 (14.77)
Ca, %[4]	0.84	0.86	0.83	0.65	0.77	0.73	0.70	0.61	0.61
P, %[5]	0.75	0.74	0.77	0.64	0.58	0.61	0.51	0.49	0.50
K, %[6]	0.99	0.96	1.01	0.85	0.56	0.59	0.78	0.54	0.60
Mg, %[7]	0.15	0.12	0.13	0.14	0.12	0.12	0.15	0.12	0.13
Na, %[8]	0.57	0.66	0.66	0.45	0.35	0.35	0.18	0.28	0.25
Lys, %	1.50 (1.53)	1.67 (1.53)	1.63 (1.53)	1.47 (1.43)	1.50 (1.43)	1.44 (1.43)	1.30 (1.24)	1.25 (1.24)	1.27 (1.24)
Arg, %	1.30 (1.21)	1.08 (0.90)	1.13 (0.90)	1.33 (1.25)	0.83 (0.68)	0.85 (0.68)	1.28 (1.22)	0.73 (0.60)	0.78 (0.60)
His, %	0.62 (0.64)	0.58 (0.53)	0.58 (0.53)	0.60 (0.61)	0.49 (0.50)	0.49 (0.50)	0.50 (0.48)	0.43 (0.43)	0.43 (0.43)
Iso, %	0.91 (0.81)	0.93 (0.78)	0.93 (0.78)	0.88 (0.78)	0.76 (0.73)	0.78 (0.73)	0.82 (0.74)	0.67 (0.64)	0.70 (0.64)
Leu, %	1.91 (1.83)	1.75 (1.53)	1.77 (1.53)	1.90 (1.77)	1.50 (1.43)	1.57 (1.43)	1.63 (1.51)	1.32 (1.25)	1.38 (1.25)
Met, %	0.46 (0.44)	0.44 (0.44)	0.44 (0.44)	0.40 (0.41)	0.38 (0.41)	0.36 (0.41)	0.38 (0.41)	0.31 (0.36)	0.32 (0.36)
Phe, %	1.14 (1.02)	1.06 (0.89)	1.07 (0.89)	1.11 (1.00)	0.89 (0.84)	0.94 (0.84)	0.97 (0.87)	0.79 (0.74)	0.83 (0.74)
Thr, %	1.04 (0.89)	1.08 (0.89)	1.08 (0.89)	0.99 (0.84)	0.91 (0.84)	0.89 (0.84)	0.82 (0.74)	0.75 (0.74)	0.77 (0.74)
Trp, %	0.32 (0.31)	0.29 (0.25)	0.30 (0.25)	0.30 (0.29)	0.24 (0.23)	0.25 (0.23)	0.24 (0.22)	0.22 (0.20)	0.21 (0.20)
Val, %	1.17 (1.17)	1.11 (0.97)	1.12 (0.97)	1.14 (1.04)	0.94 (0.91)	0.96 (0.91)	0.91 (0.80)	0.81 (0.80)	0.84 (0.80)
Glu, %	3.70 (1.81)	3.38 (1.71)	4.19 (3.34)	3.61 (2.30)	2.33 (1.89)	3.58 (3.55)	3.39 (3.15)	2.16 (1.76)	3.05 (2.90)
Gly, %	0.85	1.09	0.72	0.83	0.97	0.54	0.79	0.69	0.51

	(1.18)	(1.18)	(0.53)	(0.92)	(0.92)	(0.41)	(0.68)	(0.68)	(0.38)
Ser, %	1.10	1.07	0.97	1.10	0.93	0.68	0.95	0.82	0.61
	(1.00)	(1.00)	(0.73)	(0.97)	(0.97)	(0.50)	(0.85)	(0.85)	(0.46)
Cys, %	0.40	0.41	0.42	0.40	0.37	0.37	0.30	0.32	0.33
	(0.40)	(0.40)	(0.40)	(0.37)	(0.37)	(0.37)	(0.28)	(0.33)	(0.33)

[1] Analyzed nutrient contents specific to each experimental diet.

[2] Phase 1 (P1; 7-11 kg), Phase 2 (P2; 11-20 kg), and Phase 3 (P3; 25-30 kg) were fed for 7, 14, and 18 days, respectively.

[3] Dietary treatments were as followed: CON diet for P1, P2, and P3 (23.1, 22.7, 20.3 % CP, respectively). G+S diet for P1, P2 and P3 (21.4, 17.2, 14.8 % CP, respectively). GLU diet for P1, P2 and P3 (22.1, 17.8, 15.0 % CP, respectively). P1, P2, and P3 were fed for 7, 14 and 18 days, respectively.

[4-8] Analyzed by Agri-Food Laboratories (Guelph, ON, Canada).

[9] Calculated nutrient contents are shown in parentheses.

Table 3-3. *Growth performance and physical body composition of nursery pigs fed a low CP supplemented with G+S or GLU compared to a conventional high CP corn and soybean meal diet.*

	Treatment[1]				P-value
	CON	G+S	GLU	SEM[2]	Main effect of treatment[3]
Growth performance					
BW, kg					
Initial	7.3	7.4	7.2	0.1	-
Final	26.6[a]	24.8[ab]	24.1[b]	0.6	0.010
ADG, kg/d					
Phase 1[4]	0.13	0.13	0.14	0.01	0.504
Phase 2	0.42[a]	0.31[b]	0.32[b]	0.01	<.001
Phase 3	0.58	0.51	0.59	0.07	0.631
Overall	0.50[a]	0.46[ab]	0.43[b]	0.01	0.010
ADFI, kg/d					
Phase 1	0.19	0.18	0.17	0.01	0.619
Phase 2	0.59[a]	0.49[b]	0.44[b]	0.02	0.001
Phase 3	1.13	1.13	1.12	0.04	0.977
Overall	0.77	0.73	0.71	0.02	0.226
G:F					
Phase 1	0.68	0.68	0.78	0.06	0.392
Phase 2	0.73[x]	0.64[y]	0.70[xy]	0.03	0.079
Phase 3	0.65	0.62	0.59	0.02	0.200
Overall	0.65	0.62	0.60	0.02	0.329
Physical Composition[5]					
Carcass, kg					
Phase 2	14.3[a]	12.3[b]	12.5[b]	0.4	0.004
Phase 3	22.4[a]	19.0[b]	20.4[b]	0.6	0.001
Blood, g					
Phase 2	710[a]	569[b]	597[ab]	39	0.042
Phase 3	1159[a]	918[b]	1079[ab]	44	0.003
Liver, g					
Phase 2	409[a]	351[ab]	342[b]	18	0.037
Phase 3	593[a]	505[b]	528[ab]	22	0.029
Kidney, g					
Phase 2	85	77	87	4	0.247
Phase 3	130[a]	110[b]	124[ab]	5	0.039
Pancreas, g					

Phase 2	26[a]	22[b]	22[ab]	1	0.046
Phase 3	39[a]	29[b]	36[ab]	2	0.013
Stomach and Intestines, g					
Phase 2	1272[a]	1058[b]	1161[ab]	49	0.019
Phase 3	1886	1718	1852	84	0.348
Viscera [6], g					
Phase 2	2185[a]	1888[b]	1961[b]	80	0.040
Phase 3	3373[a]	2912[b]	3186[ab]	102	0.015

[1]Dietary treatments were as followed: CON diet for P1, P2, and P3 (23.1, 22.7, 20.3 % CP, respectively). G+S diet for P1, P2 and P3 (21.4, 17.2, 14.8 % CP, respectively). GLU diet for P1, P2 and P3 (22.1, 17.8, 15.0 % CP, respectively). P1, P2, and P3 were fed for 7, 14 and 18 days, respectively.

[2] Maximum value of the standard error of the means.

[3] Based on a one-way ANOVA for treatment differences.

[4] Phases are in correspondence to days on experimental period; Phase 1 (day 0 – 7); Phase 2 (day 7 – 21); Phase 3 (day 21 – 39)

[5] Physical composition includes weights of carcass and organs on day 21 (Phase 2) and day 35 (Phase 3)

[6] Viscera contents included heart, lungs, stomach, small and large intestine, liver, kidneys, spleen, and pancreas.

[a-b] Means followed by different superscripts in the same row are different according to a Tukey's multiple range test ($P < 0.05$)

[x-y] Means followed by different superscripts in the same row tend to be different according to a Tukey's multiple range test ($0.05 < P < 0.10$)

Table 3-4. *Whole body N retention and utilization in nursery pigs fed a low CP diet with supplemental G+S or GLU compared to a conventional high CP corn and soybean meal diet.*

	Treatment[1]				P-value
Item	CON	G+S	GLU	SEM[2]	Main effect of treatment[3]
N intake, g/d					
Phase 2[4]	51.0[a]	37.2[b]	37.6[b]	1.4	0.001
Phase 3	26.5[a]	18.7[b]	18.9[b]	0.8	0.001
Overall	38.3[a]	27.5[b]	29.1[b]	2.6	0.009
Carcass N retention, g/d					
Phase 2	9.2[a]	7.1[b]	7.0[b]	0.5	0.006
Phase 3	11.8[a]	8.6[b]	9.8[ab]	0.7	0.009
Overall	10.5[a]	7.9[b]	8.3[b]	0.6	0.003
Viscera N retention, g/d					
Phase 2	1.3	1.0	1.1	0.2	0.134
Phase 3	1.5[a]	1.2[b]	1.3[ab]	0.1	0.081
Overall	1.4[a]	1.1[b]	1.2[ab]	0.1	0.021
Whole Body N Retention, g/d					
Phase 2	10.5[a]	8.1[b]	8.1[b]	0.6	0.007
Phase 3	13.3[a]	9.9[b]	11.1[ab]	0.7	0.007
Overall	12.0[a]	9.0[b]	9.5[b]	0.6	0.003
Total N Efficiency, %					
Phase 2	21.0	22.0	21.8	1.8	0.851
Phase 3	51.4	53.3	59.5	3.5	0.264
Overall	36.9	38.4	39.2	4.7	0.940

[1]Dietary treatments were as followed: CON diet for P1, P2, and P3 (23.1, 22.7, 20.3 % CP, respectively). G+S diet for P1, P2 and P3 (21.4, 17.2, 14.8 % CP, respectively). GLU diet for P1, P2 and P3 (22.1, 17.8, 15.0 % CP, respectively). P1, P2, and P3 were fed for 7, 14 and 18 days, respectively.

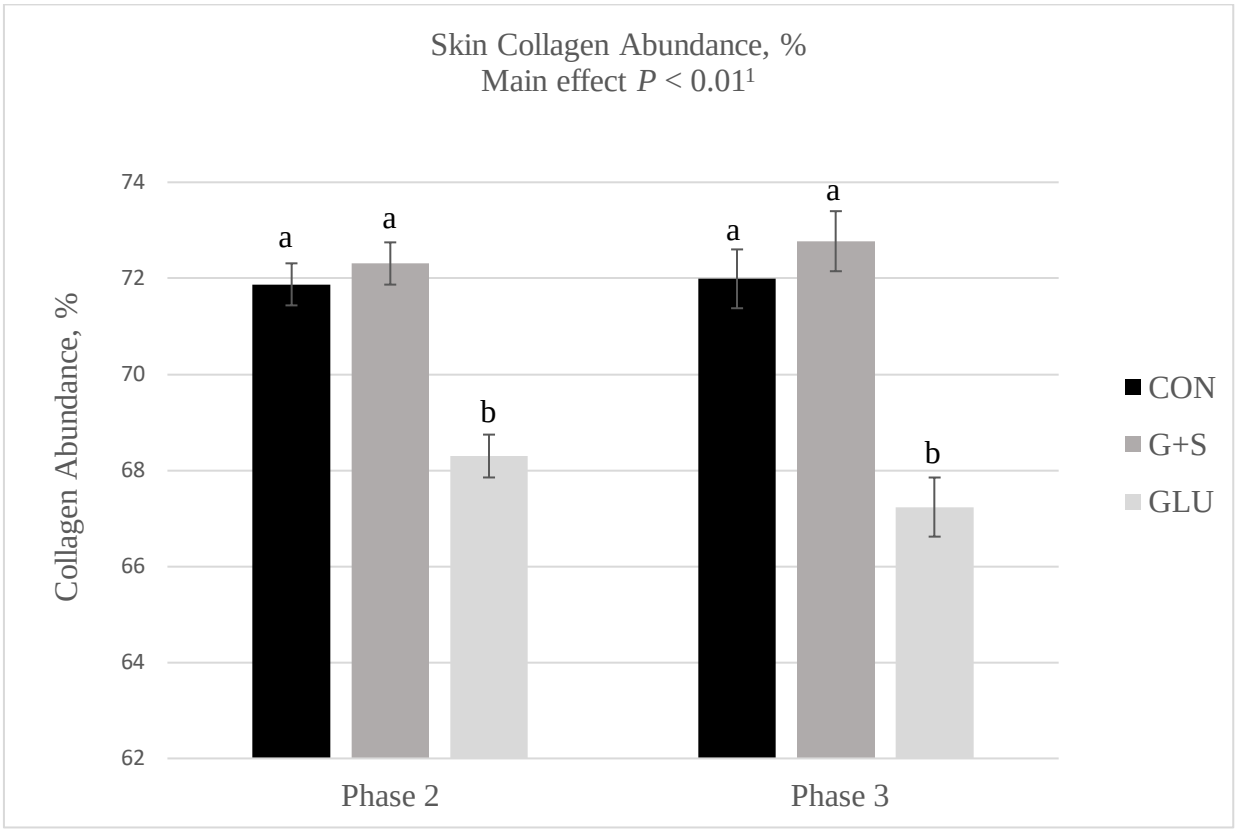
[2] Maximum value of the standard error of the means

[3] Based on a one-way ANOVA for treatment differences

[4] Phases are in correspondence to days on experimental period; Phase 1 (day 0 – 7); Phase 2 (day 7 – 21); Phase 3 (day 21 – 39)

[a-b] Means followed by different superscripts in the same row tend to be different according to a Tukey's multiple range test ($0.05 < P < 0.10$)

Figure 3-1. *Collagen abundance in the skin of nursery pigs on day 21 and 35 after weaning fed a low CP diet supplemental G+S or GLU compared to a conventional high CP corn and soybean meal diet.*



[1] Based on a two-way ANOVA within phase for treatment differences
[2] Phases are in correspondence to days on experimental period; Phase 1 (day 0 – 7); Phase 2 (day 7 – 21); Phase 3 (day 21 – 39)
[a-c] Means followed by different superscripts in the same row are significantly different according to a Tukey's multiple range test (P < 0.05)

4. THE EFFECT OF REDUCED DIETARY GLYCINE AND SERINE AND SUPPLEMENTAL THREONINE ON GROWTH PERFORMANCE AND SKIN COLLAGEN ABUNDANCE OF NURSERY PIGS FED LOW CRUDE PROTEIN DIETS

4.1. Abstract

A total of 42 barrows (initial body weight (**BW**) = 15.1 ± 0.98 kg ± SD) from the Arkell Research Station were used to determine the effect of a low CP, low Gly diet and supplementing dietary Thr above estimated requirements on growth performance, body protein amino acid retention and skin collagen abundance during the late nursery phase. Initial protein composition was determined (n = 7) and the remaining pigs (n = 7) were randomly assigned to 1 of 5 iso-nitrogenous diets: 1) Control, semi-purified diet that met estimated EAA requirements (**CON**; 12.1 % CP as-fed; analyzed contents). The remaining four diets were formulated by reducing total Gly and Ser concentrations to 60 or 20 % of the CON diet. The N that was removed with Gly and Ser was replaced with either Thr or Glu at 2 levels each, to maintain similar CP concentration as CON. Threonine was included at 1.59 % (**T1**; 12.5 % CP) or 2.34 % (**T2**; 12.2 % CP), and Glu was included at 3.47 % (**G1**; 12.7 % CP) or 4.64 % (**G2**; 12.9 % CP). All diets contained synthetic non-essential amino acids (**NEAA**) in the same proportion as found in whole-body protein of pigs to maintain an essential N to total N (**E:TN**) ratio of 0.48. Pigs were fed 2.8 × estimated ME requirements for maintenance (191 kcal/kg BW $^{0.60}$) in 3 equal meals per day over a 21-day experimental period. Pigs were weighed once per week for the determination of average daily gain (**ADG**) and the adjustment of average daily feed intake (**ADFI**). At slaughter, final physical body composition was determined and skin samples were collected for collagen analysis (n = 7). There were no differences in initial or final BW. Overall

ADG for pigs fed diets supplemented with Glu (503.7 g/d) was greater than for those fed diets supplemented with Thr (478.4 ± 9.45 g/d; $P = 0.041$), while feed efficiency for pigs fed diets supplemented with Glu (0.431) tended to be greater than for those fed diets supplemented with Thr (0.411 ± 0.01; $P = 0.074$). There was no difference in ADFI throughout the 21-day experimental period ($P > 0.05$). Pigs fed G1, G2 and T2 diets had lower skin collagen abundance compared to CON ($P < 0.05$), however skin collagen abundance was not different between pigs fed T1 versus CON diets. Reduction of dietary Gly and Ser did not affect growth performance of nursery pigs from 15 to 26 kg, however, skin collagen abundance was negatively impacted by lower dietary concentrations of Gly and Ser but was rescued by 1.59 % dietary Thr supplementation.

Key words: Non-essential amino acids, low-crude protein, threonine, glycine, collagen, nursery pigs

4.2. Introduction

Supplementing glutamate (**Glu**) to low crude protein (**CP**; 15 - 17 %, as-fed; analyzed contents) diet did not support pig final BW or ADG compared to pigs fed a high CP (20 - 22 %) corn- and soybean meal-based diet, while intermediate values were observed for G+S (Chapter 3). Therefore, either Gly and Ser endogenous synthesis was insufficient for tissue growth (i.e. protein retention) or the dietary supply was insufficient to meet the needs for Gly and Ser or total N. The current chapter will focus on the use of low CP diets supplemented with Gly and Ser or threonine (**Thr**), to determine if Thr can indirectly provide Gly via the Thr dehydrogenase pathway, since crystalline Gly is currently not available for inclusion in commercial swine diets. Whether supplying Gly directly or indirectly, sufficient quantities must

be available to maximize protein deposition and to maintain other important metabolic pathways such as DNA, RNA, glutathione, and collagen synthesis, in order to achieve optimum performance (Wu et al., 2013). Glycine is a very important precursor for collagen synthesis, implying that reduced endogenous Gly supply can influence the abundance of collagen present in the skin and the availability of Gly for other metabolic processes.

Therefore, the hypothesis of this chapter is that supplementing Thr above estimated requirements would spare Gly and Ser for protein gain and similarly skin collagen retention. The objectives are to determine the effect of a low CP, low Gly diet and supplementing dietary Thr above estimated requirements on pig performance, body protein amino acid retention and skin collagen abundance during the late nursery phase.

4.3. Methodology

4.3.1. Animals and Diets

The experimental protocol was approved by the University of Guelph Animal Care Committee and followed the Canadian Council of Animal Care guidelines (CCAC, 2009; AUP: e3786). A total of 42 barrows (initial BW = 15.1 ± 0.98 kg ± SD) from Arkell Swine Research Station (Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON: University of Guelph, Guelph, Ontario, Canada) were used and housed individually on tenderfoot floor pens (163 cm x 86 cm) at the University of Guelph (Animal Biosciences, Guelph, Ontario, Canada). Upon arrival at the University of Guelph, seven barrows were immediately euthanized via exsanguination to determine initial protein mass. After adaptation to the CON diet for 4 days,

the remaining 35 barrows were randomly assigned to one of five experimental diets and were restricted fed 2.8 x estimated ME for maintenance (191 kcal/kg $BW^{0.60}$; NRC, 2012).

Experimental diets were created at the University of Guelph using Marion (model 2030, Rapids Machinery Company, Marion, Iowa, USA) and Hobart mixers (model M-802, Hobart Manufacturing Company, Don Mills, Ontario, Canada). Each diet was formulated 10 % above the NRC (2012) estimated requirements for EAA to ensure that EAA were not limiting (Table 1). Glycine and Ser are known to be interconvertible and biosynthetically linked (Wixom et al., 1955), and therefore in this thesis these amino acids will be included together.

The total supplementation of NEAA was sufficient to reach the EAA to total N ratio (**E:TN**) of 0.48, as described by Heger et al. (1998) in all five experimental diets. The control diet was a semi-purified diet that met estimated EAA requirements (CON; 12.1 % CP as-fed; analyzed contents). The remaining four diets were formulated by reducing total Gly and Ser concentrations to 60 and 20 % of the CON diet (40 and 80 % below the CON diet). The N that was removed with Gly and Ser was replaced with either Glu or Thr at 2 levels each, to maintain a similar CP concentration as the CON diet. Threonine was included at 1.59 % (T1; 12.5 % CP; or 2.8 x estimated Thr requirements) or 2.34 % (T2; 12.2 % CP; or 4.1 x estimated Thr requirements), or Glu was included at 3.47 % (G1; 12.7 % CP) or 4.64 % (G2; 12.9 % CP). Representative diet sub-samples were collected immediately after they were mixed, and were packaged and sent to Agri-Food Laboratories for the analysis of DM, moisture, CP, calcium, phosphorus, potassium, magnesium, and sodium contents using chemical and near infra-red spectroscopic analysis (AOAC, 1997).

Pigs were weighed once per week before the consumption of their first meal to adjust individual feeding levels according to pig BW. Pigs were fed three equal meals per day at 0800, 1200, and 1700 hours and water was supplied with every meal in a water to feed ratio of 3:1.

4.3.2. Observations and analysis

Initial and final BW were determined at the beginning and at the end of the experiment, respectively. Average daily gain was calculated on a weekly basis and averaged over the experimental period. Gain to feed was calculated as the ratio between total ADG (week 1-3) and total ADFI (week 1-3). At the end of the experiment, blood samples were collected from 35 pigs via the orbital sinus (10 mL) into heparinized vacutainers (BD, Mississauga, ON, Canada) on the morning before the first meal. At the end of the 3-week experimental period and during 2 consecutive days, pigs were stunned by an electric probe behind the ears rendering them unconscious. Pigs were then killed by exsanguination for the determination of final body protein mass and physical composition and skin samples were collected. Blood was collected and weighed in a bucket then discarded, while carcasses were eviscerated and processed as described in Chapter 3.

Carcass and viscera samples for CON, G2 and T2 were analyzed for amino acid content by Evonik Nutrition & Care GmbH Analytical Services (Frankfurt, Germany) according to Llames and Fontaine (1994). Nitrogen content was determined by combustion analysis according to AOAC (1997; Method 990.03; Model FOSS Kjeltec 8200; FOSS analytical, Hillerod Denmark). Amino acid concentration was analyzed according to Llames and Fontaine (1994) using ultra performance liquid chromatography (UPLC). Skin samples were taken at the ham, and analyzed for collagen abundance as described in Chapter 3. In total 70 samples (two

skin samples per pig; n = 7 per treatment) were embedded in paraffin and were stained using Picrosirius red (PSR) staining for the analysis of collagen I and III fibers (Junquiera et al., 1979).

4.3.3. Calculations

Nitrogen retention was calculated as the difference between N mass in each pig, over the average of the initial group (n = 7), divided by the period length (21 or 22 days). Daily N intake was calculated as feed intake of the whole trial (per pig) multiplied by the N concentration of each diet. The efficiency of SID EAA deposition in the whole body was calculated as described previously by Mansilla et al. (2015).

4.3.4. Statistical Analysis

All statistical analyses were conducted using Proc glimmix of SAS 9.4 (SAS Inst. Inc., Car, NC, USA) with dietary treatment as the fixed effect and pig within dietary treatment as the random effect; initial BW was used as a covariate. When a significant treatment effect was detected, differences among individual means were assessed using the Tukey-Kramer post-hoc test. A linear pre-planned contrast was constructed to test the responses and the main effect of increasing inclusion of N sources (Thr vs. Glu; T1, T2 vs. G1, G2, respectively) for growth performance, physical composition, whole body N retention and utilization, and collagen abundance, but, no differences were found (data not shown). Statistical analyses were considered significant at $P < 0.05$ and a trend when $0.05 \leq P < 0.10$.

4.4. Results

All pigs used in this experiment remained healthy throughout the study and pigs readily consumed the experimental diets (Table 4-1). The analyzed values for dietary N and total amino acids are given in Table 4-2 and are in close agreement with the calculated values.

4.4.1. Growth Performance

In the present experiment, initial BW did not differ and final BW was not influenced by dietary treatment (Table 4-3). During week 1, ADG for pigs fed diets supplemented with Glu (G1, G2) tended to be less than those fed Thr-supplemented (T1, T2) diets ($P = 0.056$) however, dietary treatment did not influence ADG in weeks 2 or 3. The overall ADG (week 1-3) for pigs fed diets supplemented with Glu was higher than for those fed diets supplemented with Thr ($P < 0.05$). Average daily feed intake was not influenced by dietary treatment, however, G:F tended to be greater for pigs fed diets supplemented with Glu versus those fed diets supplemented with Thr ($P = 0.074$). Carcass weight for pigs fed diets supplemented with Glu tended to be higher than those fed Thr supplemented diets ($P = 0.05$).

4.4.2. Whole body N retention

Nitrogen intake was greater for pigs fed G1, G2, and CON versus those fed T1 ($P < 0.05$; Table 4-4), while intermediate values were observed for pigs fed T2 over the 21-d experimental period. Pigs fed diets supplemented with Glu had greater N intake than those fed Thr supplemented diets ($P < 0.05$). Carcass, viscera, and whole-body N retention was not influenced by dietary treatment, neither was total N efficiency.

4.4.3. Plasma amino acid concentrations

On day 21, pigs fed the T1 and T2 diets had a greater plasma Thr concentration compared to pigs fed CON, G1, and G2 ($P < 0.001$; Table 4-5). Pigs fed the CON diet had greater Gly and Lys plasma concentrations versus all other treatment groups on day 21 ($P < 0.001$ and $P = 0.061$, respectively). Other plasma amino acids were not influenced by dietary treatment.

4.4.4. Amino acid profile of carcass and visceral protein pools

The CP content and amino acid profile of carcass protein were not influenced by dietary treatment, except for a tendency for increased Thr concentration in the carcasses of pigs fed T2 versus those fed G2 and CON ($P = 0.082$; Table 4-6). The Arg and Glu concentrations in viscera protein were greater for pigs fed G2 versus those fed CON and T2 ($P < 0.05$; Table 4-7). Cysteine concentration tended to be greater in viscera protein for pigs fed G2 versus those fed CON and T2 ($P = 0.070$). Viscera Phe and Thr concentrations were greater for pigs fed T2 versus those fed CON and G2 ($P < 0.05$). There was a trend for greater Ile in the viscera of pigs fed G2 and T2 versus those fed CON ($P = 0.06$). The rest of the amino acids present in the amino acid profile of visceral protein were not influenced by dietary treatment.

The amino acid profile of deposited protein in the carcass was not influenced by dietary treatment (Table 4-8). In the amino acid profile of deposited protein in viscera, Arg was greater for pigs fed G2 versus those fed T2 and CON ($P < 0.05$; Table 4-9). Met and Glu was greater for pigs fed G2 compared to those fed CON ($P < 0.05$). There was a trend for greater Cys deposition in the viscera of pigs fed T2 versus those fed CON and G2 ($P = 0.09$). Threonine

deposition in the viscera was greater for pigs fed T2 versus those fed CON and G2 ($P < 0.05$). There was a trend for Trp and Val deposition in visceral protein to be greater for pigs fed T2 versus those fed CON and G2 ($P = 0.081$). Phenylalanine deposition in visceral protein was greater for pigs fed T2 versus those fed CON and G2 ($P = 0.002$). The efficiency of SID Thr deposition in the whole-body (carcass and viscera) above maintenance was less for pigs fed T2 versus those fed CON and G2 ($P < 0.05$; Table 4-10).

4.4.5. Skin collagen abundance

In total, 64 out of 70 samples were complete and used for statistical analysis while the rest were discarded due to uneven staining technique. Pigs fed the G1, G2, and T2 diets had reduced skin collagen abundance compared to CON ($P < 0.05$; Figure 4-1), however, skin collagen abundance was not different between pigs fed T1 versus CON diets.

4.5. Discussion

The objective of the present study was to explore the effect of reduced dietary Gly and Ser in low CP diets and supplemental Thr or Glu on growth performance, protein retention, and skin collagen abundance of nursery pigs. Data from the present study suggests that a reduction of dietary Gly and Ser does not affect growth performance (i.e. net protein gain or body weight gain) when pigs are fed low CP diets, which is in contradiction with research conducted by Powell et al. (2011). This suggests that either the dietary supply or endogenous synthesis of Gly and Ser was sufficient to meet the N requirements for lean tissue growth (i.e. protein retention) in pigs fed the reduced Gly and Ser diets in the current study. Furthermore, increased supplementation of Thr increased plasma Thr concentrations but did not increase plasma Gly

concentrations therefore implying that Thr did not produce Gly via the TDG pathway. Threonine sparing has been studied in pigs in the past by Le Floc'h et al. (1997) where Thr directly produced Gly through the action of TDG in the liver. As such, a cost-effect alternative to supplementing Gly to pig diets would be the inclusion of additional Thr above requirements in order to endogenously synthesize Gly via TDG for collagen synthesis and non-protein functions (e.g. DNA, RNA, and glutathione synthesis), but, according to this study, increasing the inclusion of Thr provided no additional benefit when feeding low CP diets.

The CON diet supplied the greatest concentrations of total Gly and Ser (1.27 and 0.62 % as-fed; analyzed contents, respectively), compared to the T2 and G2 diets which had the lowest concentrations of Gly and Ser (i.e., 80 % below the CON diet; 0.288 and 0.245 %, respectively). Supplementing the amount of Gly and Ser found in the CON was previously found in the whole-body composition of pigs by Mahan and Shields (1998) to be the ideal quantity of amino acids needed and retained by the whole body. Skin collagen abundance was negatively impacted when diets supplied Gly and Ser 40 and 80 % below the CON, respectively, however, skin collagen abundance was rescued when additional Thr was supplied at 2.8 x estimated requirements to replace N content lost from decreased Gly and Ser. Nonetheless, providing dietary Thr 4.1 x above estimated requirements did not rescue skin collagen abundance. It is noteworthy that while there was no difference in whole body N retention, the supplementation of Thr decreased ADG compared to the Glu supplemented diets possibly implying that dietary Thr at 2.8 x estimated requirements but not 4.1 x estimated requirements may have been preferentially partitioned to produce Gly for collagen synthesis through the action of TDG. The possible reason for reduced skin collagen abundance at 4.1 x estimated

requirements may be that the TDG pathway had excess Thr, which saturated the activity of the enzyme and created an inhibitory effect also known as substrate inhibition (Cleland, 1979).

As collagen represents more than 25 % of total protein in the body in man (Harkness et al., 1958; Waterlow, 1984) and Gly is the most abundant amino acid in collagen, the Gly needs for collagen synthesis during development should not be ignored. Synthesis of collagen can be compromised when lower levels of Gly and Ser are supplemented in the diet of humans (Gibson et al., 2002). A study analyzing the amino acid composition of fetal pigs by Wu et al. (1999) showed that throughout gestation, whole-body fetal Gly increased linearly from 6.2 to 11.3 g amino acids / 100 g total amino acids between day 40 to 114 of gestation. This increase in Gly during gestation is consistent with the increase in whole-body collagen as the fetus is developing (Widdowson, 1968; Devlin, 1992). The increase in collagen content as the pig is developing is a direct indication of the need for dietary Gly to sustain optimum performance. The need for dietary Gly for collagen abundance illustrates that insufficient amounts are produced endogenously within the body, indicating that there could be limitations for non-protein metabolic pathways including glutathione synthesis, in which Gly contributes to about 30 % (Ruiz-Ramirez et al., 2014).

Skin is the largest organ of the body and represents 15 % of total body weight in humans (Kolarsick et al., 2011). Skin collagen is correlated to age in humans, however the relationship between pig skin collagen and age is currently unclear due to the fast-growing genotypes presented in swine. A better representation of skin collagen is needed in order to assess differences in whole body collagen as there were slight differences found in abdominal and ventral skin collagen samples compared to dorsal and pelvic skin samples in a recent study conducted by Tzeng et al. (2018). However for this present study, skin was the appropriate

model to use since it has the highest concentration of Type III collagen which contains the highest content of Gly (Devlin, 2011). The long term implications of reduced skin collagen abundance in swine have yet to be determined.

In the amino acid profiles of viscera protein and deposited protein in viscera, Arg, Cys, and Glu increased with supplementation of Glu, however these results are not completely understood. Similarly, Thr in visceral protein and deposited protein in viscera, increased with Thr supplementation. This increase could possibly mean that Thr was liming visceral protein synthesis for pigs fed CON and G2 and/or the additional Thr was used for mucin synthesis in the gastrointestinal tract. In the small intestine, 16 % of Thr is utilized for mucin synthesis and Thr is one of the main amino acids that contributes to the protein cores of mucin, which is important for gut integrity (Bengmark and Jeppsson, 1995; Stoll et al., 1998; Faure et al., 2005). According to Stoll et al. (1998), around 50 % of the amino acids in the diet are utilized in the gastrointestinal tract. Of this, Thr accounts for 60 % of the amino acids used (Stoll et al., 1998). Nevertheless, there was an increase in visceral Glu in Glu supplemented diets, as well as an increase in visceral Thr in Thr supplemented diets, perhaps implying that during the processing of the samples on the slaughter day, organs were not completely rinsed and free of blood and digesta and this could attribute to the increase in the concentration of amino acids present.

According to the SID efficiency in the whole-body protein pools (carcass and viscera) in nursery pigs, Thr efficiency significantly decreased when Thr was supplemented at 4.1 x over the NRC (2012). These results imply that Thr was supplied over requirements and not efficiently used by the pig. It has to be considered, however, that all of the EAA in these diets were supplemented at 10 % above the estimated requirements to ensure no diet was limiting and could therefore slightly reduce the efficiency of all amino acids in the diets. This 10 %

increase in EAA supply corresponded to a general 10 % reduction in apparent AA utilization efficiencies for each EAA relative to the biological maximum outlined by the NRC (2012). Therefore, these results demonstrate that the NRC (2012) estimated EAA requirements for nursery pigs are accurate.

Low CP diets supplemented with crystalline EAA have low dietary content of NEAA and N, which can potentially decrease BW gain in pigs (Powell et al., 2011; Wang et al., 2013; Hou et al., 2015). However, studies have shown that the supplementation of Glu compared to Gly did not restore performance even though N was not limiting, suggesting that certain NEAA are specifically required for growth or other metabolic reactions (Powell et al., 2011; Wang et al., 2013). Wu (2013) suggested a new definition for amino acids that participate and regulate important metabolic pathways. This term - *functional amino acids* – recognizes that amino acids have metabolic fates beyond the synthesis of proteins, and deems all amino acids equally important in nutrition (Wu, 2013). Research conducted by Mansilla (2017) suggested that Gly was the only NEAA that was required in higher proportions compared to the amino acid profile in the whole body in order to satisfy a myriad of metabolic pathways, as noted by its positive value for minimum synthesis rate of de novo NEAA. In order to ensure that metabolic pathways have sufficient precursors for optimum performance, minimum dietary requirements for NEAA should be considered.

4.6. Conclusions and Implications

In this present experiment, the reduction of dietary Gly and Ser in low CP diets did not affect whole-body N retention of nursery pigs during a 3-week period, but, collagen abundance in the skin, was negatively influenced. Moreover, the supplementation of dietary Thr at 2.8 x

estimated requirements of NRC (2012) mitigated the reduction of collagen abundance in skin, suggesting that Thr could act as a Gly precursor, but greater Thr supplementation may be detrimental to skin collagen abundance. Skin collagen abundance can be one factor where the limitation of Gly and Ser in the diet can negatively influence performance, though the long term implications are unclear. Supplementing Thr can be an economic alternative instead of directly supplying Gly and Ser, but specific requirements still need to be determined. Therefore, in order to precisely determine the need for NEAA in low CP diets, the need to support underlying metabolic pathways should be examined in greater detail (e.g., glutathione, DNA, RNA synthesis).

Table 4-1. *Ingredient composition and nutrient content of the experimental low CP diets fed to nursery pigs with decreasing levels of Gly and Ser and supplemental Thr or Glu (as-fed basis)*

	Treatments[1]				
Item	CON	G1	G2	T1	T2
Ingredient composition, % (as-fed basis)					
Cornstarch	63.16	62.06	62.04	62.85	62.54
Casein	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	1.65	1.65	1.65	1.65	1.65
Limestone	1.23	1.23	1.23	1.23	1.23
Salt	0.44	0.44	0.44	0.44	0.44
Magnesium sulfate	0.10	0.10	0.10	0.10	0.10
Potassium sulfate	0.60	0.60	0.60	0.60	0.60
Swine premix[2]	0.60	0.60	0.60	0.60	0.60
Cellulose	3.00	3.00	3.00	3.00	3.00
Pectin	3.00	3.00	3.00	3.00	3.00
Sucrose	8.00	8.00	8.00	8.00	8.00
Soya oil	4.00	4.00	4.00	4.00	4.00
Lysine-HCl	1.15	1.15	1.15	1.15	1.15
DL-Methionine	0.40	0.40	0.40	0.40	0.40
Cysteine-HCl	0.19	0.19	0.19	0.19	0.19
Tryptophan	0.14	0.14	0.14	0.14	0.14
Isoleucine	0.44	0.44	0.44	0.44	0.44
Valine	0.54	0.54	0.54	0.54	0.54
Leucine	0.88	0.88	0.88	0.88	0.88
Histidine	0.31	0.31	0.31	0.31	0.31
Arginine	0.42	0.42	0.42	0.42	0.42
Phenylalanine	0.46	0.46	0.46	0.46	0.46
Tyrosine	0.30	0.30	0.30	0.30	0.30
Proline	0.55	0.55	0.55	0.55	0.55
Aspartate	1.35	1.35	1.35	1.35	1.35
Threonine[3]	0.56	0.56	0.56	1.57	2.58
Glutamate[4]	1.77	3.03	4.29	1.77	1.77
Glycine[5]	1.21	0.73	0.24	0.73	0.24
Serine[6]	0.55	0.33	0.11	0.33	0.11

Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrient content[7]					
CP, %	13.57	13.57	13.57	13.57	13.57
ME, kcal/kg	3450	3436	3422	3449	3449
NE, kcal/kg	2650	2650	2651	2650	2651
SID[8] Lysine, %	1.11	1.11	1.11	1.11	1.11
SID Arginine, %	0.50	0.50	0.50	0.50	0.50
SID Histidine, %	0.38	0.38	0.38	0.38	0.38
SID Isoleucine, %	0.57	0.57	0.57	0.57	0.57
SID Leucine, %	1.11	1.11	1.11	1.11	1.11
SID Methionine, %	0.47	0.47	0.47	0.47	0.47
SID Met + Cys, %	0.61	0.61	0.61	0.61	0.61
SID Cysteine, %	0.14	0.14	0.14	0.14	0.14
SID Phenylalanine, %	0.59	0.59	0.59	0.59	0.59
SID Phe + Tyr, %	1.03	1.03	1.03	1.03	1.03
SID Threonine, %	0.66	0.66	0.66	1.66	2.66
SID Tryptophan, %	0.18	0.18	0.18	0.18	0.18
SID Valine, %	0.70	0.70	0.70	0.70	0.70
SID Glutamate, %	2.27	2.89	3.52	2.27	2.27
SID Glycine, %	1.24	0.76	0.28	0.76	0.28
SID Proline, %	0.97	0.97	0.97	0.97	0.97
SID Serine, %	0.67	0.45	0.23	0.45	0.23
SID Alanine, %	1.15	1.15	1.15	1.15	1.15
SID Aspartate, %	1.50	1.50	1.50	1.50	1.50

[1] Dietary treatments consisted of: 1) CON; 12.1 % CP 2) G1; 12.7 % CP 3) G2; 12.9 % CP 4) T1; 12.5 % CP 5) T2; 12.2 % CP.

[2] Supplied per kg of complete diet: vitamin A, 10,000 IU as retinyl acetate (2.5 mg) and retinylpalmitate (1.7 mg); vitamin D3, 1,000 IU as cholecalciferol; vitamin E, 56 IU as dl-α- tocopherol acetate (44 mg); vitamin K, 2.5 mg as menadione; choline, 500 mg; pantothenic acid, 15 mg; riboflavin, 5 mg; folic acid, 2 mg; niacin, 25 mg; thiamine, 1.5 mg; vitamin B6, 1.5 mg; biotin, 0.2 mg; vitamin B12, 0.025 mg; Se, 0.3 mg from Na2SeO3; Cu, 15 mg from CuSO4.5H2O; Zn, 104 mg from ZnO; Fe, 100 mg from FeSO4; Mn, 19 mg from MnO2; and I, 0.3 mg from KI (DSM Nutritional Products Canada Inc., Ayr, ON)

[3-6] Amino acids that changed in concentration depending on the experimental diet

[7] Calculated using ingredient values according to NRC (2012).

[8] Standardized ileal digestible

Table 4-2. *Analyzed crude protein, Ca, P, K, Mg, Na (%) and total amino acid (%) content of each experimental low CP diet fed to nursery pigs with decreasing levels of Gly and Ser and supplemental Thr or Glu*

Analyzed, %[1]					
	Treatments[2]				
Item	CON	G1	G2	T1	T2
CP, %	12.11	12.73	12.88	12.46	12.24
	(13.57)[7]	(13.57)	(13.57)	(13.57)	(13.57)
Ca, %[3]	0.74	0.77	0.69	0.69	0.67
P, %[4]	0.32	0.26	0.25	0.24	0.25
K, %[5]	0.29	0.26	0.27	0.28	0.28
Mg, %[6]	0.01	0.01	0.01	0.01	0.01
Na, %[7]	0.32	0.22	0.23	0.31	0.33
Lys, %	1.06	1.13	1.15	1.13	1.06
	(1.11)	(1.11)	(1.11)	(1.11)	(1.11)
Arg, %	0.47	0.48	0.49	0.47	0.47
	(0.50)	(0.50)	(0.50)	(0.50)	(0.50)
His, %	0.35	0.36	0.38	0.36	0.37
	(0.38)	(0.38)	(0.38)	(0.38)	(0.38)
Iso, %	0.53	0.55	0.57	0.54	0.54
	(0.57)	(0.57)	(0.57)	(0.57)	(0.57)
Leu, %	1.07	1.09	1.14	1.09	1.07
	(1.11)	(1.11)	(1.11)	(1.11)	(1.11)
Met, %	0.41	0.41	0.41	0.40	0.39
	(0.47)	(0.47)	(0.47)	(0.47)	(0.47)
Phe, %	0.56	0.59	0.61	0.59	0.56
	(0.59)	(0.59)	(0.59)	(0.59)	(0.59)
Thr, %	0.58	0.62	0.75	1.59	2.34
	(0.66)	(0.66)	(0.66)	(1.66)	(2.66)
Trp, %	0.15	0.15	0.16	0.15	0.15
	(0.18)	(0.18)	(0.18)	(0.18)	(0.18)
Val, %	0.65	0.68	0.71	0.66	0.69
	(0.70)	(0.70)	(0.70)	(0.70)	(0.70)
Glu, %	2.30	3.47	4.64	2.32	2.35
	(2.29)	(3.54)	(4.79)	(2.29)	(2.29)
Gly, %	1.27	0.69	0.32	0.74	0.29
	(1.25)	(0.77)	(0.29)	(0.77)	(0.29)
Pro, %	0.78	0.78	0.79	0.77	0.76

	(0.97)	(0.97)	(0.97)	(0.97)	(0.97)
Ser, %	0.62	0.44	0.25	0.44	0.26
	(0.68)	(0.46)	(0.25)	(0.46)	(0.25)
Ala, %	1.13	1.15	1.15	1.14	1.11
	(1.15)	(1.15)	(1.15)	(1.15)	(1.15)
Asp, %	1.51	1.51	1.56	1.48	1.49
	(1.50)	(1.50)	(1.50)	(1.50)	(1.50)
Cys, %	0.11	0.14	0.14	0.12	0.13
	(0.14)	(0.14)	(0.14)	(0.14)	(0.14)

[1] Dietary treatments consisted of: 1) CON; 12.1 % CP 2) G1; 12.7 % CP 3) G2; 12.9 % CP 4) T1; 12.5 % CP 5) T2; 12.2 % CP.

[2] Analyzed nutrient contents specific to each experimental diet

[3-7] Analyzed by Agri-Food Laboratories (Guelph, ON, Canada)

[8] Calculated nutrient contents that differ between experimental diets are shown in parentheses

Table 4-3. *Initial BW, final BW, ADG, ADFI, G:F and physical composition of nursery pigs fed low CP diets with reduced levels of Gly and Ser and either additional Glu or Thr for a 3 week experimental period*

	Treatments[1]						P-value	
	CON	G1	G2	T1	T2	SEM[2]	Main effect of treatment[3]	N-source[4]
Growth performance								
Initial BW, kg	15.1	15.3	15.1	15.1	14.9	0.1	-	-
Final BW, kg	25.5	25.6	25.7	24.9	25.3	0.3	0.800	0.142
ADG, g/d Wk 1	376	348	383	378	395	15	0.200	0.056
ADG, g/d Wk 2	445	490	453	478	449	23	0.983	0.645
ADG, g/d Wk 3	669	627	675	582	636	29	0.949	0.298
Total ADG, g/d	497[b]	500[a]	508[a]	469[b]	488[b]	14	0.800	0.041
ADFI, g/d Wk 1	791.1	791.6	791.6	791.6	791.4	12.2	0.464	0.541
ADFI, g/d Wk 2	871.2	873.1	875.9	866.0	872.1	13.1	0.219	0.302
ADFI, g/d Wk 3	960.3	970.7	966.2	961.7	961.8	13.9	0.721	0.294
Total ADFI, g/d	874.2	878.5	877.9	873.1	875.1	12.8	0.488	0.353
G:F	0.43	0.43	0.43	0.40	0.42	0.1	0.857	0.074
Physical Composition[5]								
Carcass, kg	20.9	21.2	21.1	20.2	20.4	0.1	0.616	0.050
Viscera, g	3073	2938	3148	3030	3235	80	0.757	0.470
Stomach and Intestines, g	1549	1524	1666	1672	1675	61	0.237	0.263
Liver, g	435	437	453	460	441	17	0.694	0.896
Kidneys, g	115	126	125	132	121	6	0.729	0.898

[1] Dietary treatments consisted of: 1) CON; 12.1 % CP 2) G1; 12.7 % CP 3) G2; 12.9 % CP 4) T1; 12.5 % CP 5) T2; 12.2 % CP.
[2] Maximum value of the standard error of the means
[3] Based on a one-way ANOVA for treatment differences
[4] P- value of the main effect of Glu or Thr as the N source (G1, G2 vs. T1, T2)
[5] Physical composition includes weights of carcass and organs on day 35

Table 4-4. *Whole body N retention and utilization in nursery pigs fed low CP diets with reduced Gly+Ser and either additional Glu or Thr*

	Treatments[1]						P-value	
	CON	G1	G2	T1	T2	SEM[2]	Main effect of treatment[3]	N-Source[4]
N intake, g/d	16.4[a]	16.5[a]	16.6[a]	15.2[b]	15.6[ab]	0.2	0.012	0.002
Carcass N Retention, g/d	8.6	10.7	10.3	10.1	9.6	0.7	0.263	0.364
Viscera N Retention, g/d	0.7	0.7	0.8	0.6	0.8	0.1	0.242	0.668
Whole body N Retention, g/d	10.7	11.3	11.1	9.3	10.4	0.6	0.636	0.406
Total N eff, %	57.0	69.1	67.4	71.2	67.2	4.0	0.615	0.789

[1] Dietary treatments consisted of: 1) CON; 12.1 % CP 2) G1; 12.7 % CP 3) G2; 12.9 % CP 4) T1; 12.5 % CP 5) T2; 12.2 % CP.
[2] Maximum value of the standard error of the means
[3] Based on a one-way ANOVA for treatment differences
[4] P- value of the main effect of Glu or Thr as the N source (G1, G2 vs. T1, T2)

Table 4-5. *Amino acid concentrations (μM) in the plasma of nursery pigs fed low CP diets with decreasing levels of Gly+Ser, and either additional Glu or Thr on day 35*

	Treatments[1]						P-value
	CON	G1	G2	T1	T2	SEM[2]	Main effect of treatment[3]
EAA, μM							
Met	46	56	50	62	49	5	0.149
Cys	4	4	4	4	5	1	0.601
Lys	227	198	149	188	163	19	0.061
Thr	457[c]	416[c]	334[c]	2927[b]	4978[a]	247	<.001
Trp	48	45	50	54	58	5	0.273
Arg	182	176	184	211	274	83	0.914
Ile	86	76	78	77	73	6	0.633
Leu	123	123	123	121	116	7	0.954
Val	261	252	246	260	254	11	0.869
His	70	58	67	81	61	7	0.138
Phe	55	53	49	50	52	3	0.604
NEAA, μM							
Gly	2593[a]	2062[a]	1224[b]	2036[a]	1478[b]	158	<.001
Ser	150	117	92	141	117	34	0.775
Pro	197	229	213	217	216	27	0.949
Ala	527	572	549	592	568	39	0.806
Asp	4	9	10	8	6	3	0.386
Asn	27	41	37	44	43	12	0.850
Glu	168	216	210	231	213	18	0.158
Gln	352	396	424	436	379	75	0.934
Tyr	66	57	60	66	53	44	0.186

[1] Dietary treatments consisted of: 1) CON; 12.1 % CP 2) G1; 12.7 % CP 3) G2; 12.9 % CP 4) T1; 12.5 % CP 5) T2; 12.2 % CP.

[2] Maximum value of the standard error of the means

[3] Based on a one-way ANOVA for treatment differences

[a-c] Means followed by different superscripts in the same row are significantly different according to a Tukey's multiple range test ($P < 0.05$)

Table 4-6. *Crude protein content (DM basis) and amino acid profile of carcass protein at final slaughter in nursery pigs fed low CP diets with reduced Gly+Ser and either additional Glu or Thr for a 3-week experimental period*

	Carcass				
	CON	G2	T2		P-value
	n = 7	n = 7	n = 7	SEM[1]	Main effect of treatment[2]
CP, %	50.4	53.8	54.2	1.1	0.119
AA profile, (g/100g CP)					
EAA					
Arg	6.64	6.67	6.72	0.05	0.614
His	2.66	2.56	2.55	0.05	0.388
Ile	3.54	3.41	3.34	0.06	0.192
Leu	6.57	6.39	6.33	0.09	0.260
Lys	6.78	6.51	6.47	0.11	0.210
Met	1.95	1.86	1.85	0.03	0.200
Cys	1.07	1.03	1.04	0.02	0.467
Phe	3.45	3.39	3.35	0.03	0.240
Thr	3.60^{xy}	3.48^{x}	3.71^{y}	0.05	0.082
Trp	0.85	0.80	0.81	0.02	0.376
Val	4.34	4.25	4.20	0.05	0.208
NEAA					
Ala	6.43	6.53	6.58	0.06	0.318
Asp	7.93	7.76	7.77	0.08	0.310
Gly	9.52	10.03	10.47	0.33	0.235
Pro	6.37	6.67	6.93	0.19	0.220
Ser	3.77	3.70	3.72	0.03	0.316
Glu	12.96	12.76	12.74	0.12	0.431

[1] Maximum value of the standard error of the means

[2] Based on a one-way ANOVA for treatment differences

[a-c] Means followed by different superscripts in the same row are significantly different according to a Tukey's multiple range test ($P < 0.05$)

[x-y] Means followed by different superscripts in the same row tend to be different according to a Tukey's multiple range test ($P < 0.10$)

Table 4-7. *Crude protein content (DM basis) and the amino acid profile of viscera in nursery pigs fed low CP diets with reduced Gly+Ser and either additional Glu or Thr for a 3-week experimental period*

	Viscera				
	CON	G2	T2		P-value
	n = 7	n = 7	n = 7	SEM[1]	Main effect of treatment[2]
CP, %	61.6	62.1	60.2	0.78	0.255
AA profile, (g/100g CP)					
EAA					
Arg	6.44[b]	6.56[a]	6.41[b]	0.03	0.023
His	2.28	2.25	2.33	0.03	0.364
Ile	3.65	3.77	3.75	0.03	0.123
Leu	7.57	7.58	7.66	0.05	0.396
Lys	6.99	7.03	6.90	0.05	0.305
Met	1.96	2.02	1.98	0.02	0.119
Cys	1.38	1.40	1.34	0.01	0.070
Phe	4.00[b]	4.04[b]	4.14[a]	0.02	0.029
Thr	4.01[b]	4.01[b]	4.28[a]	0.03	0.003
Trp	1.08	1.10	1.12	0.01	0.119
Val	4.98	4.98	5.06	0.03	0.231
NEAA					
Ala	6.11	6.13	6.07	0.04	0.392
Asp	8.36	8.40	8.39	0.04	0.733
Gly	7.67	7.62	7.33	0.13	0.204
Pro	5.26	5.21	5.23	0.09	0.938
Ser	4.22	4.22	4.21	0.02	0.862
Glu	12.47[b]	12.72[a]	12.54[b]	0.04	0.030

[1] Maximum value of the standard error of the means

[2] Based on a one-way ANOVA for treatment differences

[a-c] Means followed by different superscripts in the same row are significantly different according to a Tukey's multiple range test ($P < 0.05$)

Table 4-8. *The AA profile of deposited protein in carcass in nursery pigs fed low CP diets with reduced Gly+Ser and either additional Glu or Thr for a 3-week experimental period*

	Carcass				
	CON	G2	T2		P-value
	n = 7	n = 7	n = 7	SEM[1]	Main effect of treatment[2]
AA profile, (g/100g CP)					
EAA					
Arg	6.47	6.45	6.50	0.13	0.966
His	2.80	2.53	2.48	0.13	0.298
Ile	3.54	3.20	2.99	0.16	0.157
Leu	6.40	5.91	5.68	0.24	0.210
Met	2.01	1.77	1.74	0.09	0.171
Cys	0.93	0.85	0.86	0.05	0.502
Phe	3.33	3.16	3.03	0.10	0.201
Thr	3.50	3.18	3.67	0.13	0.136
Lys	6.94	6.23	6.05	0.28	0.170
Trp	0.85	0.75	0.73	0.05	0.341
Val	4.23	3.99	3.81	0.12	0.164
NEAA					
Ala	6.26	6.41	6.45	0.13	0.602
Asp	7.76	7.30	7.21	0.21	0.253
Gly	9.27	10.32	11.26	0.69	0.243
Pro	6.17	6.81	7.34	0.41	0.243
Ser	3.59	3.42	3.41	0.08	0.304
Glu	12.80	12.20	11.99	0.33	0.297

[1] Maximum value of the standard error of the means
[2] Based on a one-way ANOVA for treatment differences

Table 4-9. *The AA profile of deposited protein in nursery pigs fed low CP diets with decreasing levels of Gly+Ser, and either additional Glu or Thr on day 35*

	Viscera				
	CON	G2	T2		P-value
	n = 7	n = 7	n = 7	SEM[1]	Main effect of treatment[2]
AA profile, (g/100g CP)					
EAA					
Arg	5.91[b]	6.48[a]	5.97[b]	0.01	0.021
His	2.27	2.15	2.41	0.13	0.434
Ile	2.98	3.53	3.51	0.13	0.056
Leu	6.97	7.11	7.42	0.19	0.338
Lys	6.05	6.31	5.85	0.24	0.454
Met	1.65[b]	1.96[a]	1.82[ab]	0.06	0.042
Cys	1.31[ab]	1.39[a]	1.17[b]	0.05	0.090
Phe	3.59[c]	3.94[b]	4.28[a]	0.10	0.002
Thr	3.60[b]	3.70[b]	4.75[a]	0.12	0.004
Trp	0.87	0.96	1.02	0.03	0.081
Val	4.67	4.90	5.16	0.13	0.081
NEAA					
Ala	6.15	6.12	6.05	0.18	0.921
Asp	7.77	8.07	8.06	0.16	0.421
Gly	8.16	8.14	7.18	0.44	0.227
Pro	5.56	5.43	5.72	0.32	0.823
Ser	3.81	3.84	3.87	0.08	0.878
Glu	11.32[c]	12.51[a]	11.95[b]	0.20	0.026

[1] Maximum value of the standard error of the means

[2] Based on a one-way ANOVA for treatment differences

[a-c] Means followed by different superscripts in the same row are significantly different according to a Tukey's multiple range test ($P < 0.05$)

Table 4-10. *Efficiency of SID EAA deposition in the whole-body protein pools (carcass + viscera) in nursery pigs fed low CP diets with decreasing levels of Gly+Ser, and either additional Glu or Thr on day 35*

Whole body (carcass + viscera)						
	NRC (2012)[1]	CON	G2	T2		P-value
		n = 7	n = 7	n = 7	SEM[2]	Main effect of treatment[3]
EAA						
Arg	127.0	129.0	146.2	143.8	7.9	0.277
His	86.4	73.7	75.4	73.9	5.6	0.973
Ile	65.7	63.1	65.5	61.0	4.2	0.750
Leu	64.9	57.5	60.4	57.9	3.7	0.833
Lys	64.8	60.6	62.1	59.4	3.8	0.891
Met	63.1	40.5	41.0	39.6	2.4	0.927
Met+Cys	52.1	49.4	50.4	48.8	3.0	0.929
Phe	58.0	58.0	62.5	60.0	3.6	0.678
Thr	67.1	56.7[a]	58.6[a]	15.1[b]	2.9	<.001
Val	69.1	63.1	67.6	64.4	3.9	0.714

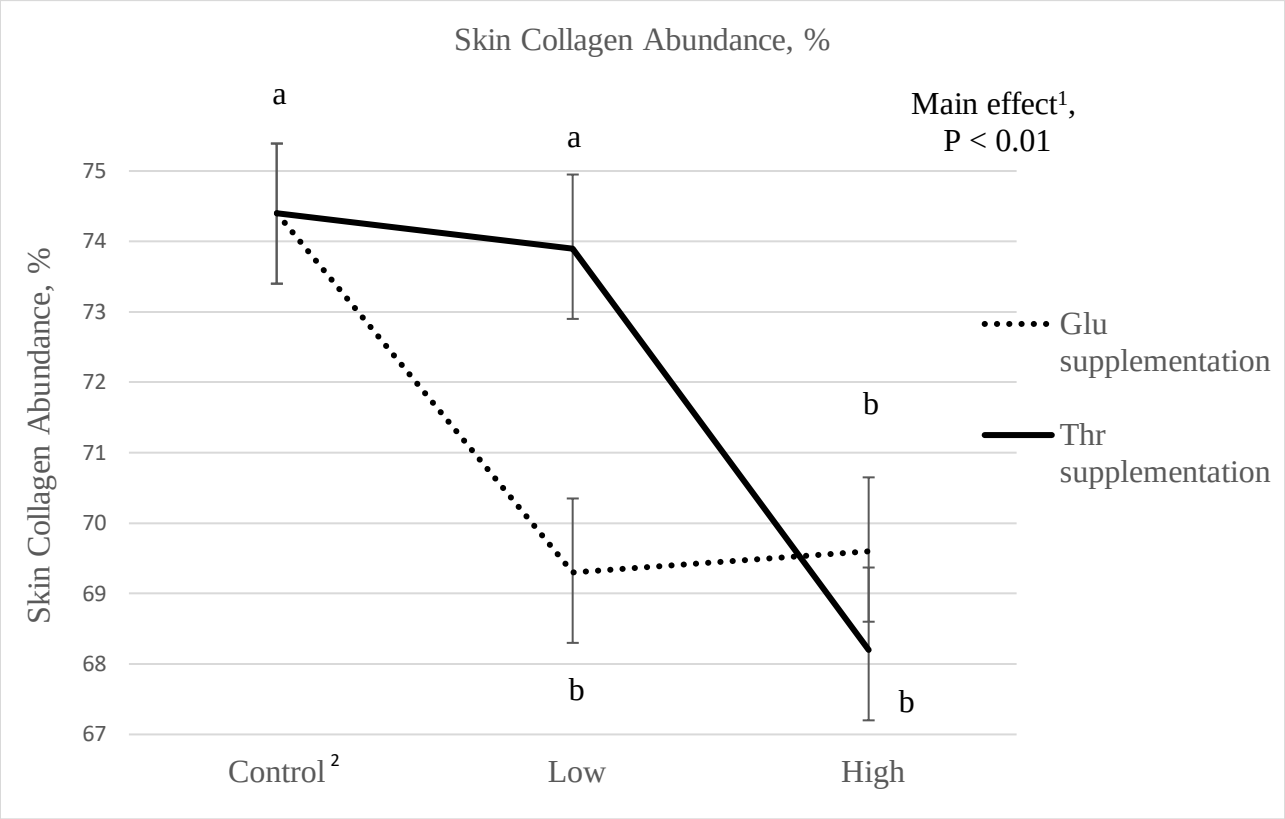
[1] Based on NRC (2012) values of maximum efficiency of amino acid deposition above maintenance for nursery pigs

[2] Maximum value of the standard error of the means

[3] Based on a one-way ANOVA for treatment differences

[a-c] Means followed by different superscripts in the same row are significantly different according to a Tukey's multiple range test (P < 0.0

Figure 4-1. *Collagen abundance in nursery pigs fed low CP diets with decreasing levels of Gly+Ser, and either additional Glu or Thr on day 35*



[a-c] Means followed by different superscripts are significantly different according to a Tukey's multiple range test (P < 0.05)

[1] Based on a one-way ANOVA for treatment differences

[2] Control, Low, High corresponds to the inclusion of Thr or Glu when replacing Gly and Ser to maintain the same CP levels

5. SUMMARY, GENERAL DISCUSSION, AND IMPLICATIONS

With the dramatic increase in global population from 7.6 billion, to almost 10 billion in 2050 (Godfray et al., 2010; Ray et al., 2013) increased competition between humans and livestock for high quality protein sources will become apparent. One way to alleviate this competition is for livestock to consume a diet that is low in CP by supplementing these diets with CAA. A benefit to reducing the dietary CP content is that pork production will have less of an impact on the environment through N losses in the urine and feces; every 1 % reduction in dietary CP with supplementation of CAA results in an 8-10 % reduction in N losses (Obrock, 1997). Supplementing CAA to a low CP diet has been extensively studied and growth performance can be maintained when reducing the dietary CP concentration by up to 4 % (Powell et al., 2011). However, lowering the CP of the diet while maintaining the EAA concentration also reduces the NEAA and total N concentrations present in the diet (Gloaguen et al., 2014). It has been shown that diets with EAA as the only source of N are used less efficiently for net protein gain and that an optimal dietary E:TN ratio must be maintained to improve performance; the optimal E:TN ratio has been suggested at 0.48 based on titrations of dietary EAA and NEAA and N retention as the primary outcome (Heger et al., 1998).

Non-essential amino acids, and in particular, Gly, play a vital role in metabolism including DNA, RNA, glutathione, and collagen synthesis, where Gly makes up 33 % of the amino acid residues. Wang and colleagues (2014) have shown that Gly is also a nutritionally essential amino acid for protein accretion in milk fed piglets. Similarly, Powell and colleagues (2011) have concluded that the addition of dietary Gly restores growth performance in pigs fed low-CP diets compared to pigs fed a conventional corn- and soybean meal-based diet. The studies presented in

this thesis hypothesized that supplementing Gly to nursery pigs fed a low CP diet will support growth performance and skin collagen abundance not different from pigs fed a commercial diet and supplementing Thr above requirements will promote a sparing effect for Gly when pigs are fed low CP diets.

The objectives of this thesis were to determine the impact of supplementing Gly and Ser to a low CP diet on growth performance and skin collagen abundance, as well as supplementing Thr above requirements to a low CP diet to determine if additional Thr could spare Gly and Ser for body protein amino acid retention and skin collagen abundance in nursery pigs.

In Chapter 3, growth performance and skin collagen abundance were analyzed to determine if additional supplementation of the NEAA Gly and Ser are required when feeding low CP diets to nursery pigs. In this chapter, it was demonstrated that performance such as final BW and ADG were negatively affected by reducing dietary CP concentration and supplementing with GLU, compared to pigs fed the CON diet, while pigs fed the G+S diet displayed intermediate values; pigs fed the CON and G+S diets had significantly higher skin collagen abundance compared to pigs fed the GLU diet, indicating that G+S play a vital role in skin collagen abundance. The partitioning of significant Gly toward skin collagen synthesis may reduce available Gly for other metabolic processes. The addition of the GLU diet was to ensure that Gly and Ser were not simply acting as N sources and the observation that the addition of Glu to a low CP diet did not restore skin collagen abundance, suggests that individual NEAA may be necessary to promote optimum metabolism. Therefore, other metabolic processes beyond protein synthesis may become limited with the reduction of dietary Gly and Ser in low CP diets and the supplementation of NEAA should be considered when formulating diets, beyond the effects on growth performance.

Crystalline Gly is currently unavailable for use in swine diets, hence an alternative method to supply Gly might be a desirable option for producers at this time. In Chapter 4, low CP diets were supplemented with either Gly+Ser, Thr, or Glu to determine if additional Thr above requirements set by the NRC (2012) can spare Gly for skin collagen in nursery pigs. In the skin collagen abundance data, when Thr was supplemented 2.8 x above estimated requirements of NRC (2012), collagen content was similar to the CON diet, indicating that Thr could have had a sparing effect on Gly. Though, when analyzing the carcass and viscera data for amino acids, the additional Thr presented in the diets did not increase the amino acid content of Gly or Ser. Therefore, it is possible that there is another mechanism that controls this preferential partitioning of amino acids toward skin collagen, which allowed skin collagen abundance to be restored without seeing improvements in whole-body N retention. The supplementation of Thr at 4.1 x estimated requirements did not restore skin collagen abundance, possibly indicating that the TDG enzyme was saturated and excess supplementation of Thr created an inhibitory effect. Determining the long-term consequences of altered skin collagen abundance and the optimum supplementation of Thr in order to effectively spare Gly when feeding low CP diets would be necessary prior to implementing these feeding strategies on farm.

As skin is the largest organ of the body, representing about 15 % of body weight, it is a great determinant of requirements for amino acids. Although in the swine industry, skin collagen has relatively little economic value. However, there is an urgent need to improve longevity in the breeding stock (i.e., gilts and sows) in order to optimize reproductive performance. The replacement rate of reproductive females is nearly 50 % before parity 4, due metabolic and nutritional stressors as well as high incidence of lameness, which can negatively impact future reproductive success and result in early culling (Tarres et al., 2006; Hoge and Bates, 2011). The

(long term) effect of altered collagen abundance in skin but also as structural support in other tissues in reproductive females has not been examined. Non-essential amino acids also have metabolic roles beyond protein synthesis (e.g., glutathione, creatine, DNA and RNA synthesis), which can consume a significant amount of the endogenous supply and, consequently, will influence the 'requirements' for those amino acids. Therefore, the need for dietary NEAA should be assessed with both long-term health and optimum performance as outcomes of interest.

Data presented in these two experiments support the conclusion that N and NEAA play an important role in metabolism and protein synthesis, and when insufficient dietary quantities are provided, endogenous supply cannot keep up with the demands for protein retention and potentially other important metabolic pathways. Although ingredients such as ammonia can be efficiently used as a N precursor to enhance growth performance in pigs fed low CP diets (Mansilla, 2017), individual NEAA like Gly have been shown to be critical in the synthesis of specific proteins such as collagen, and for other compounds (e.g., DNA, RNA, glutathione, etc.; Shemin and Rittenberg 1946). In conclusion, 'requirements' for NEAA should be established for optimum health and biologically important pathways beyond protein synthesis or net protein gain.

6. REFERENCES

Akinde, D.O., 2014. Amino acid efficiency with dietary glycine supplementation: Part 1. World's Poultry Science Journal, 70(3), pp.461-474.

Alleman, F., Michel, J., Chagneau, A.M., and Leclercq, B., 2000. The effects of dietary protein independent of essential amino acids on growth and body composition in genetically lean and fat chickens. British Poultry Science, 41(2), pp.214-218.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Baker D.H., 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. Biokyowa, 9:1–24.

Ball, R.O., Atkinson, J.L. and Bayley, H.S., 1986. Proline as an essential amino acid for the young pig. British journal of Nutrition, 55(3), pp.659-668.

Ballevre, O., Cadenhead, A., Calder, A.G., Rees, W.D., Lobley, G.E., Fuller, M.F. and Garlick, P.J., 1990. Quantitative partition of threonine oxidation in pigs: effect of dietary threonine. American Journal of Physiology-Endocrinology and Metabolism, 259(4), pp. E483-E491.

Batterham, E.S., 1992. Availability and utilization of amino acids for growing pigs. Nutrition Research Reviews, 5(1), pp.1-18.

Bengmark, S. and Jeppsson, B., 1995. Gastrointestinal surface protection and mucosa reconditioning. Journal of Parenteral and Enteral Nutrition, 19(5), pp.410-415.

Bird, M.I., and Nunn, P.B., 1983. Metabolic homoeostasis of L-threonine in the normally-fed rat. Importance of liver threonine dehydrogenase. Biochemical Journal, 214(3), pp.687-694.

Bird, M.I., Nunn, P.B., and Lord, L.A., 1984. Formation of glycine and aminoacetone from L-threonine by rat liver mitochondria. Journal of Biochemistry, 802(2), pp.229-236.

Boudier, C., Holle, C., and Bieth, J.G., 1981. Stimulation of the elastolytic activity of leukocyte elastase by leukocyte. Journal of Biological Chemistry, 256(20), pp.10256-10258.

Carleton, H. M., Drury, R. B., and Wallington, E. A., 1980. Carleton's histological technique. Oxford University Press, USA.

Cleland, W.W., 1979. Substrate inhibition. Journal of Enzymology 63, pp. 500-513.

Darling, P.B., Dunn, M., Sarwar, G., Brookes, S., Ball, R.O., and Pencharz, P.B., 1999. Threonine kinetics in preterm infants fed their mothers' milk or formula with various ratios of whey to casein. The American Journal of Clinical Nutrition, 69(1), pp.105-114.

Darling, P.B., Grunow, J., Rafii, M., Brookes, S., Ball, R.O. and Pencharz, P.B., 2000. Threonine dehydrogenase is a minor degradative pathway of threonine catabolism in adult humans. American Journal of Physiology, 278(5), pp.E877-E884.

Davis, A.T., and Austic, R.E., 1982. Threonine-degrading enzymes in the chicken. Poultry science, 61(10), pp.2107-2111.

Davis, A.J., and Austic, R.E., 1994. Dietary threonine imbalance alters threonine dehydrogenase activity in isolated hepatic mitochondria of chicks and rats. The Journal of Nutrition, 124(9), pp.1667-1677.

D'Mello, J.P.F., 1973. Aspects of threonine and glycine metabolism in the chick (Gallus domesticus). Annals of Nutrition and Metabolism, 15(6), pp.357-363.

Debessa, C.G., Maifrino, L.B.M., and de Souza, R.R., 2001. Age related changes of the collagen network of the human heart. Mechanisms of Ageing and Development, 122(10), pp.1049-1058.

Devlin, T.M., 2011. Textbook of biochemistry clinical correlations. Oxford University Press, USA.

Edmonds, M.S. and Baker, D.H., 1987. Amino acid excesses for young pigs: effects of excess methionine, tryptophan, threonine or leucine. Journal of Animal Science, 64(6), pp.1664-1671.

Edmonds, M.S., Gonyou, H.W. and Baker, D.H., 1987. Effect of excess levels of methionine, tryptophan, arginine, lysine or threonine on growth and dietary choice in the pig. Journal of Animal Science, 65(1), pp.179-185.

Figueroa, J.L., Lewis, A.J., Miller, P.S., Fischer, R.L. and Diedrichsen, R.M., 2003. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine 1 2. Journal of Animal Science, 81(6), pp.1529-1537.

Frost, D.V. and Sandy, H.R., 1951. Utilization of nonspecific nitrogen sources by the adult protein depleted rat. Journal of Biological Chemistry, 189, pp.249-260.

Fuller, M., 2012. Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. British Journal of Nutrition, 108(S2), pp.S238-S246.

Furuya, S. and Kaji, Y., 1991. Additivity of the apparent and true ileal digestible amino acid supply in barley, maize, wheat or soya-bean meal based diets for growing pigs. Animal Feed Science and Technology, 32(4), pp.321-331.

Gibson, N.R., Jahoor, F., Ware, L. and Jackson, A.A., 2002. Endogenous glycine and tyrosine production is maintained in adults consuming a marginal-protein diet. The American journal of clinical nutrition, 75(3), pp.511-518.

Gloaguen, M., Floc'h, L., Corrent, E., Primot, Y. and Van Milgen, J., 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. Journal of animal science, 92(2), pp.637-644.

Godfray, H.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science, pp.118-383.

Graber, G. and Baker, D.H., 1973. The essential nature of glycine and proline for growing chickens. Poultry science, 52(3), pp.892-896.

Green, M.L. and Elliott, W.H., 1964. The enzymic formation of aminoacetone from threonine and its further metabolism. Biochemical Journal, 92(3), pp.537.

Gregg, K. and Rogers, G.E., 1986. Feather keratin: composition, structure and biogenesis. Biology, 24(1),pp. 666-694.

Hafkenscheid, J.C.M. and Hectors, M.P.C., 1975. An enzymic method for the determination of the glycine/taurine ratio of conjugated bile acids in bile. Clinical Nutrition, 65(1), pp.67-74.

Hansen, J.A., Knabe, D.A. and Burgoon, K.G., 1993. Amino acid supplementation of low-protein sorghum-soybean meal diets for 20-to 50-kilogram swine. Journal of Animal Science, 71(2), pp.442-451.

Hartshorne, D. and Greenberg, D.M., 1964. Studies on liver threonine dehydrogenase. Archives of biochemistry and biophysics, 105(1), pp.173-178.

Heger, J., 2003. Essential to non-essential amino acid ratios. Amino acids in animal nutrition, pp.103-204.

Heger, J., Mengesha, S. and Vodehnal, D., 1998. Effect of essential: total nitrogen ratio on protein utilization in the growing pig. British Journal of Nutrition, 80(6), pp.537-544.

Hobbs, P.J., Pain, B.F., Kay, R.M. and Lee, P.A., 1996. Reduction of odorous compounds in fresh pig slurry by dietary control of crude protein. Journal of the Science of Food and Agriculture, 71(4), pp.508-514.

Hoge, M.D. and Bates, R.O., 2011. Developmental factors that influence sow longevity. Journal of Animal Science, 89(4), pp.1238-1245.

Horng, J.C., Kotch, F.W. and Raines, R.T., 2007. Is glycine a surrogate for ad-amino acid in the collagen triple helix? Protein Science, 16(2), pp.208-215.

Hou, Y., Yin, Y. and Wu, G., 2015. Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. Experimental Biology and Medicine, 240(8), pp.997-1007.

Junqueira, L.C.U., Bignolas, G. and Brentani, R.R., 1979. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. The Histochemical Journal, 11(4), pp.447-455.

Kao, Y.C. and Davis, L., 1994. Purification and structural characterization of porcine L-threonine dehydrogenase. Protein expression and purification, 5(5), pp.423-431.

Karasek, M.A. and Greenberg, D.M., 1957. Studies on the properties of threonine aldolases. Journal of Biological Chemistry, 227(1), pp.191-205.

Kephart, K.B. and Sherritt, G.W., 1990. Performance and nutrient balance in growing swine fed low-protein diets supplemented with amino acids and potassium. Journal of Animal Science, 68(7), pp.1999-2008.

Kerr, B.J. and Easter, R.A., 1995. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. Journal of Animal Science, 73(10), pp.3000-3008.

Kerr, B.J., McKeith, F.K., and Easter, R.A., 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. Journal of Animal Science, 73(2), 433-440.

Kinsey, V.E. and Grant, W.M., 1944. Adequacy of the essential amino acids for growth of the rat. Science, 99(2572), pp.303-305.

Kim, K.I., McMillan,I., and Bayley, H. S., 1983. Determination of amino acid requirements of young pigs using an indicator amino acid. British Journal of Nutrition, 50(02), 369-382.

Kolarsick, P.A.J., Kolarsick, M.A.J., Goodwin, C., 2011. Anatomy and physiology of the skin. J. Dermatol. Nurses Assoc. 3, 203213.

Kong, C. and Adeola, O., 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australasian Journal of Animal Sciences, 27(7), p.917.

Le Bellego, L., Van Milgen, J., Dubois, S. and Noblet, J., 2001. Energy utilization of low-protein diets in growing pigs. Journal of Animal Science, 79(5), pp.1259-1271.

Le Floc'h, N., Obled, C. and Sève, B., 1995. In vivo threonine oxidation rate is dependent on threonine dietary supply in growing pigs fed low to adequate levels. The Journal of Nutrition, 125(10), pp.2550-2562.

Le Floc'h, N., Obled, C. and Seve, B., 1996. In vivo threonine oxidation in growing pigs fed on diets with graded levels of threonine. British Journal of Nutrition, 75(6), pp.825-837.

Le Floc'h, N., Thibault, J.N. and Sève, B., 1997. Tissue localization of threonine oxidation in pigs. British Journal of Nutrition, 77(4), pp.593-603.

Lee, C.W., Y.J., Son, Y.S. and An, W.G., 2011. Effects of dietary protein and threonine supply on in vitro liver threonine dehydrogenase activity and threonine efficiency in rat and chicken. Asian-Australasian Journal of Animal Sciences, 24(10), pp.1417-1424.

Lenis, N.P., van Diepen, H.T., Bikker, P., Jongbloed, A.W. and van der Meulen, J., 1999. Effect of the ratio between essential and nonessential amino acids in the diet on utilization of nitrogen and amino acids by growing pigs. Journal of Animal Science, 77(7), pp.1777-1787.

Li, P. and Wu, G., 2018. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino acids, 50(1), pp.29-38.

Llames, C. R., and J. Fontaine., 1994. Determination of amino-acids in feeds: collaborative study. J. AOAC. Int. 77:1362-14602.

London, I.M., West, R., Shemin, D. and Rittenberg, D., 1950. On the origin of bile pigment in normal man. Journal of Biological Chemistry, 184(1), pp.351-358.

Luckey, T.D., Moore, P.R., Elvehjem, C.A. and Hart, E.B., 1947. Growth of chicks on purified and synthetic diets containing amino acids. Proceedings of the Society for Experimental Biology and Medicine, 64(3), pp.348-351.

Mahan, D.C. and Shields, R.G., 1998. Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. Journal of Animal Science, 76(2), pp.513-521.

Mansilla, W.D., Columbus, D.A., Htoo, J.K. and de Lange, C.F., 2015. Nitrogen absorbed from the large intestine increases whole-body nitrogen retention in pigs fed a diet deficient in dispensable amino acid nitrogen. The Journal of Nutrition, 145(6), pp.1163-1169.

Mansilla, W.D., Silva, K.E., Zhu, C.L., Nyachoti, C.M., Htoo, J.K., Cant, J.P. and de Lange, C.F., 2017. Ammonia nitrogen added to diets deficient in dispensable amino acid nitrogen is poorly utilized for urea production in growing pigs. The Journal of Nutrition, 147(12), pp.2228-2234.

Meléndez-Hevia, E., de Paz-Lugo, P., Cornish-Bowden, A. and Cárdenas, M.L., 2009. A weak link in metabolism: the metabolic capacity for glycine biosynthesis does not satisfy the need for collagen synthesis. Journal of Biosciences, 34(6), pp.853-872.

Midas, T.S., Xu,Y. and Dannenberg, J.J., 2005. Completely geometrically optimized DFT/ONIOM triple-helical collagen-like structures containing the ProProGly, ProProAla, ProProDAla, and ProProDSer triads. Journal of the American Chemical Society, 127(41), pp.14130-14131.

Millet, S., Aluwé, M., Van den Broeke, A., Leen, F., De Boever, J. and De Campeneere, S., 2018. Pork production with maximal nitrogen efficiency. Animal, 12(5), pp.1060-1067.

Mitchell, J.R., Becker, D.E., Jensen, A.H., Harmon, B.G. and Norton, H.W., 1968. Determination of amino acid needs of the young pig by nitrogen balance and plasma-free amino acids. Journal of Animal Science, 27(5), pp.1327-1331.

Mosenthin, R., Sauer, W.C., Blank, R., Huisman, J. and Fan, M.Z., 2000. The concept of digestible amino acids in diet formulation for pigs. Livestock Production Science, 64(2), pp.265-280.

Mudd, S.H., Cerone, R., Schiaffino, M.C., Fantasia, A.R., Minniti, G., Caruso, U., Lorini, R., Watkins, D., Matiaszuk, N., Rosenblatt, D.S. and Schwahn, B., 2001. Glycine N-methyltransferase deficiency: a novel inborn error causing persistent isolated hypermethioninaemia. Journal of inherited metabolic disease, 24(4), pp.448-464.

NRC. 1988. Recommended nutrient allowances for swine (No. 2). The National Academic Press.

NRC. 2012. Nutrient Requirements of Swine. 11th ed. Washington (D.C): The National Academic Press.

Obrock, H., 1997. The effects of reducing dietary crude protein concentration on odor in swine facilities. Nebraska Swine Reports, pp.201.

Ospina-Rojas, I.C., Murakami, A.E., Eyng, C., Nunes, R.V., Duarte, C.R.A. and Vargas, M.D., 2012. Commercially available amino acid supplementation of low-protein diets for broiler chickens with different ratios of digestible glycine+ serine: lysine. Poultry Science, 91(12), pp.3148-3155.

Ospina-Rojas, I.C., Murakami, A.E., Moreira, I., Picoli, K.P., Rodrigueiro, R.J.B. and Furlan, A.C., 2013. Dietary glycine+ serine responses of male broilers given low-protein diets with different concentrations of threonine. British Poultry Science, 54(4), pp.486-493.

Powell, S., Bidner, T. D., Payne, R. L., & Southern, L. L. 2011. Growth performance of 20-to 50-kilogram pigs fed low-crude-protein diets supplemented with histidine, cysteine, glycine, glutamic acid, or arginine. Journal of Animal Science, 89(11), 3643-3650.

Ray, D.K., Mueller, N.D., West, P.C. and Foley, J.A., 2013. Yield trends are insufficient to double global crop production by 2050. PloS one, 8(6), p.e66428.

Reeds, P.J., 2000. Dispensable and indispensable amino acids for humans. The Journal of Nutrition, 130(7), pp.1835S-1840S.

Rich, L. and Whittaker, P., 2005. Collagen and picrosirius red staining: a polarized light assessment of fibrillar hue and spatial distribution. Journal of Science,22(2), pp.97-104.

Ruiz-Ramírez, A., Ortiz-Balderas, E., Cardozo-Saldaña, G., Diaz-Diaz, E. and El-Hafidi, M., 2014. Glycine restores glutathione and protects against oxidative stress in vascular tissue from sucrose-fed rats. Clinical Science, 126(1), pp.19-29.

Ruiz-Torres, A. and Kürten, I., 1976. Is there a recycling of hydroxyproline? Experientia, 32(5), pp.555-556.

Russell, L.E., Cromwell, G.L. and Stahly, T.S., 1983. Tryptophan, Threonine, Isoleucine and Methionine Supplementation of a 12% Protein, Lysine-Supplemented, Corn-Soybean Meal Diet for Growing Pigs 1, 2. Journal of Animal Science, 56 (5), pp.1115-1123.

Russell, L.E., Kerr, B.J. and Easter, R.A., 1987. Limiting amino acids in an 11 % crude protein corn and soybean meal diet for growing pigs. Journal of Animal Science, 65 (5), pp. 1266-1272.

Sauer, W.C. and Ozimek, L., 1986. Digestibility of amino acids in swine: results and their practical applications. A review. Livestock Production Science, 15(4), pp.367-388.

Sayre, F.W., Jensen, D. and Greenberg, D.M., 1956. Substrate induction of threonine dehydrase in vivo and in perfused rat livers. Journal of Biological Chemistry, 219(1), pp.111-117.

Schirch, L.V. and Gross, T., 1968. Serine transhydroxymethylase identification as the threonine and allothreonine aldolases. Journal of Biological Chemistry, 243(21), pp.5651-5655.

Shemin, D. and Rittenberg, D., 1946. The biological utilization of glycine for the synthesis of the protoporphyrin of hemoglobin. Journal of Biological Chemistry, 166 (621), p.945.

Shoulders, M.D. and Raines, R.T., 2009. Collagen structure and stability. Annual review of Biochemistry, 78, pp.929-958.

Stein, H.H., Fuller, M.F., Moughan, P.J., Sève, B., Mosenthin, R., Jansman, A.J.M., Fernández, J.A. and De Lange, C.F.M., 2007a. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livestock Science, 109(1), pp.282-285.

Stein, H.H., Pedersen, C., Wirt, A.R. and Bohlke, R.A., 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. Journal of Animal Science, 83(10), pp.2387-2395.

Stein, H.H., Seve, B., Fuller, M.F., Moughan, P.J. and De Lange, C.F.M., 2007b. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. Journal of Animal Science, 85(1), pp.172-180.

Stoll, B., Henry, J., Reeds, P.J., Yu, H., Jahoor, F. and Burrin, D.G., 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. The Journal of Nutrition, 128(3), pp.606-614.

Stucki, W.P. and Harper, A.E., 1962. Effects of altering the ratio of indispensable to dispensable amino acids in diets for rats. The Journal of nutrition, 78(3), pp.278-286.

Sullivan, T.P., Eaglstein, W.H., Davis, S.C. and Mertz, P., 2001. The pig as a model for human wound healing. Wound Repair and Regeneration, 9(2), pp.66-76.

Sutton, A.L., Kephart, K.B., Verstegen, M.W., Canh, T.T. and Hobbs, P.J., 1999. Potential for reduction of odorous compounds in swine manure through diet modification. Journal of Animal Science, 77(2), pp.430-439.

Tarres, J., Bidanel, J.P., Hofer, A. and Ducrocq, V., 2006. Analysis of longevity and exterior traits on Large White sows in Switzerland. Journal of Animal Science, 84(11), pp.2914-2924.

Thompson, J.S. and Richardson, K.E., 1967. Isolation and characterization of an L-alanine: glyoxylate aminotransferase from human liver. Journal of Biological Chemistry, 242(16), pp.3614-3619.

Tuitoek, K., Young, L.G., De Lange, C.F.M. and Kerr, B.J., 1997. The effect of reducing excess dietary amino acids on growing-finishing pig performance: an elevation of the ideal protein concept. Journal of Animal Science, 75(6), pp.1575-1583.

Tzeng, S.Y., Kuo, T.Y., Hu, S.B., Chen, Y.W., Lin, Y.L., Chu, K.Y. and Tseng, S.H., 2018. Skin collagen can be accurately quantified through non-invasive optical method: Validation on a swine study. Skin Research and Technology, 24(1), pp.59-64.

Wang, W., Dai, Z., Wu, Z., Lin, G., Jia, S., Hu, S., ... & Wu, G. 2014a. Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs. Amino Acids, 46(8), 2037-2045.

Wang, W., Wu, Z., Dai, Z., Yang, Y., Wang, J. and Wu, G., 2013. Glycine metabolism in animals and humans: implications for nutrition and health. Amino acids, 45(3), pp.463-477.

Wang, W., Wu, Z., Lin, G., Hu, S., Wang, B., Dai, Z. and Wu, G., 2014b. Glycine stimulates protein synthesis and inhibits oxidative stress in pig small intestinal epithelial cells. The Journal of Nutrition, 144(10), pp.1540-1548.

Wang, X., Qiao, S., Yin, Y., Yue, L., Wang, Z. and Wu, G., 2007. A deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. The Journal of Nutrition, 137(6), pp.1442-1446.

Waterlow, J.C., 1984. Protein turnover with special reference to man. Experimental Physiology, 69(3), pp.409-438.

Whittaker, P., Kloner, R.A., Boughner, D.R. and Pickering, J.G., 1994. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. Basic research in Cardiology, 89(5), pp.397-410.

Wixom, R.L., Pipkin, G.E. and Day, P.L., 1955. Interrelationship of serine and glycine for chick growth. The Journal of Nutrition, 56(3), pp.409-422.

Wu, G., 2010. Functional amino acids in growth, reproduction, and health. Advances in Nutrition, 1(1), pp.31-37.

Wu, G., 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Journal of Animal Science and Biotechnology, 5(1), p.34.

Wu, G., Bazer, F.W., Dai, Z., Li, D., Wang, J. and Wu, Z., 2014. Amino acid nutrition in animals: protein synthesis and beyond. The Journal of Nutrition, 2(1), pp.387-417.

Wu, G., Bazer, F.W., Burghardt, R.C., Johnson, G.A., Kim, S.W., Knabe, D.A., Li, X.L., Satterfield, M.C., Smith, S.B. and Spencer, T.E., 2010. Functional amino acids in swine nutrition and production. Dynamics in animal nutrition. Wageningen Academic Publishers, The Netherlands, pp.69-98.

Wu, G., Wu, Z., Dai, Z., Yang, Y., Wang, W., Liu, C., and Yin, Y. 2013. Dietary requirements of "nutritionally non-essential amino acids" by animals and humans. Amino Acids, 44(4), 1107-1113.

Wu, G., Meininger, C.J., Knabe, D.A., Baze, F.W. and Rhoads, J.M., 2000. Arginine nutrition in development, health and disease. Current Opinion in Clinical Nutrition & Metabolic Care, 3(1), pp.59-66.

Yi, D., Li, B., Hou, Y., Wang, L., Zhao, D., Chen, H., Wu, T., Zhou, Y., Ding, B. and Wu, G., 2018. Dietary supplementation with an amino acid blend enhances intestinal function in piglets. Amino acids, pp.1-12.