Effects of vitamins, calcium, and phosphorus in nursery pig and sow diets on bone mineralization and growth performance

by

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B.S., Iowa State University, 2020
M.S., Kansas State University, 2021

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
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Abstract

This dissertation is comprised of 5 chapters consisting of a literature review on calcium (Ca) and phosphorus (P) requirements in reproducing sows, two experiments evaluating the effects of vitamin D metabolites in nursery pig diets, two experiments evaluating over-supplementing folic acid in nursery pigs, a meta-regression analysis of narasin in grow-finish pigs, and an experiment evaluating two different farrowing systems during lactation. In chapter 1, a literature review was conducted to summarize Ca and P requirements in gestation and lactating sows derived from empirical and factorial models. The large variation among results of empirical studies and factorial models makes it difficult to define precise Ca and P requirements for gestating and lactating sows. However, with the most recent data a minimum level of 6.0 g/d of STTD P during gestation and 22.1 g/d of STTD P during lactation appear to meet basal requirements. The objective of chapter 2 was to determine the effects of added $25(OH)D_3$ with three levels of STTD P on nursery pig growth performance, bone and urine characteristics, and serum vitamin D. Overall, added $25(OH)D_3$ had limited effect on growth performance; however, an increase in serum concentrations of $25(OH)D_3$ and $24,25(OH)_2D_3$ was observed. The addition of $25(OH)D_3$ to P-deficient diets increased percentage bone ash. Increasing STTD P to 100% of NRC (2012) requirement estimate increased growth and 130% of NRC maximized bone ash. The objective of chapter 3 was to determine the response to supplementation of $1,25(OH)_2D_3$-glycoside (active form of vitamin D) provided from a plant extract on nursery pig growth performance, mortality, bone characteristics, and blood measurements. Overall, supplementation of $1,25(OH)_2D_3$-glycoside had minimal impact on growth or serum parameters; however, increasing $1,25(OH)_2D_3$-glycoside increased percentage bone ash. In chapter 4, two studies were conducted to determine the effect of folic acid on growth performance, SCFA concentrations,

and serum homocysteine concentrations. Overall, the addition of folic acid resulted in reduced growth performance with the greatest negative impact being observed when pigs were fed 20 mg/kg. Folic acid supplementation altered SCFA concentrations and increased serum homocysteine. In chapter 5, a meta-regression analysis was conducted to evaluate the effects of added narasin in growing-finishing pig diets to predict growth rate, feed efficiency, and carcass yield. The models developed suggest important variables for predicting the percentage change in growth performance and carcass yield for pigs fed diets with added narasin include the narasin feeding duration, average weight, and growth performance of pigs fed diets without narasin. This meta-regression analysis provides equations to predict responses on growth performance and carcass yield. Lastly, in chapter 6, the effects of a pre-weaning socialization system during the lactation period on piglet growth and livability, pig lifetime performance, and subsequent sow performance were evaluated. Pigs raised in the conventional farrowing system had increased livability, lifetime growth performance, and carcass characteristics compared to the pre-weaning socialization system.

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Table of Contents

List of Figures xii
List of Tables xiv
Acknowledgements xvi
Preface xvii
Chapter 1 - A review of Calcium and Phosphorus requirement estimates for gestating and lactating sows 1
Abstract 1
Introduction 2
Ca and P homeostasis 3
Calcium homeostasis 4
Phosphorus homeostasis 6
Bone remodeling 7
Phytase and digestibility of Ca and P 10
Calcium and Phosphorus requirements 11
Empirical estimates of Ca and P requirements in gestation 13
Factorial estimation of gestation Ca and P requirements 17
Maintenance requirement 17
Placenta and fetus requirements 19
Maternal growth requirement 21
Model requirement estimates 22
Empirical estimation of lactation Ca and P requirements 24
Factorial estimation of Ca and P requirements in lactation 26
Maintenance requirements 27
Milk production requirements 28
Modeled requirement estimates 30
Conclusion 31
Literature Cited 32

Chapter 2 - Effects of added 25(OH)D_3 with varying standardized total tract digestible phosphorus concentrations on nursery pig performance, bone characteristics, and serum vitamin D status 3
Abstract 3
Introduction 4
Materials and Methods 6
General 6
Animals and housing 6
Blood, urine, and bone characteristics 7
Diet chemical analysis 8
Statistical analysis 9
Results 9
Growth performance 10
Blood and urine analysis 11
Bone characteristics 11
Discussion 14
Acknowledgements 21
Literature Cited 22
Chapter 3 - Influence of added 1,25$(OH)_2D_3$-glycoside on nursery pig growth performance, bone measurements, and cytokine concentrations 1
Abstract 1
Introduction 2
Materials and Methods 4
General 4
Animals and housing 4
Blood sampling 5
Bone characteristics 6
Statistical analysis 7
Results 8
Growth performance 8
Bone characteristics 8

Blood analysis 9
Discussion 9
Acknowledgements 14
Literature Cited 14
Chapter 4 - Effects of added folic acid on growth performance, short chain fatty acid concentrations, and serum homocysteine concentrations in nursery pigs 1
Abstract 1
Introduction 2
Materials and Methods 4
General 4
Experiment 1 4
Fecal characteristics 5
Experiment 2 6
Blood characteristics 6
Statistical analysis 7
Results 8
Experiment 1 8
Experiment 2 9
Discussion 10
Acknowledgements 18
Literature Cited 18
Chapter 5 - A meta-regression analysis to evaluate the effects of narasin on grow-finish pig performance 1
Abstract 1
Introduction 2
Materials and Methods 3
Database 3
Statistical analysis 5
Results 7
Overall data 7
Period data 11

Discussion 13
Implications 16
Acknowledgements 16
Literature Cited 16
Chapter 6 - Effects of a pre-weaning socialization system on piglet livability, lifetime growth performance, and subsequent sow performance 3
Abstract 3
Introduction 4
Materials and Methods 6
General 6
Animals and Housing 6
Lactation 6
Nursery 8
Finishing 10
Statistical analysis 11
Results 11
Sow performance 11
Wean-to-market performance 12
Carcass characteristics 13
Discussion 13
Acknowledgements 20
Literature Cited 20

List of Figures

Figure 1.1. Modeled standardized total tract digestibility (STTD) P (g/d) requirement estimates for fetal growth during gestation..... 45
Figure 1.2. Standardized total tract digestibility (STTD) P (g/d) requirement estimates during gestation from factorial and empirical estimates.. 46
Figure 1.3. Total Ca (g/d) requirement estimates during gestation from factorial and empirical estimates..... 47
Figure 1.4. Modeled standardized total tract digestibility (STTD) P (g/d) requirement estimates for P output in milk during lactation..... 48
Figure 1.5. Standardized total tract digestibility (STTD) P (g/d) requirement estimates during lactation from factorial and empirical estimates.. 49
Figure 1.6. Total Ca (g/d) requirement estimates during lactation from factorial and empirical estimates..... 50
Figure 2.1. Effects of added 25(OH)D_3 and STTD P on percentage de-fatted bone ash [25(OH)D_3 × STTD P, linear, $P = 0.098$]. 28
Figure 2.2. Representative growth plate sections (10th rib) from different animals.. 29
Figure 3.1. Influence of 1,25(OH)2D3-glycoside on nursery pig bone density.. 20
Figure 4.1. Fecal score frequency on d 9 and 24 by main effect of pharmacological levels of Z.. 25
Figure 4.2. Fecal score frequency by main effect of pharmacological levels of Zn for d 9 and 24. 26
Figure 5.1. Observed and predicted improvements in ADG as influenced by control ADG (pigs fed 0 mg/kg narasin) for overall data when 15 mg/kg of narasin is fed for longer than 65 days.. 21
Figure 5.2. Observed and predicted improvements in G:F as influenced by control G:F (pigs fed 0 mg/kg of narasin) for overall data when 15 mg/kg of narasin is fed.. 22
Figure 5.3. Observed and predicted improvements in carcass yield as influenced by control G:F (pigs fed 0 mg/kg of narasin) for overall data when 15 mg/kg of narasin is fed for longer than 65 days.. 23

Figure 5.4. Observed and predicted improvements in ADG as influenced by control ADG (pigs fed 0 mg/kg of narasin) for period data when 15 mg/kg of narasin is fed to pigs lighter than 105 kg..... 24
Figure 5.5. Observed and predicted improvements in G:F as influenced by control G:F (pigs fed 0 mg/kg of narasin) for period data when pigs were fed 15 mg/kg of narasin.. 25
Figure 6.1. Treatments were assigned on a farrowing room basis. A total of 40 farrowing rooms were used with 20 replications per treatment.. 28
Figure 6.2. Sub-populations were created within the pre-weaning socialization system and categorized as general, small, or critical populations.. 29

List of Tables

Table 1.1. Empirical studies evaluating Ca and P concentrations in gestation diets 1
Table 1.2. Equations for Ca and P composition of placenta, fetus, and requirement estimates for Ca and P levels in gestation diets 1
Table 1.3. Results of modeled requirement estimates for Ca and P levels in gestation[1] 1
Table 1.4. Empirical studies evaluating the effects of Ca and P in lactation diets 1
Table 1.5. Equations for Ca and P composition of milk and requirement estimates in lactation diets 2
Table 1.6. Results of modeled requirement estimates for Ca and P levels in lactation 1
Table 2.1. Diet composition of 70% STTD P treatments (as-fed basis) 30
Table 2.2. Diet analysis (as-fed basis) 32
Table 2.3. Effects of added 25(OH)D_3 and STTD P on nursery pig performance 1
Table 2.4. Effects of added 25(OH)D_3 and STTD P on nursery pig urine and blood characteristics 3
Table 2.6. Effects of added 25(OH)D_3 and STTD P on nursery pig bone characteristics 4
Table 2.7. Effects of added 25(OH)D_3 and STTD P on nursery pig bone histopathology 6
Table 3.1. Diet composition (as-fed basis) 21
Table 3.2. Influence of 1,25$(OH)_2D_3$-glycoside on nursery pig growth performance 23
Table 3.3. Influence of 1,25$(OH)_2D_3$-glycoside on nursery pig bone characteristics 24
Table 3.4. Main effects of 1,25$(OH)_2D_3$-glycoside and bone on nursery pig bone characteristics 1
Table 3.5. Influence of 1,25$(OH)_2D_3$-glycoside on nursery pig bone histopathology measurements 1
Table 3.6. Main effects of 1,25$(OH)_2D_3$-glycoside on nursery pig serum performance 1
Table 4.1. Diet composition (as-fed basis), Exp. 1 27
Table 4.2. Diet composition (as-fed basis), Exp. 2 29
Table 4.3. The effects of folic acid and pharmacological levels of Zn on nursery pig growth performance, Exp. 1 1
Table 4.4. The effects of folic acid and pharmacological levels of Zn on nursery pig fecal characteristics, Exp. 1 2
Table 4.5. Effects of folic acid on nursery pig growth performance, Exp. 2 1

Table 4.6. Effects of folic acid on nursery pig serum homocysteine concentrations, Exp. 2 1
Table 5.1. Effect of narasin on growth performance of growing-finishing pigs for overall data . 26
Table 5.2. Effects of narasin on growth performance when narasin inclusion rate changed over time of grow-finish pigs for overall data 1
Table 5.3. Regression coefficients to predict percent change in ADG, G:F and carcass yield for overall and period data 2
Table 6.1. Effects of farrowing strategy on sow and litter performance 30
Table 6.2. Effects of farrowing strategy on wean-to-finish pig growth performance 32
Table 6.3. Effects of farrowing strategy on carcass characteristics 34

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Preface

This dissertation is original work completed by the author, L. L. Becker. Chapters 1 (doi:10.1093/tas/txae087) and 5 (doi:10.1093/tas/tae099) were published in Translational Animal Science. Each of the chapters were formatted according to the required standards of the corresponding journal.

Chapter 1 - A review of Calcium and Phosphorus requirement estimates for gestating and lactating sows[1]

Abstract

Calcium (Ca) and phosphorus (P) are minerals involved in biological functions and essential structural components of the skeleton. The body tightly regulates Ca and P to maintain homeostasis. Maternal needs for Ca and P increase during gestation and lactation to support conceptus growth and milk synthesis. Litter size and litter ADG have a large effect on Ca and P requirements for sows because as they increase, the requirements increase due to a greater need from the sow. The objective of this review was to summarize published literature on Ca and P requirements in gestating and lactating sows derived from empirical data and factorial models. A total of 9 empirical studies and 7 factorial models were reviewed for determining the Ca and P requirements in gestation. For lactation, there were 6 empirical studies and 7 factorial models reviewed. Empirical studies determined requirements based on the observed effect of Ca and P on bone mineralization, sow and litter performance, and milk characteristics. Factorial models generated equations to estimate Ca and P requirements using the main components of maintenance, fetal and placental growth, and maternal retention in gestation. The main components for factorial equations in lactation include maintenance and milk production. In gestation, the standardized total tract digestible phosphorus (STTD P) requirement estimates from empirical studies range from 5.4 to 9.5 g/d with total Ca ranging from 12.9 to 18.6 g/d to maximize bone measurements or performance criteria. According to the factorial models, the

[1] This work has been published in *Translational Animal Science*: Becker, L. L., J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. M. DeRouchey.2024. A review of calcium and phosphorus requirement estimates for gestating and lactating sows. Transl. Anim. Sci. 8:txae087. doi:10.1093/tas/txae087.

requirements increase throughout gestation to meet the needs of the growing fetuses and range from 7.6 to 10.6 g/d and 18.4 to 38.2 g/d of STTD P and total Ca respectively on d 114 of gestation for parity 1 sows. During lactation, STTD P requirement estimates from empirical studies ranged from 8.5 to 22.1 g/d and total Ca ranged from 21.2 to 50.4 g/d. For the lactation factorial models, STTD P requirements ranged from 14.2 to 25.1 g/d for STTD P and 28.4 to 55.6 g/d for total Ca for parity 1 sows with a litter size of 15 pigs. The large variation in requirement estimates makes it difficult to define Ca and P requirements; however, a minimum level of 6.0 and 22.1 g/d of STTD P during gestation and lactation, respectively, appears to be adequate to meet basal requirements. The limited data and high variation indicate a need for future research evaluating Ca and P requirements for gestating and lactating sows.

Introduction

Calcium (Ca) and phosphorus (P) are macrominerals involved in biological functions and essential structural components of the skeleton (Crenshaw, 2001). During gestation and lactation, the maternal need for Ca and P increases to support conceptus growth and milk synthesis. The increased requirements of Ca and P are generally met by modifications in diet formulation, increased feed intake, and adaptations in Ca and P metabolism. Sow parity, litter size, and body weight contribute to maternal requirements because they influence the amount of nutrients transferred to the uterus, milk, and maternal retention (Auldist et al., 2000; Mahan et al., 2009). Optimal maternal nutrition during gestation and lactation is critical to ensure avoid mobilization of sow body reserves and provide adequate growth and development of the offspring.

Research efforts have been made to determine mineral requirements in growing and finishing pigs (Cromwell et al., 1970; Ekpe et al., 2002; Vier et al., 2019). However, limited research is available to summarize Ca and P requirements in gestating and lactating sows.

Common approaches for estimating nutrient requirements include empirical studies and factorial models (Hauschild et al., 2010). In empirical studies, dose-response experiments are often conducted to determine nutrient requirements within a selected population of animals. In the factorial method, nutrient requirements for individual animals are estimated or modeled from the combination of multiple components (maintenance, maternal growth, fetal and placental development, and milk production) that influence nutrient needs. However, a summary of all peer-reviewed literature for Ca and P requirement estimates is unavailable for gestating an dlactating sows. Therefore, the objective of this review is to summarize published literature on Ca and P requirements for gestating and lactating sows derived from both empirical studies and factorial models.

Ca and P homeostasis

The majority of Ca and P in the body is in the skeleton. Calcium and P are deposited in bone as Ca phosphate-based crystals (hydroxyapatite) which provide mechanical strength to the skeletal tissue and serve as a Ca and P reservoir for maintaining mineral homeostasis. The remainder of Ca and P is present in extracellular fluids and soft tissue to be available for essential roles in biological processes. Specifically, the functions of Ca include blood coagulation, muscle contraction, and cell signaling. Phosphorus is important for many organic compounds such as adenosine triphosphate, phospholipids, and nucleic acids (Dodds and Whiles, 2010).

Given their importance for several biological functions that require a delicate balance, Ca and P are tightly regulated in the body to maintain homeostasis. The main hormones involved in regulation are parathyroid hormone, 1,25-dihydroxyvitamin D [$1,25(OH)_2D_3$], and calcitonin. The secretion of these hormones is triggered by Ca and P levels in the blood and the relationship

between these hormones allows for the maintenance of homeostatic concentrations of Ca and P in circulation.

Calcium homeostasis

In swine, 96 to 99% of total body Ca is contained in the skeleton and is deposited in bones as hydroxyapatite (Crenshaw, 2001). Calcium in circulation can be found as free ionized Ca (iCa), bound to protein (predominantly albumin), or to a lesser extent in the form of complexes with anions and organic ions (Tinawi, 2021). Ionized Ca is the biologically active form of circulating Ca and is directly involved as the signal to maintain Ca homeostasis (Negrea, 2019).

Calcium-sensing receptors in tissues including the parathyroid glands and kidneys detect changes in serum iCa concentrations. Parathyroid hormone is synthesized in the parathyroid grand and can be immediately released as the Ca receptor is activated. In response to hypocalcemia (low serum iCa), an upregulation of parathyroid hormone synthesis occurs (Jacquillet and Unwin, 2019). Parathyroid hormone triggers bone resorption by stimulating osteoblasts to release receptor activator of nuclear kappa beta ligand (RANKL). The RANKL binds the receptor activator of nuclear kappa beta (RANK) on the surface of osteoclasts and promotes the differentiation of osteoclasts to start the bone resorption process (Silva and Bilezikian, 2015). As a result, the breakdown of hydroxyapatite and the subsequent release of Ca and phosphate into the bloodstream increases serum Ca levels to reach homeostasis. The circulating parathyroid hormone also stimulates the conversion of vitamin D to its active form through a two-step hydrolysis process. Vitamin D is first converted to 25-hydroxyvitamin D_3 [$25(OH)D_3$] in the liver by the enzyme 25-hydroxylase (Zhu and DeLuca, 2012). Then $25(OH)D_3$ goes through another conversion in the kidney and becomes $1,25(OH)_2D_3$ by the

enzyme 1α-hydroxylase. Increased concentrations of $1,25(OH)_2D_3$ stimulate intestinal absorption of Ca and P, specifically in the small intestine. Additionally, $1,25(OH)_2D_3$ targets the proximal convoluted tubules within the kidneys to stimulate Ca reabsorption and stimulate Ca release from bone tissues. Thus, increasing serum Ca levels.

When Ca-sensing receptors detect high serum iCa (hypercalcemia), an upregulation in the synthesis and secretion of calcitonin in the thyroid gland occurs and is available for immediate release. Calcitonin inhibits bone resorption and has the opposite metabolic effect as parathyroid hormone. The main function of calcitonin is to lower the Ca level in serum by decreasing the mobilization of bone and renal reabsorption of Ca (Davey et al., 2008). This results in a reduction in serum Ca through increased Ca excretion in urine to achieve Ca homeostasis.

During gestation, estrogen also plays a role in Ca homeostasis. Estrogen indirectly stimulates Ca absorption by increasing intestinal sensitivity to $1,25(OH)_2D_3$ (Fleet and Schoch, 2010). The increase in estrogen during gestation contributes to increased intestinal absorption of Ca.

Reproducing animals are more vulnerable to developing disorders of Ca homeostasis due to changes in Ca needs during gestation and lactation (Horst et al., 1997). Maternal need for Ca is increased after parturition to support the requirements for colostrum and milk synthesis (Horst et al., 1997). A failure to meet these physiological needs through Ca intake and metabolic adjustments may result in hypocalcemia. Among other factors, hypocalcemia is indicated as a potential risk factor for uterine prolapses in beef cattle (Richardson et al., 1981). Periparturient hypocalcemia is commonly observed in dairy cows (Reinhardt et al., 2011) which increases the susceptibility to reproductive disorders (Curtis et al., 1983). Ionized Ca is required for smooth muscle contraction and the uterine wall consists of smooth muscle. When sows and cows

experienced induced hypocalcemia under experimental conditions, a reduction in amplitude and frequency of uterine contractions occurred (Ayliffe et al., 1984; Al-Eknah and Noakes, 1989). Additionally, hypocalcemia has been associated with prolonged labor and dystocia (Risco et al., 1984; Heppelmann et al., 2015). However, hypocalcemia is rarely reported in sows (Grez-Capdeville and Crenshaw, 2020) and therefore may not impact the farrowing process.

In dairy cows, hypocalcemia is associated with feeding excess dietary Ca during late gestation. Grez-Capdeville and Crenshaw (2020) recently conducted a study to induce hypocalcemia in sows during late gestation and early lactation. However, even when fed 1.75% total Ca (0.46% standardized total tract digestible P; STTD P) in gestation and lactation they were unable to detect hypocalcemia in peripartum sows. Specific mechanisms have not been described to link uterine prolapses and hypocalcemia in sows warranting further research to understand whether there is an association between uterine prolapses and hypocalcemia in sows.

Phosphorus homeostasis

Like Ca, approximately 60 to 85% of the total P in the body is stored in bones as hydroxyapatite which serves as a P reservoir to maintain homeostasis (Crenshaw, 2001). The remaining P is intracellular, as a component of phospholipids in cell membranes. The small fraction of P that is in serum exists as circulating phospholipids and inorganic phosphate. Thus, P is required to maintain cellular structure and function and regulate metabolic processes through the activation and inhibition of phosphorylated enzymes.

In P regulation, parathyroid hormone and $1,25(OH)_2D_3$ regulate serum P concentrations. Fibroblast growth factor-23 (FGF-23) plays an important role in P homeostasis. Hypophosphatemia (low serum P) stimulates the activation of vitamin D to $1,25(OH)_2D_3$, which increases the intestinal absorption and renal reabsorption of P by stimulating the proximal

convoluted tubules in the kidneys to release P into circulation. Wubuli et al. (2020) observed that sows fed low P diets had increased expression of renal 1α-hydroxylase and higher circulating levels of $1,25(OH)_2D_3$ compared to sows fed adequate or excess dietary P. Low serum P levels are commonly associated with high circulating levels of Ca (Baylink et al., 1971), which results in a decrease in parathyroid hormone secretion.

In response to hyperphosphatemia (high serum P), FGF-23 is secreted from bone mainly by osteocytes and to a lesser extent by osteoblasts. By downregulating the sodium phosphate co-transporters in the proximal tubule of the kidneys, FGF-23 causes a decrease in P reabsorption, resulting in increased urinary P excretion. Fibroblast growth factor-23 also inhibits the activity of 1α-hydroxylase and decreases production of $1,25(OH)_2D_3$. The secretion of parathyroid hormone is also inhibited by FGF-23; however, the main regulator of parathyroid hormone secretion is serum iCa. The subsequent reduction in circulating $1,25(OH)_2D_3$ and parathyroid hormone secretion results in decreased serum P by increasing P excretion.

It is evident that FGF-23, $1,25(OH)_2D_3$, and parathyroid hormone are all involved in mineral metabolism in the intestines, bone, kidney, and parathyroid gland, with the goal of maintaining Ca and P homeostasis (Shimada et al., 2003). In summary, parathyroid hormone functions to increase serum Ca and P by activating vitamin D. Calcitonin and FGF-23 function to decrease serum Ca and P, respectively. The appropriate sensors are continuously functioning to maintain mineral homeostasis through regulation of these hormones.

Bone remodeling

Bone remodeling relies on the function of osteoclasts and osteoblasts. Osteoclasts are responsible for the breakdown of bone tissue and osteoblasts are responsible for synthesis of

bone tissue. The process of bone remodeling occurs to restore skeletal damage by deposition of new bone material and maintain mineral homeostasis.

The 5 steps of bone remodeling are: activation, resorption, reversal, formation, and termination (Kenkre and Bassett, 2018). During the activation step, osteoblasts release RANKL which triggers osteoclast differentiation. Next, the differentiated mononucleated osteoclasts migrate to the bone surface and form multinucleated osteoclasts to begin bone resorption. During the resorption step, the osteoclasts adhere to the bone surface and secrete proteolytic enzymes and hydrogen ions into the extracellular compartment formed between the bone and osteoclasts. This secretion allows degeneration of the organic matrix which is necessary to release Ca and P ions into circulation. After completion of bone resorption, the reversal phase occurs when mononuclear cells appear on the bone surface and prepare new osteoblasts to begin bone formation. The mononuclear cells provide signals for osteoblasts to differentiate and migrate. The reversal step couples the processes of bone resorption and formation. During the formation step, osteoblasts lay down bone until the previously resorbed bone is completely replaced by new bone. Once complete, the bone surface is covered with flattened lining cells and the termination step proceeds with a resting period until a new remodeling cycle is initiated.

The duration of the bone remodeling cycle can vary based on the age of the sow and stage of reproductive cycle. Bone formation is prominent during gestation, while bone resorption is prominent during lactation (van Riet et al., 2016). This indicates that during gestation, the duration of the bone formation step occurs at a faster rate compared to the bone resorption step. During lactation, the opposite takes place.

Prolactin is one of the hormones responsible for lactation and has been reported to be an important regulator of bone remodeling (VanHouten and Wysolmerski, 2003). Prolactin

upregulates RANKL resulting in increased osteoclast differentiation and activation to start the bone remodeling process (Seriwatanachai et al., 2008). Macari et al. (2018) observed a decrease in bone density in the femur and vertebrae in lactating mice because of prolactin's effect on RANKL. Mahan and Fetter (1982) observed decreased bone ash in vertebrae of lactating sows compared to pregnant sows. Maxson and Mahan (1986) also observed decreased bone ash in the femur, humerus, metatarsal, metacarpal, and vertebra in lactating sows compared to pregnant sows. However, this bone loss is reversible after weaning indicating that milk production is the main trigger for bone resorption (Liesegang et al., 2006). The sow can replenish some of her skeletal reserves during the subsequent gestation (Ardeshirpour et al., 2007); however, the degree to which she can replenish her reserves is not fully understood. In mice, bone mineral content is completely restored after weaning (Kovacs and Kronenberg, 1997).

Sow parity influences the bone remodeling process during gestation. Primiparous sows have a higher rate of bone formation relative to resorption compared to multiparous sows (van Riet et al., 2016). This is because primiparous sows are continuing to develop their skeleton whereas multiparous sows have a more mature skeleton. Multiparous sows also have a higher rate of bone formation occurring than bone resorption during gestation, but to a lower extent than primiparous sows. The high rates of bone formation occurrence for multiparous sows in gestation may be due to efforts to replenish skeletal reserves lost in previous lactation periods (van Riet et al., 2016). Due to the ability of sows to replenish their skeletal stores, it is difficult to determine Ca and P requirements in single gestation or lactation period studies, because the degree of replenishment is not fully understood along with the impact of parity on replenishment.

Bones of mature sows are larger, have greater mineral concentration, and can withstand more force compared to bones from young sows (Giesemann et al., 1998). Research has

observed that bone (metacarpals, metatarsals, and ribs) strength increased from parity 1 to parity 2 (Arthur et al., 1983a; 1983b; Maxson and Mahan, 1986). But Giesemann et al. (1998) observed a decrease in bone (ribs and metatarsals) weight from parity 5 to 6 sows. It can be concluded that bones of sows grow larger and stronger from parity 1 to 2 but may decrease in older parities. The increase in bone strength in young sows may be due to the ability to retain more Ca and P in bone and preserve body stores.

Phytase and digestibility of Ca and P

The use of microbial phytase allows for a reduction in total dietary Ca and P because it increases their digestibility. The amount of digestible P and Ca released by phytase may depend on the physiological state of the pig. The use of one value for the release of Ca and P by phytase for all stages of production can be questioned and may result in suboptimal Ca and P supply during gestation and lactation because the efficacy of phytase is less in mid-gestation compared with growing pigs or sows in late-gestation (Kemme et al., 1997; Jongbloed et al., 2004; Nyachoti et al., 2006).

The response to microbial phytase on standardized total tract digestibility (STTD) Ca and apparent total tract digestibility (ATTD) of P in Ca and P-adequate-corn-based diets fed to gestating sows is equivocal. During mid-gestation, phytase increased ATTD of Ca and P (Jongbloed et al., 2004), increased only ATTD of P (Nyachoti et al., 2006; Jongbloed et al., 2013; Jang et al., 2014), or did not affect the ATTD of Ca or P (Kemme et al., 1997; Sulabo, 2003; Liesegang et al., 2005). In late gestation, phytase increased ATTD of P (Kemme et al., 1997; Sulabo, 2003; Jongbloed et al., 2004; Nyachoti et al., 2006; Espinosa et al., 2024) or ATTD of Ca and P (Hanczakowska et al., 2009; Jongbloed et al., 2013). It is unclear why different responses to phytase are observed during various stages of gestation.

The digestibility of Ca is lower in sows compared to growing pigs and changes throughout gestation and lactation. During early gestation, sows need enough Ca to meet their maintenance requirement. On the other hand, growing pigs need enough Ca to meet their maintenance and bone development needs, which may explain the differences in digestibility of Ca between the two (Bikker and Blok, 2017; Lee et al., 2023). Lactating sows have greater digestibility of Ca compared to sows during mid- and late-gestation (Kemme et al., 1997; Jongbloed et al., 2004; Manner and Simon, 2006; Nyachoti et al., 2006). A reduction in Ca digestibility also occurs during mid-gestation compared to late-gestation (Kemme et al., 1997; Jongbloed et al., 2004, 2013; Nyachoti et al., 2006; Lee et al., 2019, 2023). Because of the changing digestibility of Ca in growing pigs and gestating and lactating sows, digestibility values for Ca from growing pigs should not be applied to sows.

Additional research is needed to understand the factors that affect absorption of Ca and P in gestating and lactating sows. For many of the studies included in this review, the requirements for STTD P and total Ca were determined before phytase was commonly used in swine diets. But today, phytase is now used in virtually all stages of production to more efficiently use mineral resources present in feed ingredients. The data described herein reports results of empirical and factorial requirement estimation techniques, and when using this information to formulate swine diets, consideration must be given to phytase and the potential release of Ca and P it provides, while recognizing there is variation in the literature regarding expected changes in mineral digestibility in gestation and lactation diets due to phytase.

Calcium and Phosphorus requirements

A literature search was conducted through the Kansas State University Libraries utilizing CAB Direct and PubMed to summarize Ca and P requirements in gestating and lactating sow

diets. Key search terms for gestation diets included sow AND gestation AND one of the following terms: minerals, phosphorus, or calcium. Key search terms for lactation diets included sow AND lactation AND one of the following terms: minerals, phosphorus, or calcium.

Nutrient requirements can be defined as the minimum amount of a nutrient required to be consumed to induce a specific response (Grez-Capdeville and Crenshaw, 2022). Empirical studies and factorial methods are the most common approaches to estimate nutrient requirements (Hauschild et al., 2010). In the empirical method, dose-response studies are conducted to determine nutrient requirements in a selected population of animals. The most common response criteria for Ca and P requirements include growth performance and bone development. Other criteria including plasma concentrations and milk production have been used (Cromwell et al., 1970; Stockland and Blaylock, 1973). Most recently, urinary P excretion has been proposed as a response criterion for determining P requirements (Grez-Capdeville and Crenshaw, 2022). The requirements determined from the empirical method reflect individuals within a population who are affected by their genetic potential, environment, time of evaluation, and the criterion used to estimate optimal responses. The empirical method does not track changes in the requirement over time and does not show the time point when the maximum response is observed (Hauschild et al., 2010).

In the factorial approach, nutrient requirements for individual animals are estimated or modeled from the combination of multiple components that influence nutrient needs. In gestation and lactation, these components include maintenance, maternal growth, fetus and placenta development, and milk production.

Empirical estimates of Ca and P requirements in gestation

Within the empirical studies evaluated, common response criteria for estimating Ca and P requirements include sow and litter performance, bone characteristics, milk components, and urinary P excretion. To compare results from different experiments used in this review, diets for each experimental treatment within trial were reformulated primarily using the NRC (2012) nutrient loading values and digestibility coefficients for ingredients to standardize dietary nutrient concentrations. Diets were reformulated to generate estimates of STTD P and Ca because older published literature only reported total P and Ca. Feed composition values for ingredients that were not reported in the NRC (2012) or the Stein (2021) feed ingredient database were cited from other published data. These ingredients included defluorinated phosphate (Bikker et al., 2016) and bone flour (Brazilian Tables, 2017). For vitamin and trace mineral premixes, no loading value was used for Ca because the exact composition of premixes was not reported. None of the studies utilized phytase in their experimental diets.

There were 9 empirical studies that evaluated Ca and P feeding levels during gestation published from 1974 to 2022 (Table 1.1). Of these 9 studies, 8 of them compared only 2 or 3 levels of Ca and P making it difficult to determine requirements of gestating sows. Harmon et al. (1974) conducted 2 experiments comparing 2 levels of P (2.5 or 5.4 g/d of STTD P from reformulated diets) by utilizing a diet with no supplemental P and 3 different P sources. During Exp. 1, gestating sows fed dicalcium phosphate had increased percentage bone P compared to those fed no added P or soft- or curacao-phosphate. In Exp. 2, ribs from sows fed the 3 P sources (dicalcium, soft or curacao phosphates) had increased weight, bending load, breaking stress, and bone ash compared to ribs from sows fed no added P. Using the reformulated diets, the feeding level of 5.4 g/d of STTD P and 13.4 g/d of total Ca (2.48:1 total Ca:STTD P) maximized bone

ash and strength. Harmon et al. (1975) conducted a third study utilizing 7.0 g/d of STTD P (from reformulated diets) from dicalcium phosphate compared to no added P (2.4 g/d STTD P)) and observed a numerical increase in bone ash for sows fed the diet containing 7 g/d of STTD P and 13.1 g/d of total Ca (1.87:1 total Ca:STTD P). No treatment differences were observed for sow and litter performance or milk Ca and P concentrations (Harmon et al., 1974; 1975).

Nimmo et al. (1981a) measured bone characteristics when comparing 2 levels of dietary P and Ca (5.6 or 12.3 g/d and 12.3 or 18.6 g/d of STTD P and total Ca, respectively). Bone strength was increased when sows were fed the high Ca and P diet, as measured by increased 4th metatarsal peak force to break the bone. No treatment differences were observed for serum Ca and P concentrations which is likely due to the regulation of these minerals to maintain homeostasis. Mahan and Fetter (1982) compared 3 levels of Ca and P (5.1, 6.6, or 8.1 g/d and 12.0, 14.4, or 16.9 g/d STTD P and total Ca, respectively from the reformulated diets) and observed a numerical linear increase in percentage bone ash in vertebrae. No differences were observed in sow and litter performance or serum and milk Ca and P concentrations.

Grandhi et al. (1986) compared 2 levels of Ca and P and observed a numerical, but not statistical, increase in femur bone ash in sows fed the high Ca and P diets. The lack of a significant response may have been due to sows being fed 100 vs 150% of the NRC (1979) requirement estimate for these minerals. Maximum bone mineralization may have been reached at the low level in this study. However, due to the numerical improvements in bone characteristics, the authors suggested a requirement of 150% of NRC (1979) estimates. When reformulating the diets, the 150% feeding levels provided 16.3 g/d of STTD P and 26.0 g/d of total Ca for parity 1 sows and 14.8 g/d of STTD P and 23.6 g/d of total Ca for older parity sows. These estimates are greater than Harmon et al. (1974; 1975), Nimmo et al. (1981a; 1981b), and

Mahan and Fetter (1982). When reformulating the diets used by Grandhi et al. (1986), the calculated total P content of the reformulated diet is substantially higher than the total P content reported in the paper (1.01% vs 0.83%). Due to this discrepancy, the results of the Grandhi et al. (1986) experiment need to be interpreted with caution.

Studies that did not collect bone measurements estimated Ca and P requirements by using sow and litter performance (Adam and Shearer, 1977; Kornegay and Kite, 1983; Miller et al., 1994; Tan et al., 2016). However, sow and litter performance, serum Ca and P levels, and milk characteristics are not as sensitive to dietary Ca and P levels compared to bone characteristics (Harmon et al., 1974). Adam and Shearer (1977) compared 3 levels of Ca and P but did not observe differences in sow and litter performance. The authors utilized meat and bone meal and bone flour in their dietary treatments. When reformulating the diets using NRC (2012) ingredient loading values, the reformulated diets were higher in Ca and P compared to those reported in the paper (0.61 vs 0.42% total P and 0.84 vs 0.53% total Ca, respectively). Because there were no differences in sow or litter performance, the lowest Ca and P concentrations were considered adequate.

Kornegay and Kite (1983) compared gestating sows fed 4.6 or 6.7 g/d of STTD P (from reformulated diets) while holding Ca constant in the diets by measuring sow and litter performance and serum mineral levels. The high P diet resulted in heavier weaning weights. Miller et al. (1994) fed 9.0, 15.7, or 22.9 g/d of total Ca while holding P constant in the diet and measured sow and litter performance as well as milk Ca concentrations. Increasing Ca levels in the diet had no effect on Ca concentration in milk, milk production, or sow performance. Providing 7.1 g/d of STTD P and 15.7 g/d of total Ca (2.21:1 total Ca:STTD P when using the reformulated diets), was concluded to be adequate because no improvements in milk Ca

concentrations were observed. Tan et al. (2016) evaluated litter performance of sows fed 8.3 and 14.5, 9.2 and 16.9, or 10.5 and 19.5 g/d of STTD P and total Ca, respectively (from reformulated diets). Differences in serum osteocalcin (for bone formation) and pyridinoline (for bone resorption) were not different among treatments. The authors suggested 9.2 g/d of STTD P and 16.9 g/d of total Ca with 1.84:1 total Ca:STTD P (for reformulated diets) was adequate which agrees with NRC (2012) estimates.

More recently, Grez-Capdeville and Crenshaw (2022) determined a total P requirement estimate of 10.3 g/d (6.0 g/d of STTD P) by comparing 6 different concentrations of P and utilizing urinary P excretion as the primary response criteria. The authors suggested a total Ca requirement of 12.9 g/d by setting a total Ca:total P ratio of 1.25:1. The P content in urine reflects P intake and post-absorptive utilization of P as a result of adjustments in tubular P reabsorption to maintain P homeostasis (Vipperman et al., 1974). Grez-Capdeville and Crenshaw (2022) observed low excretion of P in urine until the concentration of dietary P increased, at which P maintenance needs were met. When sows are fed low P diets, a narrow Ca:P ratio is desired to improve the efficiency of dietary P utilization resulting in lower urinary P excretion (Grez-Capdeville and Crenshaw, 2022). Urinary Ca excretion was constant and independent of dietary P levels in gestation. The authors also measured bone biomarkers in plasma samples (carboxyl-terminal propeptide of type I collagen for bone formation and carboxyl-terminal collagen type I crosslinks for bone resorption) but did not observe any statistical differences among treatments. There is limited data available to directly relate urinary P excretion to bone mineralization in gestating sows. Further work must be conducted to understand how P requirements based on urinary P excretion compare to requirements to maximize other biological processes such as bone mineralization or growth.

In summary for the empirical gestation studies, the STTD P level that maximized bone measurements or performance ranged from 5.4 to 9.5 g/d with total Ca ranging from 12.9 to 18.6 g/d with total Ca:STTD P ratios ranging from 1.84:1 to 2.48:1. There is considerable variation in the published literature on feeding levels required to maximize response criteria which makes it difficult to define precise STTD P and total Ca requirements for gestating sows. This is partially due to the treatment design of trials that have been conducted as most studies only tested 1 or 2 Ca or P concentrations. For 6 out of the 9 empirical studies, the authors observed improvements in bone characteristics at the highest dietary concentration of Ca and P. The treatment diet with the highest level of Ca and P may have been above or below the true requirement to maximize bone characteristics, and caution needs to be taken when interpreting these data. However, with the most recent data from Grez-Capdeville and Crenshaw (2022), a minimum level of 6.0 g/d of STTD P is adequate to meet maintenance needs of gestating sows. Without a direct measurement of bone mineralization, from a formulation standpoint, a margin of safety might be applied to support bone mineralization.

Factorial estimation of gestation Ca and P requirements

A total of 7 factorial models published from 1999 to 2021 were reviewed for determining the Ca and P requirements in gestation. During gestation, Ca and P requirements are determined by calculating the sum of the amount needed for maintenance (to replace minimum urinary and fecal losses; Bikker et al., 2017a), fetal and placental growth, and retention in the body for maternal growth (Table 1.2). The units for phosphorus requirement are reported in g/d of STTD P. Both total Ca and STTD Ca are reported in g/d because the STTD Ca system is relatively new and needs continued research efforts to understand the STTD Ca requirement of gestating sows.

Maintenance requirement

The daily maintenance requirements are determined by accounting for endogenous losses as a proportion of BW. Greater losses occur through feces than in urine (6 mg STTD P/kg BW for feces and 1 mg STTD P/kg BW for urine in gestation; Bikker and Blok, 2017). These values are adopted from growing pigs because. However, endogenous losses increase as feed intake increases. The impact of feed intake on endogenous losses is not great in gestation because intake is relatively constant; however, the effect is demonstrated in lactation where maintenance requirements from endogenous losses account for 10 mg STTD P/kg BW. Some researchers assume a constant level of endogenous losses throughout all stages of production in their model at either 7 mg STTD P/kg BW (Jongbloed et al., 1999; 2003) or 10 mg STTD P/kg BW (Jondreville and Dourmad, 2005; Quiniou et al., 2021). Greater endogenous losses occur for Ca compared to P with 8 mg/kg BW of STTD Ca and 2 mg/kg BW of STTD Ca for fecal and urinary endogenous losses, respectively, in gestation.

While several publications use the same basic structure for determining Ca and P requirements with the factorial approach, the structure can differ between models. For example, Quiniou et al. (2021) factored in conceptus weight in the maintenance requirement for STTD P. No other models include it due to the conceptus weight being accounted for in sow BW because as the conceptus grows, the sow BW will increase. Quiniou et al. (2021) also adopted a partial efficiency utilization of absorbed Ca and P of 96% in both their gestation and lactation models.

For the factorial models, the maintenance requirements for STTD P increase from 1.0 g/d in early gestation to 2.4 g/d in late gestation due to an increase in sow BW (for a parity 1 sow with a litter size of 15 piglets; Table 1.3). The maintenance requirement for Ca follows the same increase as P (4.9 to 8.4 g/d total Ca and 1.4 to 2.2 g/d STTD Ca). The Ca requirement for maintenance is greatest when using the factorial equation developed by Quiniou et al. (2021)

because they estimated 32 and 3 mg Ca/kg BW for endogenous fecal and urinary losses, respectively. These values were derived from growing pigs (Misiura et al., 2018). As a result, their estimated total Ca requirement is greater throughout all of gestation compared to the other factorial estimates and empirical studies.

The maintenance requirements for P and Ca are greater for later parity sows compared to parity 1 sows due to an increase in sow BW. Because the maintenance requirement is a function of sow BW, older, heavier sows have slightly higher P and Ca requirements than lighter sows.

Placenta and fetus requirements

The placenta is responsible for the exchange of nutrients, metabolites, and respiratory gases between the dam and fetus. However, the transfer of minerals to the fetus in utero is not greatly affected by the dietary levels of minerals provided in the gestation diet (Peters et al., 2010). Formation of the placenta initiates with implantation and undergoes rapid expansion and development from d 18 to 30 of gestation. The formation and development of the placenta continues from d 30 to 60 of gestation and is complete in weight and surface area by d 70 of gestation (Knight et al., 1977). The Ca and P requirements for placental growth reflect this as the P requirement increases from 0 to 0.09 g/d from d 15 to approximately d 60 of gestation. After d 60 of gestation, the P requirement decreases from 0.09 to 0.06 or 0 g/d depending on the factorial equation used. Although litter size is an input for calculating the P requirement for placental growth, the requirement is relatively insensitive to increasing litter size because of the small amount of P required for placental growth during gestation. Although Ca and P are required for placental growth in gestation, their contribution to the STTD P and total Ca requirements are low (0 to 0.09 g/d for P and Ca).

After approximately d 69 of gestation, the requirement for fetal growth and development contributes the greatest to the P requirement. Fetal weight gain accelerates greatly during late gestation resulting in increased requirements of STTD P and total Ca to meet this need (Figure 1.1). As genetic improvement has occurred, the rate of fetal growth during gestation has also increased. McPherson et al. (2004) reported fetal weights at d 100 to 114 of gestation that were approximately 28 to 30% greater than previously reported by Wise et al. (1997), Wu et al. (1999), and Leenhouwers et al. (2002). Because of the change in fetal growth over time, the increase in fetal weight has been addressed in factorial mineral requirement equations. Jongbloed et al. (1999) first addressed the differences in fetal weight and utilized a correction factor of 1.285 when estimating the P requirement for fetuses. Jongbloed et al. (2003) also used a correction factor of 1.227 for parity 1 sows and 1.216 for parity 2+ sows. The correction factor corrects for higher litter birth weight and greater P content in piglets as genetics progress increases over time. The base equation is used to estimate the STTD P requirement for fetal growth, and then is multiplied by the correction factor to estimate the updated STTD P requirement accounting for changes in litter birth weight.

The total Ca requirement for placental and fetal growth follows the same pattern during gestation as STTD P because they are calculated using a ratio relative to STTD P requirements. Quiniou et al. (2021) used a ratio of 0.80:1 STTD Ca:STTD P and calculated the STTD P requirement for placenta growth by using the protein content of the placenta. Gaillard et al. (2019) used a ratio of 1.759:1 STTD Ca:STTD P and calculated the placenta STTD P requirement factoring in litter size and litter birth weight. Although the ratios are different, the Ca requirement for placental growth is within 0.02 g/d for the two models throughout gestation because the requirement for placental growth is very small. Bikker and Blok (2017) used a ratio

of 1.759:1 STTD Ca:STTD P to estimate the Ca requirement for fetal growth whereas Quiniou et al. (2021) used the fetal weight and Ca content in the fetus to calculate the requirement. Like the Ca requirement for placental growth, the requirements for fetal growth are very similar.

Litter size and litter birth weight have an important effect on P and Ca requirements for fetal growth. Therefore, late parity sows with large litter sizes have slightly increased P and Ca for fetal growth to provide nutrients to more pigs compared to parity 1 sows with smaller litter sizes.

Maternal growth requirement

The other component for estimating Ca and P requirements in gestation is the requirement for maternal growth. As parity 1 sows continue to grow, they deposit maternal soft tissue and bone tissue over subsequent parities (Bikker and Blok, 2017). Maternal gain is required to replenish tissue mobilized during the previous lactation period including protein and lipid (Bikker and Blok, 2017). For young sows, the mineral requirements for maternal growth include the need for continued growth during gestation to reach mature BW. The P requirement for maternal body reserves is calculated according to sow BW gain and its P content. Quiniou et al. (2021) used a STTD P content in maternal gain of 5.5 g/kg. Bikker and Blok (2017) used a STTD P content of 4.1 g/kg for parity 1 sows and 5.5 g/kg for older parity sows. An increase in P content in the body was observed with increasing parity (first to 6^{th} parity) by Peters et al. (2010).

Another strategy to estimate P required for maternal growth is to assume a proportion to protein deposition by considering a P to protein ratio of 0.96% (Jongbloed et al., 2003; NRC, 2012; Gaillard et al., 2019). The NRC (2012) used protein deposition in maternal tissue to calculate the P required for maternal growth and utilized an adjustment factor to account for

changes in sow BW and backfat thickness. The used can change the adjustment factor to account for their observed sow BW and backfat thickness compared to the NRC (2012) estimate. For this review, an adjustment factor of 0.109 was used for calculating the requirements. The NRC (2012) also accounts for P retention in bone tissue within the maternal P requirement. The STTD P retention in bone tissue decreases from 2 to 0.8 g/d as parity increases from 1 to 4. Jongbloed et al. (1999; 2003) also accounted for STTD P retention in bone tissue with a decrease from 1.5 to 0.1 g/d as parity increases from 1 to 5. It is known that demineralization of bone occurs during lactation (van Riet et al., 2016) but the rate at which it is restored during the subsequent gestation is not fully understood.

The Ca requirement for maternal growth is determined using a ratio to STTD P. Quiniou et al. (2021) used a ratio of 1.75:1 (STTD Ca:STTD P) for sows of all parities. Bikker and Blok (2017) used a ratio of 1.60:1 (STTD Ca:STTD P) for parity 1 and 1.75:1 (STTD Ca:STTD P) for older parities because the Ca:P ratio in the body increases as parity increases (Peters et al., 2010). Gaillard et al. (2019) used a ratio of 1.65:1 (STTD Ca:STTD P) for all parities. The variation in the ratios selected indicates that sows may adapt Ca and P retention based on dietary supply. The variation may also be due to limited research to fully understand the Ca and P requirements and the appropriate ratio to be used. The Ca and P requirements for maternal growth decrease as the sow matures. This is because as sows reach maturity in terms of BW and skeleton, their growth rates decrease, and they require less Ca and P for growth.

Model requirement estimates

The STTD P and total Ca or STTD Ca estimates are determined by using the sum of the requirements for maintenance, and fetus, placenta, and maternal growth. The requirements increase as gestation progresses due to the increased need for the growing fetuses. The

maintenance and maternal growth remain relatively constant throughout gestation and the requirement for placental growth is a very small contributor to the STTD P requirement. In summary, the STTD P requirements determined from factorial estimates in gestation for parity 1 sows with a litter size of 15 range from 2.8 to 5.1, 4.9 to 8.5, 6.9 to 10.6 g/d in early, mid, and late gestation, respectively. The total Ca requirements range from 8.5 to 17.7, 12.1 to 29.2, 17.1 to 38.2 g/d in early, mid, and late gestation, respectively. Jongbloed et al. (1999; 3.60:1), Jongbloed et al. (2003; 4.00:1), and NRC (2012; 2.30:1 total Ca:STTD P) determined the total Ca requirements by setting a ratio of total Ca:STTD P. Jondreville and Dourmad (2005) did not estimate requirements for Ca in gestation or lactation.

When feeding older parity sows, these requirements would be very similar to younger parity sows; however, the maintenance and fetal requirements would increase as parity increases and the requirement for maternal gain would decrease. Together, the net effect is that older parity sows have slightly lower requirements compared to parity 1 sows. Therefore, when feeding a sow herd to accommodate parity 1 sows, the requirements will be met for later parity sows by default.

These factorial estimates are very similar to requirements determined by empirical studies (STTD P of 5.4 to 9.5 g/d and total Ca of 12.9 to 18.6 g/d; Figure 1.2 and 1.3). However, the range of total Ca requirement is greater in late gestation for the factorial estimates compared to the empirical studies. This could be due to different approaches of estimating dietary Ca levels by setting a ratio to P or using the sum of each component in the factorial equations. The high estimate of 30.3 g Ca/d was calculated using a ratio of 4.0 (Jongbloed et al., 2003) for total Ca:STTD P by calculating the STTD P using the factorial approach. The highest total Ca:STTD P ratio from empirical studies was 2.49:1.

Empirical estimation of lactation Ca and P requirements

Like empirical gestation studies, common response criteria for estimating nutrient requirements in lactation include sow and litter performance, bone characteristics, milk components, and urinary P excretion. The same procedures were followed as described above for reformulating diets to compare empirical studies from the literature. There is limited research to determine Ca and P requirements in lactation with only 6 empirical trials published from 1974 to 2022 (Table 1.4).

Harmon et al. (1974) compared 2 levels of P (5.1 or 11.1 g/d of STTD P from reformulated diets) by utilizing a diet with no supplemental P or the high P concentration with 3 different P sources (dicalcium, soft or curacao phosphates). Ribs from sows fed the different P sources had increased bone weight, bending load, breaking stress, and ash compared to ribs from sows fed the low P diet. Harmon et al. (1975) conducted a second study utilizing 3 dietary levels of P from dicalcium phosphate during lactation (6.2, 8.5, or 10.8 g/d of STTD P from reformulated diets) and observed numerical increases in bone ash as STTD P increased. The authors did not observe any treatment effects on sow or litter performance, or milk Ca and P concentrations (Harmon et al., 1975). The highest concentrations of STTD P and total Ca (at least 10.8 g of STTD P and 21.0 g/d of total Ca) maximized bone ash.

Mahan and Fetter (1982) observed a numerical linear increase in percentage bone ash of vertebrae when comparing 3 different levels of Ca and P (15.4 and 36.6, 23.7 and 52.1, or 26.9 and 56.3 g/d of STTD P and total Ca, respectively). No treatment differences were observed for sow and litter performance or serum and milk Ca and P concentrations. The lowest STTD P levels fed by Mahan and Fetter (1982) were greater than the highest levels of Harmon et al.

(1974, 1975). Thus, the differences in results from these studies may be more of a reflection of treatment design than differences in bone mineral deposition.

Maxson and Mahan (1986) evaluated 5 levels of dietary Ca and P but did not observe differences in sow and litter performance, serum Ca and P concentrations, bone characteristics, or milk Ca and P concentrations. There were no improvements in response criteria above 12.3 g/d of STTD P and 31.7 g/d of total Ca for parity 1 sows and 12.8 g/d of STTD P and 33.1 g/d total Ca for parity 2 sows. Miller et al. (1994) evaluated 3 levels of Ca in the diet (24.8, 42.7, or 62.3 g/d of total Ca) at a constant P concentration (19.7 g of STTD P) and measured sow and litter performance as well as milk Ca concentrations. Increasing dietary Ca had no effect on milk Ca concentration, milk production, or sow performance. During early lactation, sows fed the highest level of Ca had the greatest litter ADG compared to sows fed the lowest or intermediate Ca level. However, 42.7 g/d of total Ca and 19.7 g/d of STTD P (when using the reformulated diets) was selected as a requirement because no improvements in milk Ca concentration was observed compared to 24.8 g/d Ca.

Equivocal results have been observed for Ca and P requirements for milk production. Miller et al. (1994) observed no statistical differences in milk Ca and P concentrations and concluded that minor dietary changes in Ca have little effect on Ca concentrations in milk because of how tightly Ca is regulated. Tan et al. (2016) observed an increase in milk Ca as dietary Ca and P concentrations increased. However, the range in milk Ca concentration was only 0.03%. The small differences are most likely not important from a biological standpoint and imply the importance of the homeostatic mechanism in maintaining the Ca and P concentrations in blood and milk, particularly when the diet is not severely deficient in Ca or P (Maxson and Mahan, 1986; Tan et al., 2016).

Grez-Capdeville and Crenshaw (2022) determined a total P requirement of 31.1 g/d (16.6 g/d of STTD P) in early lactation (d 0 to 5) and 40.3 g/d (22.1 g/d of STTD P) in late lactation (d 12 to 19) by measuring urinary P excretion. They suggested a total Ca requirement of 38.9 g/d in early lactation and 50.4 g/d in late lactation by setting a total Ca:total P ratio of 1.25:1. The optimal dietary P concentration was determined by using a plateau linear model for urinary P excretion because bone mineralization was not measured. Further research is needed to fully understand the relationship between urinary P excretion and bone mineralization.

In summary, empirical lactation feeding studies observed that 8.5 to 22.1 g/d of STTD P and 21.2 to 50.4 g/d of total Ca with total Ca:STTD P ratios ranging from 2.17:1 to 2.50:1 concentrations maximized bone characteristics or litter performance. The large variation in requirement estimates makes it difficult to define precise STTD P and total Ca requirements for lactating sows. However, from the most recent data from Grez-Capdeville and Crenshaw (2022), a minimum of 16.6 and 22.1 g/d of STTD P during early and late lactation, respectively, appear to be adequate to meet basal requirements. The increase in requirements during late lactation is necessary for the sow to meet the need for increasing milk production. However, without direct correlation to bone mineralization, a margin of safety might be warranted. Additional data are needed to determine dietary Ca and P requirements for bone characteristics of today's high-producing sows.

Factorial estimation of Ca and P requirements in lactation

The 2 main components leading to overall Ca and P requirements in lactation diets are the requirement for sow maintenance and milk production. In addition, some models account for the Ca and P mobilized from body tissue (Jongbloed et al., 1999; 2003; NRC, 2012; Bikker and Blok, 2017).

Maintenance requirements

The daily STTD P requirement for maintenance is determined by accounting for 10 mg STTD P/kg BW of endogenous losses (9 mg STTD P/kg BW for fecal losses and 1 mg STTD P/kg BW for urinary endogenous losses (Table 1.5). However, some models consider 7 mg STTD P/kg BW of endogenous losses and use the same equation as used in gestation (Jongbloed et al., 1999; 2003; NRC, 2012). Bikker and Blok (2017) considered 10 mg STTD P/kg of BW when assuming no sow BW loss in lactation and 9.5 mg STTD P/kg of BW when assuming a sow BW loss of 22.5 kg in lactation. The estimated requirements suggested by Bikker and Blok (2017) used a coefficient of 9.5 mg STTD P/kg of BW for the maintenance requirement. Endogenous losses are influenced by BW and feeding level. More Ca and P are excreted with increased dietary intake because of increased secretion of digestive enzymes (Adeola et al., 2016). The increase in feed intake in lactation, hence greater endogenous losses, compared to gestation is accounted for in the maintenance requirements by utilizing greater coefficients (9.5 mg STTD P/kg of BW by Bikker and Blok, 2017 and 10 mg STTD P/kg of BW by Gauthier et al., 2019 and Quiniou et al., 2021).

As a result, the STTD P requirement for maintenance is relatively constant throughout lactation and is primarily influenced by sow BW. The STTD P for maintenance ranges from approximately 1.3 to 1.9 g/d throughout lactation depending on the equation (for a parity 1 sow with a litter size of 15 piglets (Table 1.6). Older parity sows have slightly increased STTD P requirement for maintenance due to an increase in sow BW compared to younger parity sows.

The daily Ca requirement for maintenance is calculated similarly to P estimates. The Ca estimates for endogenous losses is approximately 14 mg/kg BW of Ca (Bikker and Blok, 2017; Gauthier et al., 2019). Like the P estimate for maintenance, Bikker and Blok (2017) adjusts Ca

requirements from 14 to 13.25 mg/kg BW with a lactation weight loss of 22.5 kg. Like the P estimate, the maintenance Ca requirements decrease as lactation progresses. The STTD Ca for maintenance is 2.69 g/d on d 7 of lactation, 2.62 g/d on d 14, and 2.52 g/d on d 21 (for a parity 1 sow with a litter size of 15 piglets). Quiniou et al., 2021 models total Ca for maintenance of 6.45 g/d on d 7, 6.28 g/d on d 14, and 6.05 g/d on d 21 of lactation. The decrease in maintenance mineral requirements is a function of increased sow weight loss during lactation. Older parity sows have slightly increased Ca requirements for maintenance compared to early parity sows due to the increased BW.

Milk production requirements

Litter ADG and litter size are the key factors for determining P requirements for milk production. The Ca and P content increases from colostrum to milk (Hurley, 2015). The average Ca is 0.80 g/kg in colostrum (range of 0.48 to 1.52 g/kg) and 2 g/kg in milk (range of 1.51 to 2.54 g/kg; d 9 to 28 of lactation). The average P concentration in colostrum is 1.08 g/kg (range of 0.52 to 1.58 g/kg) and 1.42 g/kg in milk (range of 0.87 to 1.83 g/kg; d 9 to 28 of lactation; Hurley, 2015). The greater mineral concentration in milk vs colostrum is because of greater immunoglobulin concentrations in colostrum. Quiniou et al. (2021) incorporate the Ca (2 g/kg) and P (1.42 g/kg) content of milk into the factorial equation for determining the requirement for Ca. Jondreville and Dourmad (2005) use a P content in milk of 1.55 g/kg to estimate the P requirement (Gueguen and Perez, 1981).

Other approaches can be used to estimate the P requirement for milk production. For example, a P:N ratio of 0.196 can be factored into the model to estimate the P needed for milk production (NRC, 2012; Quiniou et al., 2021). The P and Ca content in newborn pigs is also a factor that can be utilized to predict the P requirements for milk (5.4 and 8.0 g/kg P and Ca,

respectively; Bikker and Blok, 2017). Phosphorus digestibility in milk (91%; Jongbloed et al., 2003; Bikker and Blok, 2017) and partial P efficiency (98%; Bikker and Blok, 2017) can also be used to estimate P requirements for milk production. The Ca and P requirements for milk are influenced by changes in milk production throughout lactation. While several researchers developed factorial static equations for Ca and P for milk during lactation, Gauthier et al. (2019) and Quiniou et al. (2021) developed dynamic requirement estimates throughout lactation (Figure 1.4). Gauthier et al. (2019) utilized a milk production curve developed by Wood (1967) and adapted by Hansen et al. (2012) and determined peak milk production on approximately d 14 of lactation. Quiniou et al. (2021) estimated Ca and P secretion in milk by using a litter growth rate curve determined by the number of nursing pigs. This approach determined peak milk production at approximately d 19 of lactation. However, peak milk production is affected by the number of nursing piglets as sows with larger litters have been reported to reach peak milk production earlier in lactation compared to sows with smaller litters (Hansen et al., 2012).

Although there are different approaches to determine Ca and P requirements in milk, all the factorial equations are sensitive to litter size and litter ADG. Factorial models developed by Jongbloed et al. (1999; 2003) are more sensitive to litter size compared to the other models (NRC, 2012; Biker and Blok, 2017). For example, when increasing the litter size from 10 to 15, the STTD P requirement for milk increases by 33% (14.1 to 21.2 g/d Jongbloed et al., 1999; 13.6 to 20.4 g/d Jongbloed et al., 2003) compared to an increase of only 0.7% (14.1 to 14.2 g/d; Bikker and Blok, 2017) or 3.2% (12.4 to 12.8 g/d; NRC, 2012). The large increase in requirement estimates could be due to the increases in litter size observed in more recent models and when they were developed. Models were developed using 10 to 11 pigs per litter by Jongbloed et al. (1999) compared to 15 to 16 pigs by Bikker and Blok (2017). The input values

into the factorial equations can have large effects on the sow's Ca and P requirements for milk production.

Litter ADG also influences the requirement estimates for Ca and P. For example, when increasing the litter ADG from 2.1 to 2.5 kg/d, the dynamic STTD P for milk requirement increases by 17.1% (13.3 to 16.0 g/d; Gauthier et al., 2019) or 14.6% (14.1 to 16.5 g/d; Quiniou et al., 2021). Similar increases were observed for static STTD P requirements for output in milk (15.9%; Jongbloed et al., 1999; 2003). Based on the models, the range of STTD P requirements for milk production is approximately 13.8 to 18.5 g/d during lactation for a parity 1 sow with a litter size of 15 piglets.

The Ca requirement for milk is generally determined using a ratio relative to P. Gauthier et al. (2019) used a ratio of 1.37:1 (STTD Ca:STTD P). Calcium requirements for fetal and placental growth, and milk output are commonly calculated as a ratio to P because little research is available to determine Ca requirement estimate.

Modeled requirement estimates

The STTD P and total Ca or STTD Ca requirement estimates are determined by using total requirements for maintenance, milk production, and tissue mobilization (Jongbloed et al., 1999; 2003; NRC, 2012; Bikker and Blok, 2017). However, the amount of Ca and P mobilized is often provided with little explanation of how it was determined. Total Ca requirements can be estimated using a ratio relative to STTD P, but there is wide variation in suggested Ca:P ratios (3.20:1 total Ca:STTD P for Jongbloed et al., 1999; 3.30:1 for Jongbloed et al., 2003; 2.00:1 for NRC, 2012). But if a 3.20:1 total Ca:STTD P ratio is used with a STTD P requirement of 25.1 g/d, the total Ca requirement would be calculated to be approximately 80 g/d (Jongbloed et al., 1999; 2003). However, determining the appropriate ratio is important because excess Ca

decreases P digestibility potentially causing an increased P requirement (Lee et al., 2023). These total Ca estimates are greater than all the empirical and factorial estimates collected in this literature review.

In summary, the STTD P requirements during lactation range from 14.2 to 25.1 g/d for STTD P and 28.4 to 55.6 g/d for total Ca in parity 1 sows with a litter size of 15 piglets. These are similar to requirements determined by empirical studies (8.5 to 22.1 g/d for STTD P and 21.1 to 50.4 g/d of total Ca; Figures 1.5 and 1.6).

Conclusion

The large variation among results of empirical studies and factorial models makes it difficult to define precise Ca and P requirements for gestating and lactating sows. However, with the most recent data from Grez-Capdeville and Crenshaw (2022), a minimum level of 6.0 STTD P g/d during gestation and 22.1 g/d STTD P during lactation appear to meet basal requirements. This is similar to the estimate proposed by the NRC (2012) of 6.0 g/d of STTD P during the first 90 d of gestation. After d 90, sows may have to mobilize maternal stores to meet the need of the growing fetuses (van Riet et al., 2016). During lactation, 22.1 g/d of STTD P meets the requirement estimates provided by NRC (2012).

Once a P requirement estimate is established, the studies in this meta-analysis frequently estimate a Ca requirement based on a ratio relative to P. The NRC (2012) suggests a 2.3:1 and 2.0:1 ratio of total Ca:STTD P for gestation and lactation, respectively. Empirical studies and factorial models in this review had a range of total Ca:STTD P in gestation of 1.84:1 to 4.0:1 and 2.2:1 to 3.3:1 in lactation. In conclusion, at least 6.0 and 22.1 g/d of STTD P appears to meet minimum requirements during gestation and lactation, respectively. The limited data and

variation between studies emphasize the need for future research evaluating Ca and P requirements for reproducing sows.

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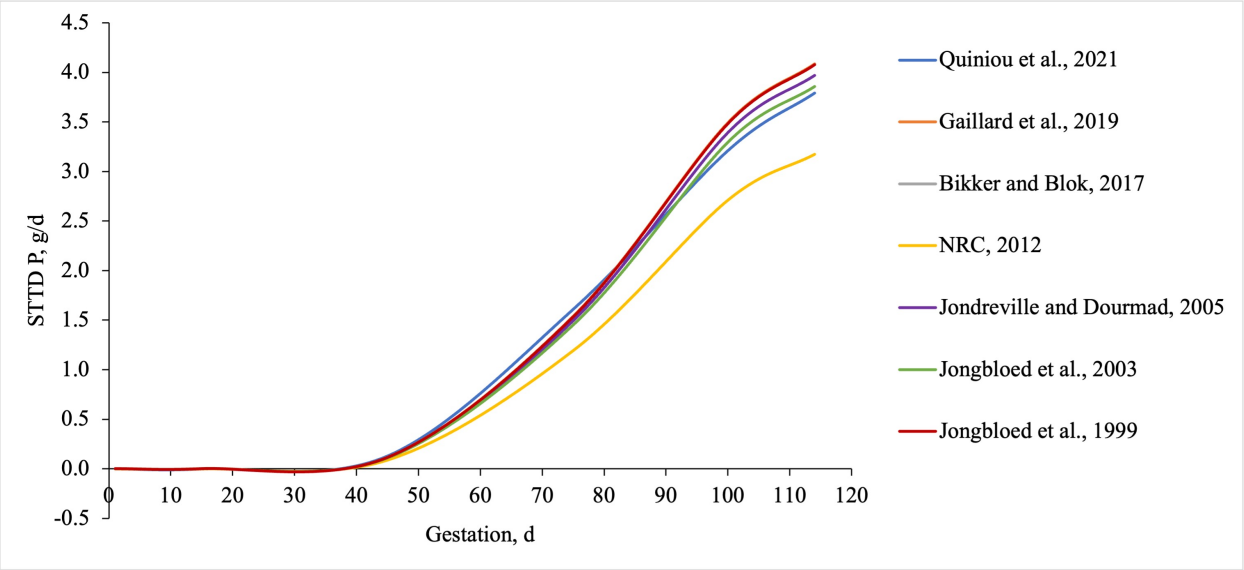

Figure 1.1. Modeled standardized total tract digestibility (STTD) P (g/d) requirement estimates for fetal growth during gestation. Estimated requirements were determined using a litter size of 15**.**

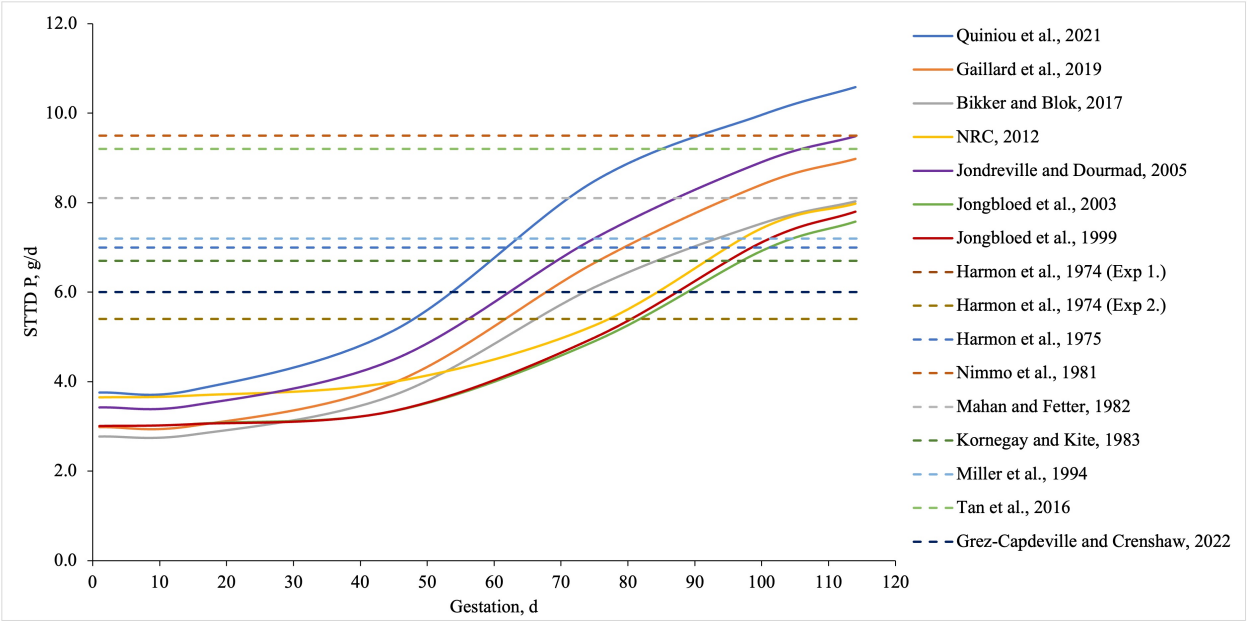

Figure 1.2. Standardized total tract digestibility (STTD) P (g/d) requirement estimates during gestation from factorial and empirical estimates. Factorial estimates are displayed by solid lines and empirical estimates are displayed by dashed lines. Factorial requirement estimates were determined using a litter size of 15 for parity 1 sows. Sow BW (140, 146, 164, 190, 210, 221 kg for d 1, 15, 45, 75, 100, 114, respectively) and sow ADG (0.44, 0.44, 0.60, 0.87, 0.80, 0.79, for d 1, 15, 45, 75, 100, 114, respectively) were estimated using values representative of weight gain during gestation (Bikker and Blok, 2017).

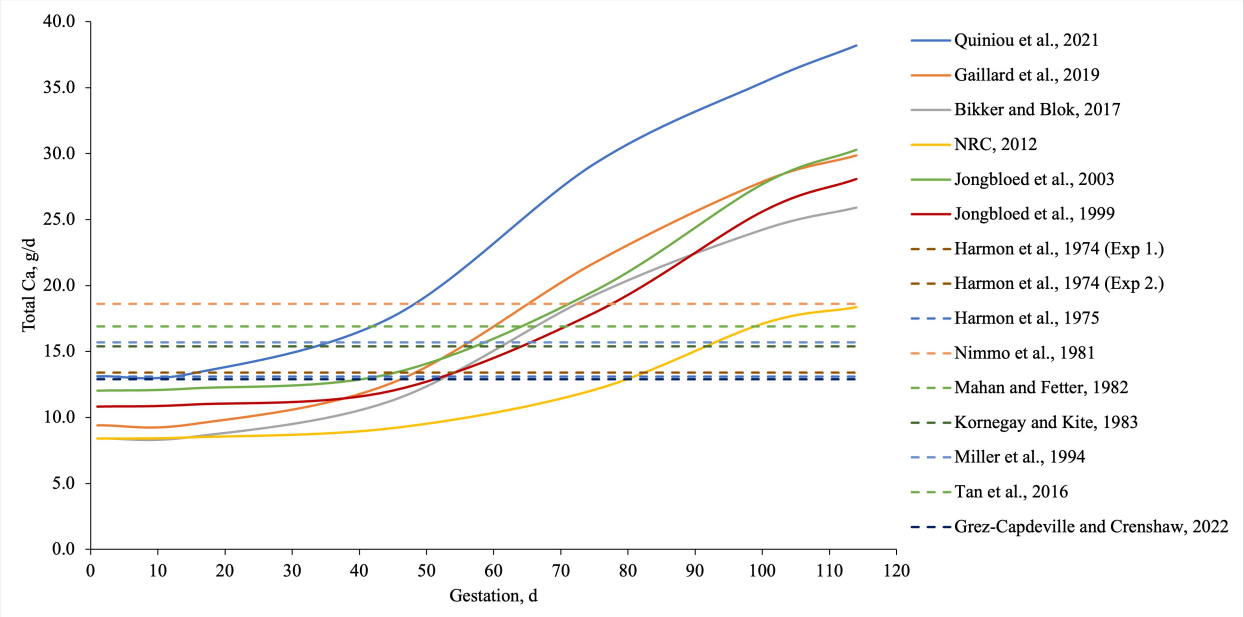

Figure 1.3. Total Ca (g/d) requirement estimates during gestation from factorial and empirical estimates. Factorial estimates are displayed by solid lines and empirical estimates are displayed by dashed lines. Factorial estimated requirements were determined using a litter size of 15 for parity 1 sows. Sow BW (140, 146, 164, 190, 210, 221 kg, for d 1, 15, 45, 75, 100, 114, respectively) and sow ADG (0.44, 0.44, 0.60, 0.87, 0.80, 0.79 kg, for d 1, 15, 45, 75, 100, 114, respectively) were estimated using values representative of weight gain during gestation (Bikker and Blok, 2017).

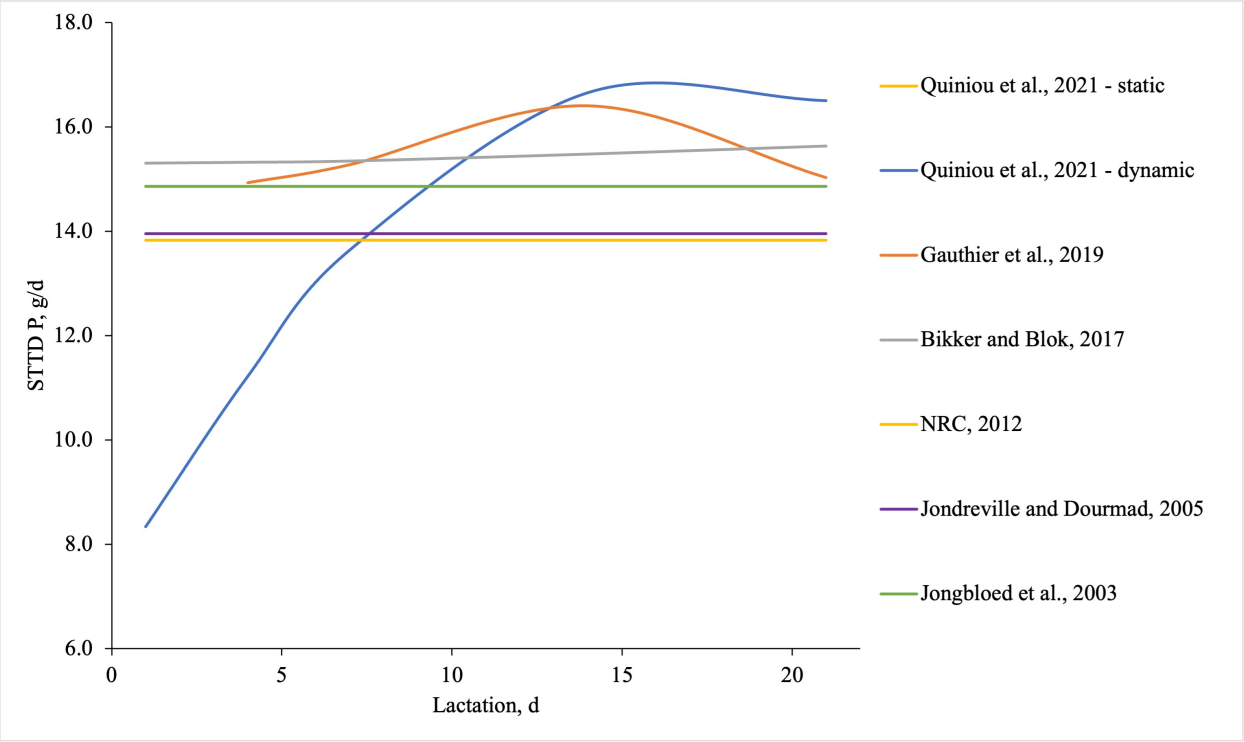

Figure 1.4. Modeled standardized total tract digestibility (STTD) P (g/d) requirement estimates for P output in milk during lactation.

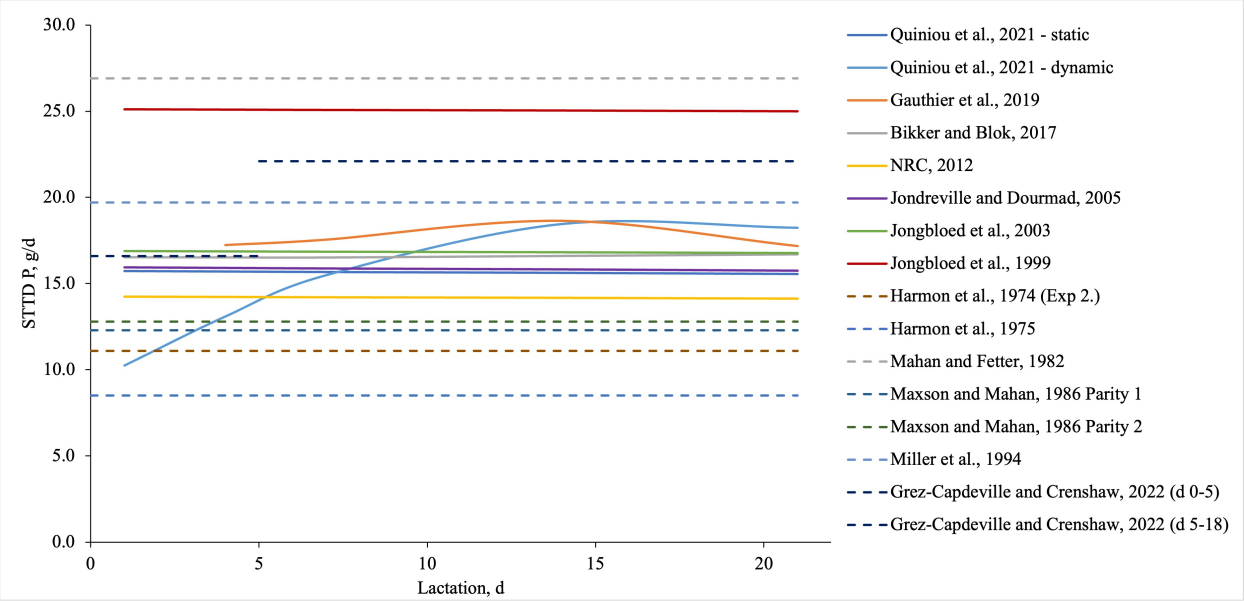

Figure 1.5. Standardized total tract digestibility (STTD) P (g/d) requirement estimates during lactation from factorial and empirical estimates. Factorial estimates are displayed by solid lines and empirical estimates are displayed by dashed lines. Factorial estimated requirements were determined using a litter size of 15 and litter ADG of 2.5 kg for parity 1 sows. Sow BW (198, 195, 192, 187, and 180 kg, for d 1, 4, 7, 14, 21, respectively) was estimated using values representative of weight gain during lactation (Bikker and Blok, 2017).

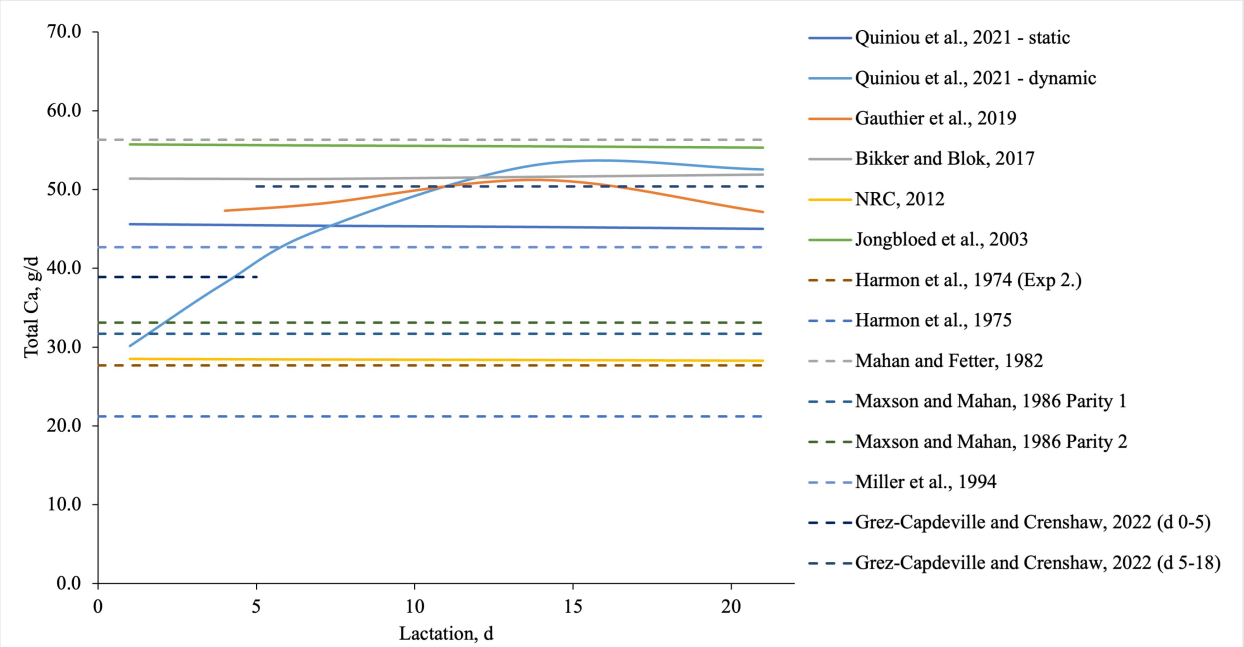

Figure 1.6. Total Ca (g/d) requirement estimates during lactation from factorial and empirical estimates. Factorial estimates are displayed by solid lines and empirical estimates are displayed by dashed lines. Factorial estimated requirements were determined using a litter size of 15 and litter ADG of 2.5 kg for parity 1 sows. Sow BW (198, 195, 192, 187, and 180 kg, for d 1, 4, 7, 14, 21, respectively) was estimated using values representative of weight gain during lactation (Bikker and Blok, 2017). Jongbloed et al. (1999) determined a total Ca requirement of 75.6, 75.5, 75.4, 75.3, and 75.2 g/d, for d 1, 4, 7, 14, 21, respectively as a result of a 3.20:1 total Ca:STTD P ratio which are not included in the figure.

Table 1.1. Empirical studies evaluating Ca and P concentrations in gestation diets[1]

		Range				Feeding level at maximum response, g/d[1]			
Study	Treatments	Total Ca, g/d	Total Ca,%	STTD P, g/d	STTD P,%	Total Ca	STTD Ca	Total P	STTD P
Harmon et al., 1974									
Exp 1.	4	13.4	0.71	2.5 or 5.4	0.13 or 0.28	13.4	9.1	9.9	5.4
Exp 2.	4	13.4	0.71	2.5 or 5.4	0.13 or 0.28	13.4	9.1	9.9	5.4
Harmon et al., 1975	2	13.1	0.73	2.4 or 7.0	0.13 or 0.39	13.1	9.2	11.8	7.0
Nimmo et al., 1981	2	12.3 or 18.6	0.72 or 1.09	5.6 or 9.5	0.33 or 0.56	18.6	13.0	14.5	9.5
Mahan and Fetter, 1982	3	12.0, 14.4, or 16.9	0.67, 0.80, or 0.94	5.1, 6.6, or 8.1	0.28, 0.36, or 0.45	16.9	11.7	13.0	8.1
Kornegay and Kite, 1983	2	15.4	0.84	4.6 or 6.7	0.25 or 0.37	15.4	8.2	13.9	6.7
Miller et al., 1994	3	9.0, 15.7, or 22.9	0.45, 0.78, 1.14	7.1 or 7.2	0.36	15.7	7.2	12.0	7.2
Tan et al., 2016 (d 0-100)	3	14.5, 16.9, or 19.5	0.60, 0.70, or 0.81	8.3, 9.2, or 10.5	0.34, 0.38 or 0.43	16.9	10.8	14.6	9.2
Grez-Capdeville and Crenshaw, 2022									
Early (d 7-77)	6	10.0, 12.0, 14.0, 16.0, 18.0, or 20.0	0.50, 0.60, 0.70, 0.80, 0.90, or 1.00	3.8, 5.2, 6.6, 8.2, 9.6, or 11.0	0.19, 0.26, 0.33, 0.41, 0.48, or 0.55	12.9	6.5	10.3	6.0
Late (d 77-112)	6	10.0, 12.0, 14.0, 16.0, 18.0, or 20.0	0.50, 0.60, 0.70, 0.80, 0.90, or 1.00	3.8, 5.2, 6.6, 8.2, 9.6, or 11.0	0.19, 0.26, 0.33, 0.41, 0.48, or 0.55	12.9	6.5	10.3	6.0

[1]Total Ca and STTD P feeding levels (g/d) that maximized response criteria within the treatment design utilized.

Table 1.2. Equations for Ca and P composition of placenta, fetus, and requirement estimates for Ca and P levels in gestation diets[1]

STTD P requirement, g/d		
	Quiniou et al., 2021	Maintenance + Fetus + Placenta + Maternal
	Gaillard et al., 2019	Maintenance + (Maternal + Fetus + Placenta) ÷ 0.98
	Bikker and Blok, 2017	Maintenance + Fetus + Maternal
	NRC, 2012	Maintenance + Fetus + Placenta + Maternal
	Jondreville and Dourmad, 2005	Maintenance + Fetus + Placenta + Maternal
	Jongbloed et al., 2003[2]	Maintenance + Fetus + Placenta + Maternal
	Jongbloed et al., 1999[2]	Maintenance + Fetus + Placenta + Maternal
Components, g/d		
Maintenance		
	Quiniou et al., 2021[3]	0.010 × (sow BW + conceptus weight) × 0.96
	Gaillard et al., 2019	(7 × sow BW) ÷ 1000
	Bikker and Blok, 2017	(7 × sow BW) ÷ 1000
	NRC, 2012	(7 × sow BW) ÷ 1000
	Jondreville and Dourmad, 2005	(10 × sow BW) ÷ 1000
	Jongbloed et al., 2003	(7 × sow BW) ÷ 1000
	Jongbloed et al., 1999	(7 × sow BW) ÷ 1000
Placenta		
	Quiniou et al., 2021[4]	(Protein placenta (d) - protein placenta (d - 1)) × 0.016
	Gaillard et al., 2019	[exp (7.34264 - 1.40598 × exp (-0.0625 × (d - 45)) + 0.00759 × d + 0.06339 × litter size) × 0.0096 ÷ 23.8] - [exp (7.34264 - 1.40598 × exp (-0.0625 × ((d - 1) - 45)) + 0.00759 × (d - 1) + 0.06339 × litter size) × 0.0096 ÷ 23.8]
	Bikker and Blok, 2017	---
	NRC, 2012[5]	(Protein content of placenta and fluids (d) - protein content of placenta and fluids (d - 1)) × 0.096
	Jondreville and Dourmad, 2005	[exp (7.34264 - 1.40598 × exp (-0.0625 × (d - 45)) + 0.00759 × d + 0.06339 × litter size) × 0.0096 ÷ 23.8] - [exp (7.34264 - 1.40598 × exp (-0.0625 × ((d - 1) - 45)) + 0.00759 × (d - 1) + 0.06339 × litter size) × 0.0096 ÷ 23.8]
	Jongbloed et al., 2003	[exp (7.34264 - 1.40598 × exp (-0.0625 × (d - 45)) + 0.000253 × ME intake × d + 0.06339 × litter size) × 0.0096 ÷ 23.8] - [exp (7.34264 - 1.40598 × exp (-0.0625 × ((d - 1) - 45)) + 0.000253 × ME intake × d + 0.06339 × litter size) × 0.0096 ÷ 23.8]

	Jongbloed et al., 1999	[exp (7.34264 - 1.40598 × exp (-0.0625 × (d - 45)) + 0.00759 × d + 0.06339 × litter size) × 0.0096 ÷ 23.8] - [exp (7.34264 - 1.40598 × exp (-0.0625 × ((d - 1) - 45)) + 0.00759 × (d - 1) + 0.06339 × litter size) × 0.0096 ÷ 23.8]
Fetus		
	Quiniou et al., 2021[6,7]	[Fetus weight (d) × P content in fetus (d)] - [Fetus weight (d - 1) × P content in fetus (d - 1)]
	Gaillard et al., 2019	[exp (4.591 - 6.389 × exp (-0.02398 × (d - 45)) + 0.0897 × litter size) × 6.25 × litter birth weight ÷ exp (4.591 - 6.389 × exp (-0.02398 × (114 - 45)) + 0.0897 × litter size)] - [exp (4.591 - 6.389 × exp (-0.02398 × ((d - 1) - 45)) + 0.0897 × litter size) × 6.25 × litter birth weight ÷ exp (4.591 - 6.389 × exp (-0.02398 × (114 - 45)) + 0.0897 × litter size)]
	Bikker and Blok, 2017	[exp (4.591 - 6.389 × exp (-0.02398 × (d - 45)) + (0.0897 × litter size))] - [exp (4.591 - 6.389 × exp (-0.02398 × ((d - 1) - 45)) + (0.0897 × litter size))]
	NRC, 2012	[exp (4.591 - 6.389 × exp (-0.02398 × (d - 45)) + (0.0897 × litter size))] - [exp (4.591 - 6.389 × exp (-0.02398 × ((d - 1) - 45)) + (0.0897 × litter size))]
	Jondreville and Dourmad, 2005	[exp (4.591 - 6.389 × exp (-0.023998 × (d - 45)) + 0.0897 × litter size) × litter birth weight × 6.25 ÷ exp (4.591 - 6.389 × exp (-0.023998 × (115-45)) + 0.0897 × litter size)] - [exp (4.591 - 6.389 × exp (-0.023998 × ((d - 1) - 45)) + 0.0897 × litter size) × litter birth weight × 6.25 ÷ exp (4.591 - 6.389 × exp (-0.023998 × (115-45)) + 0.0897 × litter size)]
	Jongbloed et al., 2003	[(exp (4.591 - 6.389 × exp (-0.02398 × (d - 45)) + 0.0897 × litter size)) × 1.216] - [(exp (4.591 - 6.389 × exp (-0.02398 × ((d - 1) - 45)) + 0.0897 × litter size)) × 1.216]
	Jongbloed et al., 1999	[(exp (4.591 - 6.389 × exp (-0.02398 × (d - 45)) + 0.0897 × litter size)) × 1.285] - [(exp (4.591 - 6.389 × exp (-0.02398 × ((d - 1) - 45)) + 0.0897 × litter size)) × 1.285]
Maternal		
	Quiniou et al., 2021	5.5 × (sow BW (d) - sow BW (d - 1))
	Gaillard et al., 2019	Sow ADG × 0.96 × (5.4199 - 2 × 0.002857 × sow BW)
	Bikker and Blok, 2017[8]	4.1 × (sow BW (d) - sow BW (d – 1))
	NRC, 2012[9,10]	0.0096 × protein deposition in the maternal body + parity-dependent daily P retention in bone tissue
	Jondreville and Dourmad, 2005	[-0.002857 × sow BW (d)^2 + 5.4199 × sow BW (d)] - [-0.002857 × sow BW (d - 1)^2 + 5.4199 × sow BW (d - 1)]
	Jongbloed et al., 2003	0.0096 × 55
	Jongbloed et al., 1999	0.0096 × 55
STTD Ca requirement, g/d		
	Gaillard et al., 2019	Maintenance + (Maternal + Fetus + Placenta) ÷ 0.98
	Bikker and Blok, 2017	Maintenance + Fetus + Maternal

Total Ca, g/d		
	Quiniou et al., 2021	Maintenance + (Fetus + Placenta + Maternal) ÷ (fecal digestibility of Ca ÷ 100)
	Gaillard et al., 2019	STTD Ca ÷ 0.50
	Bikker and Blok, 2017	STTD Ca ÷ 0.50
	NRC, 2012[11]	2.30 × STTD P
	Jongbloed et al., 2003[11]	4.00 × STTD P
	Jongbloed et al., 1999[11]	3.60 × STTD P
Components, g/d		
Maintenance		
	Quiniou et al., 2021	0.035 × (sow BW + conceptus weight) × 0.96
	Gaillard et al., 2019	(10 × sow BW) ÷ 1000
	Bikker and Blok, 2017	(10 × sow BW) ÷ 1000
Placenta		
	Quiniou et al., 2021	P placenta × 0.80
	Gaillard et al., 2019	P placenta × 1.759
Fetus		
	Quiniou et al., 2021[6,12]	[(Fetus weight (d) × Ca content in fetus (d))] - [(fetus weight (d - 1) × Ca content in fetus (d - 1))]
	Gaillard et al., 2019	P fetus × 1.759
	Bikker and Blok, 2017	P fetus × 1.75
Maternal		
	Quiniou et al., 2021	P maternal × 1.75
	Gaillard et al., 2019	P maternal × 1.65
	Bikker and Blok, 2017[13]	P maternal × 1.60

[1]Units for the following components: sow BW (kg), day of gestation (d), litter size (n), litter birth weight (kg), and sow ADG (kg).

[2]Accounted for P retention in bones: parity 1 = 1.5, parity 2 = 0.8, parity 2 = 0.4, parity 4 = 0.2, and parity 5 = 0.1 g/d.

[3]Conceptus weight (kg) = (Litter birth weight × 1.329 + 0.3) × exp(8.74519 - 1.59844 × exp(-0.05407 × (d - 45)) + 0.00006 × ME × d + 0.09745 × Litter size) ÷ exp(8.74519 - 1.59844 × exp(-0.05407 × (115 - 45)) + 0.00006 × ME × d + 0.09745 × Litter size).

[4]Protein in placenta (kg) = exp(7.34264 - 1.40598 × exp(-0.0625 × (d - 45)) + 0.000253 × ME × d + 0.06339 × Litter size) ÷ 23.8.

[5]Protein content of placenta and fluids (g) = ((38.53) × (d ÷ 54.969)$^{7.5036}$) ÷ (1 + (d ÷ 54.969)$^{7.5036}$).

[6]Fetus weight (kg) = Litter birth weight × exp(8.72962 - 4.07466 × exp(-0.03318 × (d - 45)) + 0.000154 × ME × d + 0.06774 × Litter size) ÷ exp(8.72962 - 4.07466 × exp(-0.03318 × (115 - 45)) + 0.000154 × ME × 115 + 0.06774 × Litter size).

[7]P content in fetus (g/kg) = (0.0565 × d - 0.736) ÷ (0.0565 × 115 - 0.736) × 6.2.

[8]Use coefficient of 4.1 in for parity 1 and 5.5 for higher parities.

[9]Protein deposition in maternal body (g/d) = coefficient a × (ME intake - Maintenance ME requirement on d 1 of gestation (kcal/d)) × adjustment. Coefficient a = (2.75 - 0.5 × parity) × adjustment. Adjustment > 0.

[10]P retention in bones for parity 1 = 2.0, parity 2 = 1.6, parity 3 = 1.2, and parity 4+ = 0.8 g/d.

[11]Calculated by using a set total Ca:STTD P ratio.

[12]Ca content in fetus (g/kg) = (0.1244 × d – 4.039) ÷ (0.1244 × 115 - 4.039) × 11.

[13]Use coefficient of 1.60 for parity 1 and 1.75 for parity 1+.

Table 1.3. Results of modeled requirement estimates for Ca and P levels in gestation[1]

Gestation, d	15	45	75	100	114
Sow BW, kg	146	164	190	210	221
Sow ADG, kg/d	0.44	0.60	0.87	0.80	0.79
Phosphorus, g/d					
Quiniou et al., 2021					
Maintenance	1.4	1.6	2.0	2.3	2.4
Fetus	0.0	0.1	1.6	3.2	3.8
Placenta	0.00	0.09	0.08	0.06	0.05
Maternal	2.4	3.3	4.8	4.4	4.3
STTD P	3.8	5.1	8.5	10.0	10.6
Gaillard et al., 2019[2]					
Maintenance	1.0	1.1	1.3	1.5	1.5
Fetus	0.0	0.1	1.6	3.6	4.2
Placenta	0.00	0.04	0.05	0.04	0.03
Maternal	2.0	2.6	3.7	3.3	3.2
STTD P	3.0	3.9	6.6	8.4	9.0
Bikker and Blok, 2017					
Maintenance	1.0	1.1	1.3	1.5	1.5
Fetus	0.0	0.1	1.2	2.8	3.3
Maternal	1.8	2.5	3.6	3.3	3.2
STTD P	2.8	3.7	6.1	7.5	8.0
NRC, 2012					
Maintenance	1.0	1.1	1.3	1.5	1.5
Fetus	0.0	0.1	1.2	2.8	3.3
Placenta	0.00	0.09	0.03	0.00	0.00
Maternal	2.7	2.7	2.7	3.2	3.2
STTD P	3.7	4.0	5.2	7.4	8.0
Jondreville and Dourmad, 2005					
Maintenance	1.5	1.6	1.9	2.1	2.2
Fetus	0.0	0.1	1.5	3.4	4.0
Placenta	0.00	0.05	0.05	0.03	0.03
Maternal	2.0	2.7	3.8	3.4	3.3
STTD P	3.5	4.5	7.2	8.9	9.5
Jongbloed et al., 2003					
Maintenance	1.0	1.1	1.3	1.5	1.5
Fetus	0.0	0.1	1.5	3.4	4.0
Placenta	0.00	0.05	0.05	0.03	0.03
Maternal	2.0	2.0	2.0	2.0	2.0

STTD P	3.1	3.3	4.9	6.9	7.6
Jongbloed et al., 1999					
Maintenance	1.0	1.1	1.3	1.5	1.5
Fetus	0.0	0.1	1.6	3.6	4.2
Placenta	0.00	0.05	0.05	0.03	0.03
Maternal	2.0	2.0	2.0	2.0	2.0
STTD P	3.1	3.3	5.0	7.1	7.8
Calcium, g/d					
Quiniou et al., 2021[3]					
Maintenance	4.9	5.7	7.1	8.0	8.4
Fetus	0.0	0.3	5.3	11.9	14.5
Placenta	0.00	0.14	0.13	0.09	0.09
Maternal	8.4	11.5	16.7	15.4	15.1
Total Ca	13.3	17.7	29.2	35.4	38.2
Gaillard et al., 2019					
Maintenance	1.5	1.6	1.9	2.1	2.2
Fetus	0.0	0.2	2.7	6.1	7.2
Placenta	0.00	0.09	0.09	0.06	0.06
Maternal	3.2	4.3	6.0	5.3	5.2
STTD Ca	4.7	6.3	10.9	13.9	14.9
Bikker and Blok, 2017					
Maintenance	1.4	1.6	1.8	2.0	2.1
Fetus	0.0	0.2	2.1	4.9	5.7
Maternal	2.9	3.9	5.7	5.2	5.2
STTD Ca	4.3	5.7	9.6	12.1	13.0
NRC, 2012					
Total Ca	8.5	9.2	12.1	17.1	18.4
Jongbloed et al., 2003					
Total Ca	12.2	13.4	19.6	27.7	30.3
Jongbloed et al., 1999					
Total Ca	11.0	12.0	17.9	25.6	28.1

[1]Estimated requirements were determined using a first parity sow with a litter size of 15. Sow BW and ADG were estimated using values representative of weight gain during gestation (Bikker and Blok, 2017).

[2]Maternal, fetus, and placenta components were divided by 0.98 (Maintenance + (Maternal + Fetus + Placenta) ÷ 0.98).

[3]Fetus, placenta, and maternal components were divided by 0.50 (Maintenance + (Fetus + Placenta + Maternal) ÷ (fecal digestibility of Ca ÷ 100)).

Table 1.4. Empirical studies evaluating the effects of Ca and P in lactation diets[1]

		Range				Feeding level at maximum response, g/d			
Study	Treatments	Total Ca, g/d	Total Ca,%	STTD P, g/d	STTD P,%	Total Ca	STTD Ca	Total P	STTD P
Harmon et al., 1974 (Exp 2.)	4	27.7	0.71	5.1 or 11.1	0.13 or 0.28	27.7	18.8	20.4	11.1
Harmon et al., 1975	3	21.0	0.71	6.2, 8.5, or 10.8	0.21, 0.28 or 0.36	21.2	14.5	15.7	8.5
Mahan and Fetter, 1982	3	36.6, 52.1, or 56.3	0.67, 0.80, or 0.94	15.4, 23.7, or 26.9	0.28 or 0.45	56.3	39.1	43.2	26.9
Maxson and Mahan, 1986									
Parity 1	5	31.7, 37.7, 43.7, 49.7, or 55.7	0.72, 0.86, 0.99, 1.13, or 1.27	12.3, 15.9, 19.6, 23.3, or 27.0	0.28, 0.36, 0.45, 0.55, or 0.61	31.7	21.5	22.5	12.3
Parity 2	5	33.1, 39.4, 45.7, 51.9, or 58.2	0.72, 0.86, 0.99, 1.13, or 1.27	12.8, 16.7, 20.5, 24.4, or 28.2	0.28, 0.36, 0.45, 0.55, or 0.61	33.1	22.5	23.6	12.8
Miller et al., 1994	3	24.8, 42.7, or 62.3	0.46, 0.79, or 1.15	19.7	0.35	42.7	29.4	33.1	19.7
Grez-Capdeville and Crenshaw, 2022									
Early (d 0-5)	6	28.5, 37.2, 44.1, 46.4, 55.8, or 66.0	0.50, 0.60, 0.70, 0.80, 0.90, or 1.00	10.3, 15.5, 20.2, 22.6, 28.5, or 35.0	0.18, 0.25, 0.32, 0.39, 0.46, or 0.53	38.9	19.4	31.1	16.6
Late (d 5-18)	6	38.0, 49.2, 53.2, 62.4, 62.1, or 77.0	0.50, 0.60, 0.70, 0.80, 0.90, or 1.00	13.7, 20.5, 24.3, 30.4, 31.7, or 40.8	0.18, 0.25, 0.32, 0.39, 0.46, or 0.53	50.4	25.2	40.3	22.1

[1]Total Ca and STTD P feeding levels (g/d) that maximized response criteria within the treatment design utilized.

Table 1.5. Equations for Ca and P composition of milk and requirement estimates in lactation diets[1]

STTD P, g/d		
	Quiniou et al., 2021	Maintenance + Milk
	Gauthier et al., 2019	Maintenance + (Milk ÷ 0.98)
	Bikker and Blok, 2017	Maintenance + (Milk ÷ 0.98) - Mobilized
	NRC, 2012	Maintenance + Milk - Mobilized
	Jondreville and Dourmad, 2005	Maintenance + Milk
	Jongbloed et al., 2003	Maintenance + Milk - Mobilized
	Jongbloed et al., 1999	Maintenance + Milk - Mobilized
Components, g/d		
Maintenance		
	Quiniou et al., 2021	0.010 × sow BW × 0.96
	Gauthier et al., 2019	(10 × sow BW) ÷ 1000
	Bikker and Blok, 2017[2]	(9.5 × sow BW) ÷ 1000
	NRC, 2012	(7 × sow BW) ÷ 1000
	Jondreville and Dourmad, 2005	(10 × sow BW) ÷ 1000
	Jongbloed et al., 2003	(7 × sow BW) ÷ 1000
	Jongbloed et al., 1999	(7 × sow BW) ÷ 1000
Milk		
Static		
	Quiniou et al., 2021	(0.0257 × (litter ADG ÷ 1000) + 0.42 × litter size) × 0.196
	Bikker and Blok, 2017	((litter ADG × 5.4 + piglet mean BW × 0.007) × litter size) ÷ (0.91 × 0.98)
	NRC, 2012	(0.0257 × (litter ADG ÷ 1000) + 0.42 × litter size) × 0.1955
	Jondreville and Dourmad, 2005	(0.0257 × (litter ADG ÷ 1000) + 0.42 × litter size) × 6.38 × 1.55 ÷ 50
	Jongbloed et al., 2003	(5.877 × piglet ADG + 0.0112) × litter size
	Jongbloed et al., 1999	(0.6096 × litter ADG + 0.0115) × litter size
Dynamic		

	Quiniou et al., 2021	(0.0257 × (litter ADG ÷ 1000) + 0.42 × litter size) × (2.763 - 0.014 × d) × exp(-0.025 × d) × exp(-exp(0.5 - 0.1 × d)) × 0.196
	Gauthier et al., 2019[3]	Protein in milk × 1.55 ÷ 50
Mobilized from tissue		
	Bikker and Blok, 2017[4]	-0.87
	NRC, 2012	-0.96
	Jongbloed et al., 2003	-(litter ADG × 9.6 ÷ 28)
	Jongbloed et al., 1999	-0.80
STTD Ca, g/d		
	Gauthier et al., 2019	Maintenance + Milk ÷ 0.98
	Bikker and Blok, 2017	Maintenance + (Milk ÷ 0.98) - Mobilized
Total Ca, g/d		
	Quiniou et al., 2021	Maintenance + Milk ÷ (fecal digestibility of Ca ÷ 100)
	Gauthier et al., 2019	STTD Ca ÷ 0.50
	Bikker and Blok, 2017	STTD Ca ÷ 0.50
	NRC, 2012[5]	2.00 × STTD P
	Jongbloed et al., 2003[5]	3.30 × STTD P
	Jongbloed et al., 1999[5]	3.20 × STTD P
Components, g/d		
Maintenance		
	Quiniou et al., 2021	0.035 × sow BW × 0.96
	Gauthier et al., 2019	14 × sow BW
	Bikker and Blok, 2017[6]	13.25 × sow BW
Milk		
Static		
	Quiniou et al., 2021	P milk, static ÷ 1.42 × 2.0
	Bikker and Blok, 2017	((litter ADG × 8.0 + (piglet mean BW × 0.010) × litter size) ÷ (0.91 × 0.98)
Dynamic		

	Quiniou et al., 2021	P milk, dynamic ÷ 1.42 × 2.0
	Gauthier et al., 2019	P milk, dynamic × 1.37
Mobilized from tissue		
	Bikker and Blok, 2017[7]	0.06

[1]Units for the following components: sow BW (kg), day of lactation (d), litter size (n), litter ADG (kg), piglet ADG (kg), and piglet mean BW (kg).

[2]Use coefficient of 10 if assuming no sow BW loss in lactation. Use coefficient 9.5 if assuming sow BW loss of 22.5 kg in lactation.

[3]Protein in milk (g/d) = (0.0257 × (Litter ADG ÷ 1000) + 0.42 × Litter size) × 6.38 × milk production factor.

Milk production factor = milk production (kg) ÷ average milk production for the lactation period (kg/d).

Milk production (kg) = $a \times t^b \times \exp(-c \times t)$.

a = exp(1 ÷ 3 × (-ly20 × log(128 ÷ 27) - 3 × log(20) × ly30 + 5 × log(20) × ly20 - 2 × log(20) × ly5 + 4 × ly5 × log(128 ÷ 27) + 12 × ly30 × log(5) - 20 × log(5) × ly20 + 8 × log(5) × ly5) ÷ log(128 ÷ 27))

b = -(3.23352 × ly30 - 5 × ly20 + 2 × ly5) ÷ log(128 ÷ 27)

c = 1 ÷ 15 × (ly5 × log(128 ÷ 27) - ly20 × log(128 ÷ 27) - 3 × log(20) × ly30 + 5 × log(20) × ly20 - 2 × log(20) × ly5 + 3 × ly30 × log(5) - 5 × log(5) × ly20 + 2 × log(5) × ly5) ÷ log (128÷27)

Natural log of milk yield on d 5 of lactation: ly5 = 1.93 + 0.07 × (litter size - 9.5) + 0.04 × (litter ADG - 2.05)

Natural log of milk yield on d 20 of lactation: ly20 = 2.23 + 0.05 × (litter size - 9.5) + 0.23 × (litter ADG - 2.05)

Natural log of milk yield on d 30 of lactation: ly30 = 2.15 + 0.02 × (litter size - 9.5) + 0.31 × (litter ADG - 2.05)

[4]Use 0.87, 0.97, 0.68, 0.68, or 0.39 g/d P from mobilized body tissue if assuming BW loss of 22.5 kg in lactation for parity 1, 2, 3, 4, and 5, respectively. P from mobilized body tissue is disregarded if assuming no sow BW loss in lactation.

[5]Calculated by using a set total Ca:STTD P ratio.

[6]Use coefficient of 14 if assuming no sow BW loss in lactation. Use coefficient 13.25 if assuming sow BW loss of 22.5 kg in lactation.

[7]Use 0.06, 0.05, or 0.03 g/d Ca from mobilized body tissue if assuming BW loss of 22.5 kg in lactation for parity 1 and 2, 3 and 4, and 5, respectively. Ca from mobilized body tissue is disregarded if assuming no sow BW loss in lactation.

Table 1.6. Results of modeled requirement estimates for Ca and P levels in lactation[1]

Lactation, d	7	14	21
Sow BW, kg	192	187	180
Piglet mean BW, kg	1.75	2.91	4.25
STTD P, g/d			
Quiniou et al., 2021			
Maintenance	1.8	1.8	1.7
Milk (static)	13.8	13.8	13.8
Milk (dynamic)	13.6	16.7	16.5
STTD P (static milk)	15.6	15.6	15.6
STTD P (dynamic milk)	15.5	18.5	18.2
Gauthier et al., 2019[2]			
Maintenance	1.9	1.9	1.8
Milk (dynamic)	15.6	16.9	15.6
STTD P	17.6	18.8	17.4
Bikker and Blok, 2017			
Maintenance	1.8	1.8	1.7
Milk (static)	15.3	15.5	15.6
Mobilized from body tissue	-0.87	-0.87	-0.87
STTD P	16.5	16.7	16.7
NRC, 2012			
Maintenance	1.3	1.3	1.3
Milk (static)	13.8	13.8	13.8
Mobilized from body tissue	-0.96	-0.96	-0.96
STTD P	14.2	14.2	14.1
Jondreville and Dourmad, 2005			
Maintenance	1.9	1.9	1.8
Milk (static)	14.0	14.0	14.0
STTD P	15.9	15.8	15.8
Jongbloed et al., 2003			
Maintenance	1.3	1.3	1.3
Milk (static)	14.9	14.9	14.9
Mobilized from body tissue	-0.86	-0.86	-0.86
STTD P	15.3	15.3	15.3
Jongbloed et al., 1999			
Maintenance	1.3	1.3	1.3
Milk (static)	23.0	23.0	23.0
Mobilized from body tissue	-0.80	-0.80	-0.80
STTD P	23.5	23.5	23.5

Calcium, g/d			
Quiniou et al., 2021[3]			
Maintenance	6.5	6.3	6.0
Milk (static)	39.0	39.0	39.0
Milk (dynamic)	38.4	46.9	46.5
Total Ca (static milk)	45.4	45.2	45.0
Total Ca (dynamic milk)	44.9	53.2	52.5
Gauthier et al., 2019[2]			
Maintenance	2.7	2.6	2.5
Milk (dynamic)	21.9	23.7	21.8
STTD Ca	24.6	26.3	24.3
Bikker and Blok, 2017[4]			
Maintenance	2.5	2.5	2.4
Milk (static)	23.2	23.4	23.6
Mobilized from body tissue	-0.06	-0.06	-0.06
STTD Ca	25.7	25.8	25.9
NRC, 2012			
Total Ca	28.4	28.4	28.3
Jongbloed et al., 2003			
Total Ca	50.5	50.5	50.5
Jongbloed et al., 1999			
Total Ca	75.2	75.2	75.2

[1]Estimated requirements were determined using a first parity sow with a litter size of 15 and litter ADG of 2.5 kg. Sow BW was estimated using values representative of weight gain during lactation (Bikker and Blok, 2017).

[2]Milk component was divided by 0.98 (Maintenance + (Milk ÷ 0.98)).

[3]Milk component was divided by 0.50 (Maintenance + Milk ÷ (fecal digestibility of Ca ÷ 100)).

[4]Milk component was divided by 0.98 (Maintenance + (Milk ÷ 0.98) – Mobilized).

Chapter 2 - Effects of added 25(OH)D_3 with varying standardized total tract digestible phosphorus concentrations on nursery pig performance, bone characteristics, and serum vitamin D status

Abstract

A total of 360 pigs (DNA 600 × 241; initially 5.8 kg) were used in a 45-d growth study to evaluate the effects of adding 25(OH)D_3 with three levels of standardized total tract digestible (STTD) P on nursery pig growth performance, bone and urine characteristics, and serum vitamin D. Pigs were weaned at 19 d of age and randomly allotted to 1 of 6 dietary treatments with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of 25(OH)D_3 (0 or 50 μg/kg equivalent to 2,000 IU/kg of vitamin D_3; Hy-D, dsm-firmenich, Plainsboro, NJ) and STTD P (70, 100, or 130% of the NRC (2012) requirement estimate on a dietary percentage basis). All diets contained 1,653 IU/kg of vitamin D_3. On d 45, 1 pig per pen was euthanized to collect the right fibula, metacarpal, and 2nd and 10th ribs. Overall, increasing STTD P increased (quadratic, $P \leq 0.003$) ADG, ADFI, and G:F with minimal improvement above 100% of the NRC STTD P requirement estimate. Added 25(OH)D_3 had no effect on growth performance. Increasing STTD P decreased urinary Ca concentration (linear, $P < 0.001$) and increased urinary P concentration (quadratic, $P < 0.001$). When pigs were fed added 25(OH)D_3, serum 25(OH)D_3 increased (quadratic, $P = 0.005$) as STTD P increased but no differences were observed when 25(OH)D_3 was not added and STTD P increased (25(OH)D_3 × STTD P interaction, $P = 0.032$). When pigs were fed 25(OH)D_3, serum 1,25$(OH)_2D_3$ increased (quadratic, $P < 0.001$) as STTD P decreased but the increase was not significant when no 25(OH)D_3 was fed (STTD P × 25(OH)D_3 interaction, $P = 0.002$). Bone ash percentage and

weight increased (quadratic, $P \leq 0.065$) in all bones as STTD P increased. Added 25(OH)D_3 had no effect on bone density or bone ash weight; however, the reduction in bone ash percentage observed with reducing STTD P level tended to be less when 25(OH)D_3 was provided (linear interaction, $P = 0.098$). Increasing STTD P decreased the likelihood of abnormal histologic bone lesions in the 10th rib. In summary, added 25(OH)D_3 had limited effect on growth performance; however, an increase in serum concentrations of 25(OH)D_3 and 24,25(OH)$_2D_3$ was observed. The addition of 25(OH)D_3 to P-deficient diets increased percentage bone ash. Increasing STTD P to 100% of NRC (2012) requirement estimate increased growth and 130% of NRC maximized bone ash.

Key Words: bone characteristics, nursery pig, phosphorus, vitamin D, 25(OH)D_3

Introduction

Calcium and P concentrations are tightly regulated to maintain homeostasis in the body through a complex interaction of multiple factors. The primary hormones involved in the regulation of Ca and P are parathyroid hormone, 1,25-dihydroxycholecalciferol [1,25(OH)$_2D_3$], calcitonin, and fibroblast growth factor-23 (Crenshaw, 2001; Shimada et a., 2003). When vitamin D_3 is consumed, it undergoes a two-step hydroxylation process for bioactivation which is regulated by the hormone fibroblast growth factor-23 (Hewison et al., 2000; Shimada et a., 2003). Following absorption in the small intestine, vitamin D_3 is primarily stored in the liver where it is hydroxylated to produce 25-hydroxyvitamin D_3 [25(OH)D_3], the major circulating metabolite of vitamin D. When needed, 25(OH)D_3 undergoes a second hydroxylation step, primarily in the kidney, to become 1,25-dihydroxycholecalciferol [1,25(OH)$_2D_3$], the most

biologically active form of vitamin D in the body (Hewison et al., 2000). Additionally, the kidney and some extra-renal tissues convert $25(OH)D_3$ into other vitamin D metabolites, including 24,25-dihydroxycholecalciferol [$24,25(OH)_2D_3$], which regulates cartilage and bone healing and mineralization, and $1,25(OH)_2D_3$ (Boyan et al., 2001). A key role of $24,25(OH)_2D_3$ is the regulation of $1,25(OH)_2D_3$ production to protect against vitamin D toxicity (St-Arnaud and Glorieux, 1998; Tang et al., 2019).

Low digestible P in nursery diets may lead to reduced growth, lameness, and decreased bone P reserves for subsequent growing and finishing phases (Buhler et al., 2010; She et al., 2017; Wensley et al., 2020). Research has demonstrated that achieving maximum bone mineralization requires higher P levels than those necessary for optimal growth performance (Vier et al., 2019a; 2019b). This is because P retention in soft tissues is prioritized over P retention in bones when low P diets are fed (Misiura et al., 2020).

When diets are deficient in P, an increase in the conversion of vitamin D_3 to $1,25(OH)_2D_3$ occurs which involves the previously described hydroxylation steps (Bikle, 2021). By feeding $25(OH)D_3$, it can bypass the first hydroxylation step in the liver and be directly available to the kidney to be hydroxylated to $1,25(OH)_2D_3$. The bioactivation to $1,25(OH)_2D_3$ increases intestinal P absorption and decreases P excretion in the urine to increase P in the body to be utilized for bone mineralization (Crenshaw, 2001). Additionally, the supplementation of $25(OH)D_3$ is needed for pigs to elevate their serum $25(OH)D_3$ concentrations above recommended levels (Lauridsen, 2014). When supplementing with vitamin D_3 alone in the feed, pigs are unable to maintain serum $25(OH)D_3$ concentrations even when fed at levels well above the NRC (2012) requirements (Flohr et al., 2014). Thus, in our experiment we used levels of vitamin D_3

commonly being fed to commercial swine and evaluated the response to STTD P level and 25(OH)D_3 supplementation in diets containing adequate concentrations of vitamin D_3.

The hypothesis of our experiment was that supplementing 25(OH)D_3 would increase growth performance and bone development when pigs are fed diets deficient or marginally deficient in P compared to pigs fed P-deficient diets without supplementation of 25(OH)D_3. The objective was to determine the effects of added 25(OH)D_3 with three levels of STTD P on nursery pig growth performance, bone and urine characteristics, and serum vitamin D.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (1.52 × 1.52 m) contained a 4-hole, dry self-feeder, and nipple waterer for *ad libitum* access to feed and water. The facilities are completely enclosed, environmentally controlled, and mechanically ventilated. At weaning, the facilities were set at 32°C, then beginning two weeks after placement the set point was reduced by approximately 0.28°C per day until reaching 21°C where it was maintained for the remainder of the experiment.

Animals and housing

A total of 360 mixed-sex pigs (DNA 600 × 241; initially 5.8 ± 0.54 kg) were used in a 45-d growth study. Pigs were weaned at approximately 19 d of age and placed in pens of 5 pigs each. At weaning, pigs were randomly allotted to 1 of 6 dietary treatments with 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of 25(OH)D_3 (0 or 50 µg/kg equivalent to 2,000 IU/kg of vitamin D_3; Hy-D, dsm-firmenich,

Plainsboro, NJ) and STTD P (70, 100, or 130% of the NRC (2012) requirement estimate on a dietary percentage basis). Diets were corn-soybean meal-based and fed in 3 phases (Tables 2.1 and 2.2). Phase 1 diets were fed from approximately d 0 to 10 (6 to 8 kg). Phase 2 diets were fed from approximately d 10 to 22 (8 to 12 kg). Phase 3 diets were fed from approximately d 22 to 45 (12 to 26 kg). All diets contained 1,653 IU/kg of vitamin D_3 provided by the vitamin premix, but the premixes did not contain 25(OH)D_3. Pigs were weighed on d 0, 10, 22, and 45 to determine ADG, ADFI, and feed efficiency.

Blood, urine, and bone characteristics

On d 45, a blood sample was taken from the jugular vein of 1 average weight pig per pen (72 pigs total; sex was represented equally across all treatments). Pigs were restrained and blood samples were collected using a red-top, no anti-coagulant blood tube. Ten mL of blood was centrifuged (3000 × g, 30 min) within 2 h after collection, and serum was stored at −20°C until analysis. Serum was analyzed for 25(OH)D_3, 24,25$(OH)_2D_3$, and 1,25$(OH)_2D_3$ (Heartland Assays, Ames, IA).

After blood collection, the same pig in each pen was euthanized via penetrating captive bolt and used for the analysis of urine and bones. Approximately 10 mL of urine was collected via cystocentesis using a 20 gauge × 2.5 cm needle and a 12 mL syringe, then frozen at −20°C until analyzed for Ca and P (Iowa State University Veterinary Diagnostic Laboratory). The right metacarpal, fibula, and 2nd and 10th ribs were collected from each pig to determine bone density and percentage bone ash. After removal, bones were stored at -20°C until analysis. The leftover extraneous soft tissues and cartilage were removed from the bones prior to assessment. Bone density was measured on each bone based on Archimedes principle (Williams et al., 2023). A dry bone weight was collected, then bones were submerged in ultra-purified water under a

negative pressure vacuum with 1.06 kg per cubic centimeter for a minimum of 4 h. Bones were then weighed while suspended in a vessel of ultra-purified water, and the weight was used to calculate bone density. For defatted bone ash, all bones were placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat (Wensley et al., 2020). Bones were dried at 105°C for 7 d in a drying oven and then ashed in a muffle furnace at 600°C for 24 h to determine total bone ash weight and percentage ash relative to dried bone weight.

Additionally, the left 10th rib was processed for histological examination. Briefly, the bones were fixed in 10% neutral buffered formalin for at least 24 h and decalcified using DeltaCAL (Delta Medical, Inc., Aurora, IL, USA) for at least 4 to 6 h. The costochondral junction and the body of the 10^{th} rib were embedded in paraffin blocks, sectioned at 4 μm thickness, and stained with hematoxylin and eosin. Microscopic examination was performed by a board-certified Anatomic Pathologist blinded to treatment structure. Bones were scored for failure of endochondral ossification of the physis and microscopic fractures (infractions). Histological grading of each bone consisted of physis grading, infraction and cortical fractures, fibrosis grading, and cortical metaphysis score. A full description of the scoring system is presented in Supplemental Table 1.

Diet chemical analysis

Complete diet samples were collected during the bagging of experimental diets and pooled into one homogenized sample per dietary treatment for each phase. Samples were stored at -20°C until they were submitted for analysis. Diet samples were submitted for triplicate Ca (AOAC 985.01, 2006) and P (AOAC 985.01, 2006) analysis at the Kansas State University Soils Testing Laboratory in Manhattan, KS. All diets were analyzed for vitamin D_3 and 25(OH)D_3 concentrations by dsm-firmenich Technical Marketing Analytical Services (Belvidere, NJ).

Statistical analysis

Growth performance, vitamin D status in blood, urine concentrations, and bone characteristic data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Pen was considered the experimental unit. Treatment was used as the fixed effect and weight block was used as the random intercept. Linear and quadratic contrasts were evaluated within increasing STTD P treatments as well as the main effect of 25(OH)D_3 and the interaction between 25(OH)D_3 and STTD P. For bone characteristics, treatment, bone, and the associated interactions were considered fixed effects, with weight block and pig serving as random intercepts. For histological grading analysis, data were analyzed as ordinal outcomes using a generalized linear mixed model using a multinomial distribution using a cumulative logit link function with weight block included as a random intercept. Data were summarized using the FREQ procedure of SAS and reported as a percentage of observations within each score category by treatment. This model was a good fit for physeal score and cortical metaphysis score. However, for infraction score and fibrosis score, model fit was not satisfactory due to all observations within a treatment scoring in a single category. Therefore, infraction score and fibrosis score are reported using descriptive statistics. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Analysis of complete diet Ca, P, vitamin D_3, and 25(OH)D_3 were all within reasonable analytical variation. Variation is often observed when performing analysis of complete diets for Ca and Vitamin D_3. The analyzed levels in the current experiment indicate successful diet manufacturing (Table 2.2).

Growth performance

No added 25(OH)D_3 × STTD P interactions were observed for any growth performance criteria (Table 2.3). Furthermore, added 25(OH)D_3 had no effect on growth performance during the 45-d study with the exception of decreased ($P = 0.036$) G:F from d 22 to 45. For phase 1 (d 0 to 10), increasing STTD P increased (linear, $P \leq 0.031$) BW, ADG, and G:F but did not influence ADFI. For phase 2 (d 10 to 22), increasing STTD P increased (quadratic, $P \leq 0.015$) BW, ADG, ADFI, and G:F up to 100% of the NRC (2012) STTD P requirement estimate with little benefit thereafter. For phase 3 (d 22 to 45), increasing STTD P increased (quadratic, $P \leq 0.022$) BW, ADG, ADFI, and G:F with the greatest response as STTD P increased from 70 to 100% of the NRC (2012) requirement estimate with a smaller improvement in ADG and G:F as STTD P increased from 100 to 130% of the NRC (2012) STTD P requirement estimate.

Overall (d 0 45), increasing STTD P increased (quadratic, $P \leq 0.003$) BW, ADG, ADFI, and G:F with minimal improvement as STTD P increased from 100 to 130% of the NRC (2012) STTD P requirement estimate. Increasing STTD P increased (quadratic, $P \leq 0.002$) Ca and STTD P intake (g/d). Added 25(OH)D_3 had no effect on Ca and P intake g/d.

A total of 3 pigs died during the 45-d growth trial (2 pigs fed 70% of NRC STTD P requirement and no 25(OH)D_3 and 1 pig fed 100% of NRC STTD P requirement with added 25(OH)D_3). A total of 8 pigs were removed during the trial (1 pig fed 100% of NRC STTD P requirement and no 25(OH)D_3, 3 pigs fed 130% of NRC STTD P requirement and no 25(OH)D_3, 1 pig fed 70% of NRC STTD P requirement with added 25(OH)D_3, 1 pig fed 100% of NRC STTD P requirement with added 25(OH)D_3, and 2 pigs fed 130% of NRC STTD P requirement with added 25(OH)D_3. There were no apparent correlation with dietary treatment and

removal/mortality, although this was not a primary response criteria due to the experimental sample size.

Blood and urine analysis

No added 25(OH)D_3 × STTD P interactions were observed for urinary concentrations (Table 2.4). Pigs fed increasing STTD P tended to have decreased urinary Ca excretion (quadratic, $P = 0.078$) and increased urinary P excretion (quadratic, $P < 0.001$), with all pigs fed 70% of the NRC (2012) STTD P requirement having non-detectable levels of P in the urine. For pigs fed 130% of the NRC (2012) STTD P requirement estimate, 8 out of 22 samples had non-detectable levels of Ca. Added 25(OH)D_3 had no effect on urine Ca and P concentrations.

For serum 25(OH)D_3, a quadratic 25(OH)D_3 × STTD P interaction was observed ($P = 0.032$). When pigs were fed added 25(OH)D_3, serum 25(OH)D_3 increased as STTD P increased (quadratic, $P = 0.005$). However, when no 25(OH)D_3 was added, no differences were observed for 25(OH)D_3 concentrations. A quadratic 25(OH)D_3 × STTD P interaction ($P = 0.002$) was also observed for 1,25(OH)$_2$$D_3$ concentrations. When pigs were fed added 25(OH)D_3, serum 1,25(OH)$_2$$D_3$ increased (quadratic, $P < 0.001$) as STTD P decreased. However, when no 25(OH)D_3 was added, no differences were observed for 1,25(OH)$_2$$D_3$ concentrations ($P > 0.10$). No added 25(OH)D_3 × STTD P interaction was observed for 24,25(OH)$_2$$D_3$. Pigs fed diets with added 25(OH)D_3 had increased ($P < 0.001$) 24,25(OH)$_2$$D_3$ concentrations.

Bone characteristics

For bone density, a marginally significant linear 25(OH)D_3 × STTD P × bone interaction was observed ($P = 0.058$; Table 2.5). For fibulas, bone density increased (quadratic, $P = 0.0001$) when STTD P increased to 100% of NRC with no further increase thereafter when 25(OH)D3 was added, whereas bone density continued to increase (linear, $P < 0.0001$) as STTD P increased

to 130% of NRC when no 25(OH)D3 was added. For other bones, the response to increasing STTD P was similar whether 25(OH)D3 was added or not. When evaluating the effect of STTD P within bone, increasing STTD P increased bone density for metacarpals (linear, $P = 0.001$) as well as for 2nd and 10th ribs (quadratic, $P \leq 0.055$). A main effect of bone was observed with fibulas having the greatest bone density followed by 10th and 2nd ribs with metacarpals having the lowest bone density ($P < 0.001$).

No added $25(OH)D_3$ × STTD P × bone interactions were observed for dry bone weight. For all bones, bone ash weight increased (linear, $P < 0.001$) as STTD P increased with pigs fed 130% of the NRC (2012) STTD P requirement estimate having the greatest dry bone weight for all bones. Added $25(OH)D_3$ had no effect on dry bone weight for any of the bones. A main effect of bone was observed with metacarpals having the heaviest dry bone weight followed by 10th ribs, fibulas, and 2nd ribs having the lightest dry bone weight ($P < 0.001$).

A marginally significant $25(OH)D_3$ × STTD P interaction (linear, $P = 0.098$; Figure 2.1) was observed for percentage bone ash, where percentage bone ash increased numerically in diets with added $25(OH)D_3$ when pigs were fed diets at 70 or 100% of the NRC (2012) STTD P requirement estimate, but not when added to diets at 130% of NRC (2012) STTD P requirement estimate. Additionally, percentage bone ash increased (quadratic, $P < 0.001$) as STTD P increased with pigs fed 130% of the NRC (2012) STTD P requirement estimate having the greatest bone ash for all bones. A main effect of bone was observed with fibulas, metacarpals, and 2nd ribs having greater percentage bone ash compared to 10th ribs ($P < 0.001$).

No added $25(OH)D_3$ × STTD P × bone interactions were observed for bone ash weight. For all bones, bone ash weight increased (linear, $P < 0.001$) as STTD P increased with pigs fed 130% of the NRC (2012) STTD P requirement estimate having the greatest bone ash weight for

all bones. Added 25(OH)D_3 had no effect on bone ash weight for any of the bones. A main effect of bone was observed with metacarpals having the heaviest bone ash weight followed by 10th ribs, fibulas, and 2nd ribs having the lightest bone ash weight ($P < 0.001$).

For physeal score and cortical metaphysis score, no added 25(OH)D_3 × STTD P interactions were observed (Table 2.6; Figure 2.2). For physeal score, there was a greater probability of having a higher score (indicating abnormal bone architecture) as the level of STTD P decreased (quadratic, $P = 0.002$), with all bones sampled from pigs fed 70% of the NRC (2012) STTD P requirement estimate having moderate or extensive histologic abnormalities (scores 2 and 3). Added 25(OH)D_3 had no effect on physeal score or cortical metaphysis score.

For cortical metaphysis, there was a higher frequency of a score of 2 (moderate loss of cortical trabeculae with resorption, remodeling, or fibrosis) for pigs fed 70% of the NRC (2012) STTD P requirement estimate. As STTD P increased from 70 to 130% of the NRC (2012) STTD P requirement estimate, a linear reduction ($P < 0.001$) in the probability of having a more abnormal histologic score was observed indicating more normal cortical metaphyseal structure histologically.

Statistical analysis was not performed for infraction and fibrosis scores due to poor statistical model fit caused by all observations within a treatment scoring in a single category. For infraction score, there was a higher frequency of a score of 3 (cortical fractions) for pigs fed 70% of the NRC (2012) STTD P requirement estimate. As STTD P increased from 70 to 130% of the NRC (2012) STTD P requirement estimate, a reduction in the frequency of a score 3 was observed with more pigs having a score of 0 (no evidence of infractions).

For fibrosis score, there was a higher frequency of a score of 1 (fibrosis in medullary bone) for pigs fed 70% of the NRC (2012) STTD P requirement estimate. As STTD P increased

from 70 to 130% of the NRC (2012) STTD P requirement estimate, a reduction in the frequency of a score 1 was observed with almost all pigs having a score of 0 (no evidence of fibrosis).

Discussion

Calcium and P are tightly regulated in the body to maintain homeostasis which is a complicated interaction of multiple pathophysiologic factors. The main hormones involved in Ca and P regulation are parathyroid hormone, $1,25(OH)_2D_3$, and calcitonin (Crenshaw, 2001). The secretion of these hormones is triggered by Ca and P levels in the blood and the relationship between them allows for the maintenance of homeostatic concentrations of Ca and P in circulation. Dietary vitamin D_3 must undergo a hydroxylation step in the liver to be converted to $25(OH)D_3$ facilitated by the enzyme 25-hydroxylase (Hewison et al., 2000). Once converted to $25(OH)D_3$ it enters circulation or is stored and can be further hydroxylated in the kidney to the bioactive form, $1,25(OH)_2D_3$, when needed and facilitated by the enzyme 1α-hydroxylase (Hewison et al., 2000). The $25(OH)D_3$ metabolite can also be converted to $24,25(OH)_2D_3$ which is important for bone mineralization and plays a role in regulating the amount of $1,25(OH)_2D_3$ produced (Boyan et al., 2001). The regulation of $1,25(OH)_2D_3$ is important because if too much of this metabolite is produced, toxicity may occur (Boyan et al., 2001; Bikle, 2014). By feeding $25(OH)D_3$, it can bypass the hydroxylation step in the liver and be directly available to the kidney to be hydroxylated to the bioactive form of vitamin D_3 and be utilized by the pig.

All diets in the current study contained 1,653 IU/kg of vitamin D_3 provided from the vitamin premix. Faccin et al. (2023) surveyed nutritionists in commercial production and found a wide range of vitamin D_3 levels in nursery diets from 1,389 to 10,494 IU/kg with a weighted average of 2,397 IU/kg. By providing 1,653 IU/kg of vitamin D_3, our diets were within the 25th percentile according to the survey (Faccin et al., 2023) and were 7.5 to 8.3 times greater than the

NRC (2012) requirement estimate depending on the weight range being referenced. However, the NRC (2012) requirement is likely not representative of pigs raised indoor in the swine industry today and used in the current experiment. Pigs raised outdoor have significantly greater serum 25(OH)D_3 concentrations compared to pigs raised indoor (Arnold et al., 2015). Therefore, it is recommended to feed vitamin D_3 that are much greater than recommended by the NRC (2012).

Previous research has indicated that serum 25(OH)D_3 concentrations are not always maintained above recommendations (Lauridsen, 2014) when supplementing with vitamin D_3 alone in the feed even when fed at levels well above the NRC (2012) requirements (Flohr et al., 2014). The primary metabolite used to assess an animal's vitamin D status is 25(OH)D_3 (Flohr et al., 2014). The supplementation of 25(OH)D_3 allows pigs to elevate their serum 25(OH)D_3 concentrations compared to unsupplemented levels (Zhang et al., 2021; Williams et al., 2023). In the current study, serum 25(OH)D_3 concentrations were deficient when 25(OH)D_3 was not added to the diets because concentrations were below the threshold of 10 to 15 ng/mL based on Lauridsen (2014), which is commonly used as the reference value and adapted from humans. Arnold et al. (2015) reported reference values for serum 25(OH)D_3 concentrations between 18 and 30 ng/mL in 2- to 4-week-old pigs which further supports our pigs being deficient in circulating 25(OH)D_3 concentrations. This deficiency is likely due to pigs being raised indoor and having less exposure to sunlight. When the skin is exposed to sunlight, 7-dehydrocholesterol is converted to vitamin D_3 (Crenshaw, 2001). Animals raised indoor require supplementary vitamin D to increase circulating 25(OH)D_3 concentrations and prevent diseases such as rickets and osteomalacia (Arnold et al., 2015). When 25(OH)D_3 was added to the diets, serum 25(OH)D_3 concentrations were no longer considered deficient and were comparable to other

researchers where 25(OH)D_3 was supplemented on top of basal vitamin D_3 levels (Zhang et al., 2021; Zhao et al., 2022; Williams et al., 2023).

The supplementation of 25(OH)D_3 also increased circulating concentrations of 24,25$(OH)_2D_3$. When circulating levels of 25(OH)D_3 increase, it signals to the body that there is an excess of vitamin D_3 available. In response, the enzyme 24-hydroxylase becomes activated and converts 25(OH)D_3 into 24,25$(OH)_2D_3$ as a regulatory mechanism to maintain balance and protect against vitamin D_3 toxicity (St-Arnaud and Glorieux, 1998; Tang et al., 2019). The vitamin D_3 metabolite, 24,25$(OH)_2D_3$, has not been as extensively researched compared to other metabolites; however, it plays a role in bone mineralization (Boyan et al., 2001). To our knowledge, there are no reference values for 24,25$(OH)_2D_3$ serum concentrations in swine. However, Thayer et al. (2019) measured serum concentrations of 24,25$(OH)_2D_3$ at weaning and observed a concentration of 2.4 ng/mL when fed diets with 1,500 IU of vitamin D_3 and 50 µg of 25(OH)D_3. In our experiment, serum samples were collected 45 d postweaning with concentrations of 9.5 ng/mL of 24,25$(OH)_2D_3$ when 50 µg/kg of 25(OH)D_3 was supplemented. Further research is needed to establish reference values for serum 24,25$(OH)_2D_3$ concentrations in pigs. However, concentrations of 24,25$(OH)_2D_3$ is normally higher when 25(OH)D_3 is increased (Wagner et al., 2011).

In the current experiment, pigs fed P-deficient diets had the highest levels of 1,25$(OH)_2D_3$ in serum. When diets are low in P, endocrine levels such as parathyroid hormone and 1,25$(OH)_2D_3$ are impacted. Parathyroid hormone regulates the activation of 1,25$(OH)_2D_3$ through the enzyme 1α-hydroxylase (Lütke-Dörhoff et al., 2022) indicating that feeding a P-deficient diet increases the activation of 1α-hydroxylase causing 25(OH)D_3 to be converted to 1,25$(OH)_2D_3$. The activation of 1,25$(OH)_2D_3$ increases the intestinal P absorption, renal

reabsorption of P by stimulating the proximal convoluted tubules in the kidneys to decrease urinary P excretion, and releases P from bone tissue into circulation as a means to increase P in the body. Similar results were observed in other research where serum concentrations of $1,25(OH)_2D_3$ increased in pigs fed low P diets (Oster et al., 2018; Williams et al., 2023).

In the current study, the supplementation of $25(OH)D_3$ did not improve growth performance. However, Zhang et al. (2021) and Zhao et al. (2022) observed an increase in ADG with the supplementation of $25(OH)D_3$ to diets deficient in Ca and P with 2,500 or 2,000 IU/kg of vitamin D_3, respectively. This may have been due to our diets being more deficient in Ca and P compared to their diets (30% vs 22 to 23% reduction in Ca and P compared to requirement). Conversely, Williams et al. (2023) found similar results to the current experiment with no improvement in growth performance when $25(OH)D_3$ was supplemented to diets containing 1,653 IU/kg of vitamin D_3 and excess P.

When P-deficient diets were supplemented with $25(OH)D_3$, the negative effect on bone ash was reduced compared to P-deficient diets without $25(OH)D_3$ supplementation which supports our hypothesis. In the current experiment, an increase in percentage bone ash for the 2nd rib was observed when pigs were fed P-deficient diets (70% of the NRC (2012) STTD P requirement estimate) and supplemented with $25(OH)D_3$ compared to no supplementation (52.1 vs 50.2% bone ash). Reference values used by diagnosticians for bone mineralization range from 58 to 62% for defatted-bone ash percentage for a rib (Puls, 1994). In the current study, the supplementation of $25(OH)D_3$ to a diet formulated to 100% of the NRC (2012) requirement estimate was necessary to reach 58% bone ash for 2nd ribs compared to no supplementation (58.5 vs 57.1% bone ash). Additionally, for percentage bone ash data as an average of all 4 bones, an increase in percentage bone ash (51 to 52%) occurred when $25(OH)D_3$ was

supplemented to P-deficient diets. A similar increase in percentage bone ash (averaged across all 4 bones) was also observed when 25(OH)D_3 was supplemented to a P-adequate diet (100% of NRC (2012) STTD P requirement estimate) compared to no 25(OH)D_3 supplementation. However, percentage bone ash (averaged across all 4 bones) was not increased when 25(OH)D_3 was supplemented to a diet with excess P (130% of NRC (2012) STTD P requirement estimate). These results indicate that supplementing P-deficient diets with 25(OH)D_3 allows pigs to efficiently utilize P and deposit it into bones supporting our hypothesis that 25(OH)D_3 supplementation would increase bone development when pigs are fed diets deficient or marginally deficient in P. Zhao et al. (2022) also observed an increase in percentage bone ash when supplementing 25(OH)D_3 to Ca- and P-deficient diets. However, their increase was only observed when 25(OH)D_3 was supplemented alongside phytase. In the current study, no phytase was added to the experimental diets.

In the current experiment, three different levels of STTD P were fed. The first was a P-deficient diet which was 70% of the NRC (2012) STTD P requirement estimate. The next P level was at the NRC (2012) requirement estimate and the last level in the experiment was at 130% of the NRC (2012) requirement estimate. The highest level was based on recent findings by Vier et al. (2019a; 2019b) and Williams et al. (2023) in order to observe differences in bone mineralization among treatments. Additionally, the P required to maximize bone mineralization is greater than the P required to maximize growth performance (Vier et al., 2019a; 2019b). According to the NRC (2012), the total Ca and STTD P requirements are 2.3 and 1.2, 3.8 and 1.9, and 6.3 and 3.0 g/d for 5 to 7, 7 to 11, and 11 to 25 kg BW pigs, respectively. Thus, the average NRC (2012) total Ca and STTD P requirements for 5 to 25 kg BW pigs is 4.1 and 2.0 g/d, respectively. Pigs fed 100% of the NRC (2012) STTD P requirement estimate in the current

study consumed slightly more total Ca and STTD P (4.6 and 2.5 g/d, respectively) compared to the NRC (2012) requirement, but still weren't able to maximize bone mineralization. Thus, our research continues to show that the P requirement to maximize bone mineralization is greater than the P required to maximize growth and is greater than the requirement estimate reported by NRC (2012).

Results from the current experiment demonstrated that growth performance was reduced for pigs fed diets at 70% of estimated P requirement compared to pigs fed 100 or 130% of the P requirement. Pigs fed P-deficient diets had a 19% reduction in ADG and a 9% reduction in G:F compared to pigs fed adequate or increased levels of STTD P (100% or 130% of NRC (2012) STTD P requirement estimate). Additionally, pigs fed P-deficient diets had 53% reduced bone ash weight and 14% reduced percentage bone ash compared to pigs fed increased STTD P levels (130% of NRC (2012) STTD P requirement estimate). These results are similar to Lee et al. (2021) and Williams et al. (2023) indicating that a greater proportion of dietary Ca and P is used for growth and soft tissues and a lower proportion for bone tissue synthesis when STTD P is deficient in the diet.

Our experimental diets were fed immediately after weaning and fed for 45 days allowing differences in bone mineralization and growth observations due to the duration of feeding P-deficient diets. Because the experimental diets were fed starting at weaning, the pigs did not have the opportunity to build P reserves. Thus, the reduction in BW, ADG, and G:F began during the first ten days of the experiment for pigs fed 70% compared to 130% of the NRC (2012) STTD P requirement estimate. The reduction in performance continued throughout the experiment and became more detrimental between d 22 and 45 of the experiment because the pigs were continually fed the P-deficient diet.

Percentage bone ash is typically used to measure bone mineralization (Wu et al., 2019; Viet et al., 2019a, 2019b; Lee et al., 2021). However, other analyses can be used to characterize bone composition including bone density and histopathology to help support a diagnosis of metabolic bone disease in pigs (Williams et al., 2023). Increasing STTD P increased bone density for all four bones measured in the current study. Based on the findings of Williams et al. (2023), tenth ribs were sampled for histopathology because they are more sensitive compared to other ribs and show lesions of failure of endochondral ossification and infraction in pigs fed P-deficient or vitamin D-deficient diets. In the current experiment, reducing STTD P from 130 to 70% of the NRC (2012) STTD P requirement increased the likelihood of having abnormal histological findings. Williams et al. (2023) observed a similar response for physeal score indicating that P-deficient diets result in more lesions and abnormal rib bones compared to diets adequate or excess P. When pigs were fed 100% of the NRC (2012) STTD P requirement, we did observe some bone abnormalities. These abnormalities were also observed when pigs were fed 130% of the NRC (2012) STTD P requirement indicating that bone abnormalities still occur even when pigs are fed diets formulated above the requirement. Therefore, when bone abnormalities occur, it doesn't necessarily mean that a P deficiency is causing the observed pathologic changes in bone structure. Other factors play a role in bone abnormalities including health challenges and pre-weaning growth. The supplementation of $25(OH)D_3$ to P-deficient diets appeared to lessen the likelihood of having abnormalities as observed by a numerically lower frequency of severe abnormalities compared to no supplementation of $25(OH)D_3$ (58 vs 75%).

Dietary changes in Ca and P levels are more sensitive to detect in the urine compared to the serum (Hagemoser et al., 2000). Recent research has shown that urine Ca and P can be used

to monitor dietary excesses or deficiencies of these minerals (Grez-Capdeville and Crenshaw, 2021; 2022). When pigs were fed P-deficient diets in the current study, they excreted non-detectable levels of P in the urine. This occurs because when pigs have low serum P concentrations caused by P-deficient diets, the kidney decreases the amount of P excreted and intestinal P absorption increases. At the same time, the pigs are tightly regulating Ca and P homeostasis resulting in increased Ca excretion. The opposite occurs when pigs were fed 130% of the NRC (2012) STTD P requirement estimates where pigs excreted between 77 and 85 mg/dL of P in the urine and very little Ca. Again, this occurs because the body is trying to maintain homeostasis and deposits the Ca and P into bone tissue when high levels are provided in the diet. This response was observed regardless of 25(OH)D_3 supplementation. A similar relationship between urinary Ca and P excretions in response to dietary levels of P has been observed in sows and grow-finish pigs (Hagemoser et al., 2000; Gutierrez et al., 2015; Grez-Capdeville and Crenshaw, 2022).

In summary, increasing STTD P increased growth to 100% of NRC (2012) requirement estimate and bone ash to 130% of NRC requirements. These results further reinforce that the STTD P requirement for bone mineralization is greater than for growth performance. Additionally, added 25(OH)D_3 had limited effect on growth performance, urine Ca and P, or bone characteristics; however, added 25(OH)D_3 increased serum concentrations of 25(OH)D_3 and 24,25$(OH)_2D_3$. The addition of 25(OH)D_3 to diets deficient in P for bone mineralization (70 or 100% of the NRC (2012) STTD P requirement estimate) increased percentage bone ash.

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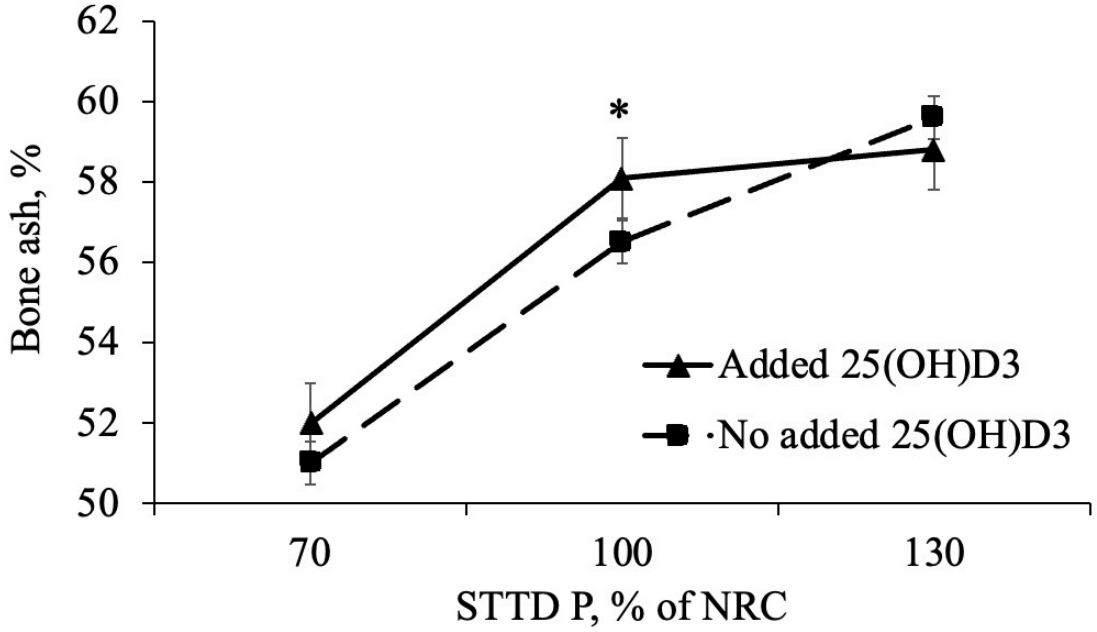

Figure 2.1. Effects of added 25(OH)D_3 and STTD P on percentage de-fatted bone ash [25(OH)D_3 × STTD P, linear, $P = 0.098$]. Values reported are the interactive means of 25(OH)D_3 × STTD P averaged across all 4 bones (fibula, 2nd and 10th ribs, and metacarpals). Added 25(OH)D_3 (Rovimix Hy-D; dsm-firmenich, Plainsboro, NJ) at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3. *, effect of 25(OH)D_3 within STTD P level, $P < 0.05$. Error bars represent ± 1 standard error of the mean.

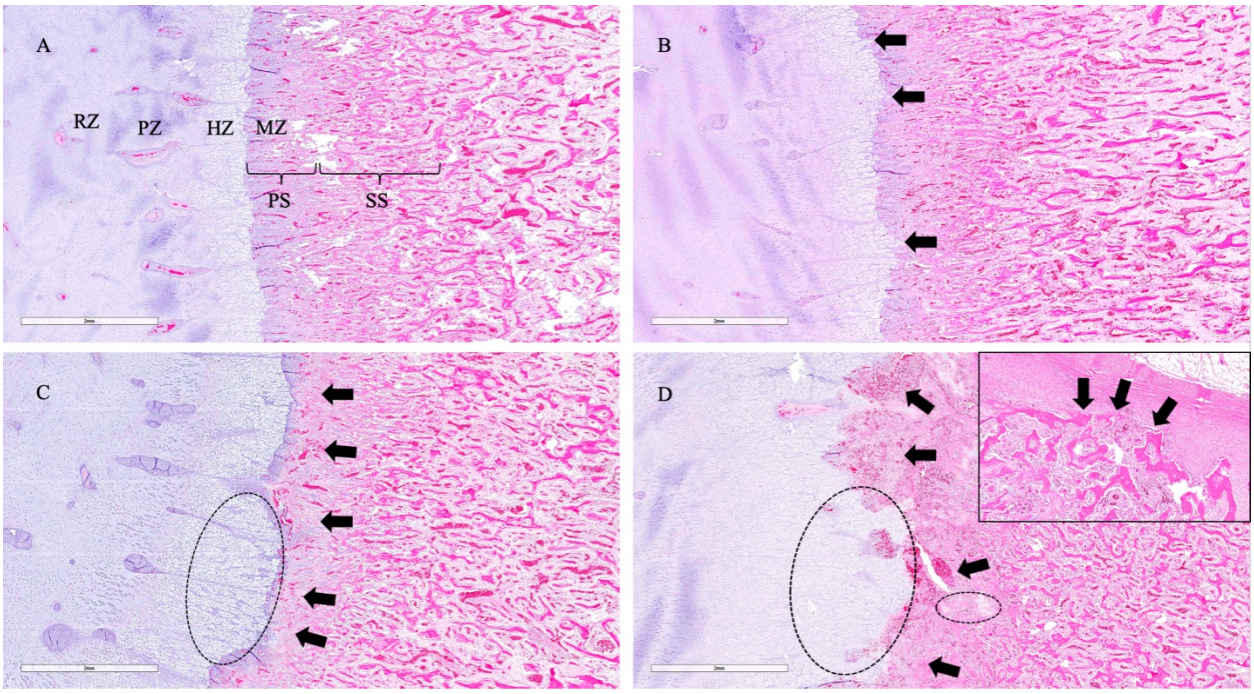

Figure 2.2. Representative growth plate sections (10th rib) from different animals. A) grade 0 with a resting zone (RZ), proliferating zone (PZ), and hypertrophic zone (HZ) of chondrocytes. Note the uniform mineralization zone (MZ), and primary and secondary spongiosa (PS, SS) in an unaffected pig. B) Small, multifocal tongues or islands of retained chondrocytes that extend into the primary spongiosa (arrows). C) Moderately sized, broad-based tongues of retained chondrocytes extend into the primary and secondary spongiosa (dashed oval). Infraction lines (arrows) covering less than 50% of the provided section were observed. D) Large, extensive, broad-based tongues of retained chondrocytes extend into the secondary spongiosia (dashed ovals) with large, extensive infraction lines (arrows) and evidence of cortical fracture (inset with arrows).

Table 2.1. Diet composition of 70% STTD P treatments (as-fed basis)[1]

	Phase		
Ingredient, %	1[2]	2[3]	3[4]
Corn	41.05	54.72	64.43
Soybean meal, 47.7% CP	22.59	27.10	31.36
Whey powder	25.00	10.00	---
Enzymatically treated soybean meal[5]	5.00	2.50	---
Soybean oil	2.00	1.00	---
Calcium carbonate	0.49	0.58	0.64
Monocalcium P, 21% P	0.14	0.32	0.35
Salt	0.34	0.55	0.60
L-Lys-HCl	0.40	0.48	0.45
DL-Met	0.22	0.22	0.20
L-Thr	0.19	0.23	0.22
L-Trp	0.03	0.04	0.03
L-Val	0.14	0.17	0.13
Vitamin and trace mineral premixes[6]	0.40	0.40	0.40
Zinc oxide	0.41	0.27	---
25(OH)D_3[7]	+/-	+/-	+/-
Sand[8]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.30
Ile:Lys	59	57	57
Leu:Lys	113	113	118
Met:Lys	36	37	37
Met and Cys:Lys	58	58	58
Thr:Lys	65	65	65
Trp:Lys	19.4	19.6	19.1
Val:Lys	72	72	72
Total Lys, %	1.48	1.49	1.44
ME, kcal/kg	3,388	3,320	3,267
NE, kcal/kg	2,544	2,471	2,411
SID Lys:ME, g/Mcal	5.31	5.46	5.39
CP, %	20.6	20.9	21.1
Ca, %	0.56	0.54	0.51
STTD P, %	0.32	0.28	0.23
Total P, %	0.51	0.49	0.47
Total Ca:Total P	1.10	1.10	1.10

[1]Dietary treatments were arranged in a 2 × 3 factorial with main effects of 25(OH)D_3 and standardized total tract digestibility (STTD) P (70, 100, or 130% of NRC (2012) requirement estimates on a dietary percentage basis). Diet composition is reported for the 70% STTD P treatment diets.

[2]Phase 1 diets were fed from approximately d 0 to 10 (6 to 8 kg). Calcium carbonate increased to 0.59 and 0.69% for 100 and 130% STTD P treatments, respectively. Monocalcium P increased to 0.82 and 1.56% for 100 and 130% STTD P treatments, respectively.

[3]Phase 2 diets were fed from approximately d 10 to 22 (8 to 12 kg). Calcium carbonate increased to 0.67 and 0.76% for 100 and 130% STTD P treatments, respectively. Monocalcium P increased to 0.95 and 1.58% for 100 and 130% STTD P treatments, respectively.

[4]Phase 3 diets were fed from approximately d 22 to 45 (12 to 26 kg). Calcium carbonate increased to 0.72 and 0.79% for 100 and 130% STTD P treatments, respectively. Monocalcium P increased to 0.90 and 1.40% for 100 and 130% STTD P treatments, respectively.

[5]HP 300; Hamlet Protein; Findlay, OH.

[6]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin. Provided per kg of diet: 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30mg I from calcium iodate; 0.30 mg Se from sodium selenite. No phytase was added in any treatment diets.

[7]Rovimix Hy-D (dsm-firmenich, Parsippany, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[8]Sand was used to equalize hand-add batch including the addition of calcium carbonate and monocalcium P.

Table 2.2. Diet analysis (as-fed basis)[1,2]

Added 25(OH)D_3[3]	No			Yes		
STTD P, % of NRC	70	100	130	70	100	130
Phase 1						
Calculated analysis						
Ca, %	0.56	0.72	0.89	0.56	0.72	0.89
P, %	0.51	0.65	0.81	0.51	0.65	0.81
Vitamin D_3, IU/kg	1,653	1,653	1,653	1,653	1,653	1,653
25(OH)D_3, µg/kg	0	0	0	50	50	50
Analyzed composition						
Ca, %	0.61	0.65	0.87	0.50	0.75	0.86
P, %	0.50	0.60	0.78	0.48	0.68	0.82
Vitamin D_3, IU/kg	1,224	1,750	1,295	1,789	1,074	1,993
25(OH)D_3, µg/kg	NMA	NMA	NMA	41	48	49
Phase 2						
Calculated analysis						
Ca, %	0.54	0.69	0.84	0.54	0.69	0.84
P, %	0.49	0.63	0.76	0.49	0.63	0.76
Vitamin D_3, IU/kg	1,653	1,653	1,653	1,653	1,653	1,653
25(OH)D_3, µg/kg	0	0	0	50	50	50
Analyzed composition						
Ca, %	0.53	0.66	0.83	0.50	0.64	0.83
P, %	0.47	0.65	0.76	0.52	0.67	0.80
Vitamin D_3, IU/kg	1,317	1,685	1,313	1,703	1,325	1,535
25(OH)D_3, µg/kg	NMA	NMA	NMA	38	41	48
Phase 3						
Calculated analysis						
Ca, %	0.51	0.64	0.76	0.51	0.64	0.76
P, %	0.46	0.58	0.69	0.46	0.58	0.69
Vitamin D_3, IU/kg	1,653	1,653	1,653	1,653	1,653	1,653
25(OH)D_3, µg/kg	0	0	0	50	50	50
Analyzed composition						
Ca, %	0.64	0.63	0.70	0.46	0.65	0.83
P, %	0.45	0.54	0.68	0.46	0.58	0.67
Vitamin D_3, IU/kg	1,310	1,374	1,559	2,661	1,834	2,116
25(OH)D_3, µg/kg	NMA	NMA	NMA	45	51	45

[1]Complete diet samples were collected during bagging of experimental diets and pooled into one homogenized sample per dietary treatment for each phase. Samples were stored at -20°C until they were submitted for analysis of Ca and P in triplicate (K-State Research and

Extension Soil Testing Laboratory, Manhattan, KS). Samples were analyzed at dsm-firmenich Technical Marketing Analytical Services (Belvidere, NJ) for vitamin D_3 and 25(OH)D_3.

[2]NMA = no measurable amount.

[3]Rovimix Hy-D (dsm-firmenich, Parsippany, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

Table 2.3. Effects of added 25(OH)D_3 and STTD P on nursery pig performance[1]

	Added 25(OH)D_3[2]							P =[3]		
	No			Yes					STTD P	
STTD P, % of NRC[4]	70%	100%	130%	70%	100%	130%	SEM	25(OH)D_3	Linear	Quadratic
BW, kg										
d 0	5.8	5.8	5.8	5.8	5.8	5.8	0.54	0.984	0.872	0.710
d 10	7.4	7.5	7.6	7.5	7.6	7.6	0.59	0.232	0.029	0.671
d 22	11.7	12.4	12.0	11.7	12.6	12.1	0.83	0.471	0.051	< 0.001
d 45	23.2	27.8	27.6	23.4	27.3	27.6	1.38	0.682	< 0.001	< 0.001
d 0 to 10 (phase 1)										
ADG, g	156	170	185	172	183	183	9.7	0.233	0.031	0.707
ADFI, g	192	190	199	200	202	198	11.3	0.413	0.803	0.930
G:F, g/kg	813	897	932	857	904	928	21.7	0.324	< 0.001	0.297
d 10 to 22 (phase 2)										
ADG, g	356	403	362	350	405	371	22.7	0.841	0.300	0.001
ADFI, g	497	523	487	499	524	487	28.8	0.952	0.452	0.015
G:F, g/kg	716	768	741	702	774	761	13.1	0.732	0.002	0.001
d 22 to 45 (phase 3)										
ADG, g	501	668	662	505	639	661	28.6	0.455	< 0.001	< 0.001
ADFI, g	832	1022	994	869	1000	1011	53.2	0.522	< 0.001	< 0.001
G:F, g/kg	603	654	669	580	639	656	11.2	0.036	< 0.001	0.022
d 0 to 45										
ADG, g	383	485	473	389	472	477	20.9	0.879	< 0.001	< 0.001
ADFI, g	597	702	676	620	691	690	36.0	0.422	< 0.001	< 0.001
G:F, g/kg	644	692	701	627	684	694	9.5	0.111	< 0.001	0.003
Ca intake, g/d	3.10	4.59	5.30	3.22	4.53	5.41	0.247	0.517	< 0.001	0.001
P intake, g/d	1.47	2.48	3.09	1.53	2.45	3.15	0.136	0.566	< 0.001	0.002

[1]A total of 360 pigs (initially 5.8 ± 0.54 kg) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P.

[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[3]No added 25(OH)D_3 × STTD P interactions were observed ($P > 0.10$) unless otherwise noted.

[4]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC (2012) requirement estimate treatment diet in phases 1, 2, and 3 respectively.

Table 2.4. Effects of added 25(OH)D_3 and STTD P on nursery pig urine and blood characteristics[1]

	Added 25(OH)D_3[2]							P =[3]		
	No			Yes					STTD P	
STTD P, % of NRC[4]	70%	100%	130%	70%	100%	130%	SEM	25(OH)D_3	Linear	Quadratic
Urine										
Calcium										
Count of samples > LOD[5]	10	11	7	12	12	7	---	---	---	---
Concentration, mg/dL	103.9	37.7	7.4	126.0	47.9	12.0	13.00	0.242	< 0.001	0.078
Phosphorus										
Count of samples > LOD[6]	0	5	10	0	2	11	---	---	---	---
Concentration, mg/dL	< 5.5	8.0	76.6	< 5.5	6.1	84.9	9.18	0.770	< 0.001	< 0.001
Serum										
25(OH)D_3, ng/mL[7]	7.2	7.2	8.2	18.7	29.2	25.0	2.07	< 0.001	0.082	0.057
24,25$(OH)_2D_3$, ng/mL	1.6	1.3	2.0	9.8	10.0	8.6	0.52	< 0.001	0.455	0.777
1,25$(OH)_2D_3$, pg/mL[8]	339	257	181	394	203	180	14.8	0.986	< 0.001	0.001

[1]A total of 360 pigs (initially 5.8 ± 0.54 kg) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P. Urine samples were collected from 1 pig per pen (72 pigs total) on d 45. Serum vitamin D analysis was conducted at Heartland Assays (Ames, IA).

[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[3]No added 25(OH)D_3 × STTD P interactions were observed ($P > 0.10$) unless otherwise noted.

[4]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC (2012) requirement estimate treatment diet in phases 1, 2, and 3 respectively.

[5]The limit of detection (LOD) was 1 mg/dL. When the concentration was below the LOD, a value of 1 mg/dL was used.

[6]The LOD was 5.5 mg/dL. When the concentration was below the LOD, a value of 5.5 mg/dL was used.

[7]Quadratic 25(OH)D_3 × STTD P interaction, $P = 0.032$. Quadratic STTD P within added 25(OH)D_3, $P = 0.005$. Quadratic STTD P within no 25(OH)D_3, $P = 0.860$.

[8]Quadratic 25(OH)D_3 × STTD P interaction, $P = 0.002$. Quadratic STTD P within added 25(OH)D_3, $P < 0.001$. Quadratic STTD P within no 25(OH)D_3, $P = 0.877$. Linear STTD P within added 25(OH)D_3 and no 25(OH)D_3, $P < 0.001$.

Table 2.5. Effects of added 25(OH)D_3 and STTD P on nursery pig bone characteristics[1]

	Added 25(OH)D_3[2]						*P* =		
	No			Yes				STTD P	
STTD P, % of NRC[3]	70%	100%	130%	70%	100%	130%	25(OH)D_3	Linear	Quadratic
Bone density, g/mL[4]									
Fibula	1.21	1.32	1.35	1.23	1.33	1.29	0.656	< 0.001	< 0.001
2nd rib	1.13	1.20	1.25	1.11	1.23	1.25	0.789	< 0.001	0.055
10th rib	1.15	1.22	1.27	1.14	1.24	1.27	0.944	< 0.001	0.039
Metacarpal	1.13	1.17	1.19	1.13	1.17	1.19	0.918	0.001	0.290
Dry bone, g[5]									
Fibula	1.20	1.97	2.20	1.18	1.91	2.22	0.777	< 0.001	0.009
2nd rib	0.82	1.41	1.75	0.72	1.39	1.64	0.388	< 0.001	0.064
10th rib	1.48	2.79	3.13	1.52	2.65	3.11	0.616	< 0.001	< 0.001
Metacarpal	2.14	2.91	3.09	2.02	2.81	3.14	0.506	< 0.001	0.004
Bone ash, %[6]									
Fibula	52.6	56.9	59.9	53.2	58.8	59.3	0.250	< 0.001	0.006
2nd rib	50.2	57.1	60.3	52.1	58.5	59.6	0.103	< 0.001	0.001
10th rib	49.2	54.9	58.6	49.1	56.8	57.6	0.620	< 0.001	0.001
Metacarpal	51.9	57.2	59.5	53.4	58.2	58.6	0.337	< 0.001	0.001
Bone ash, g[7]									
Fibula	0.63	1.12	1.32	0.63	1.12	1.32	0.931	< 0.001	0.009
2nd rib	0.41	0.81	1.06	0.37	0.82	0.98	0.501	< 0.001	0.065
10th rib	0.73	1.54	1.84	0.75	1.51	1.80	0.715	< 0.001	< 0.001
Metacarpal	1.11	1.66	1.84	1.08	1.64	1.84	0.720	< 0.001	0.001

[1]A total of 360 pigs (initially 5.8 ± 0.54 kg) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P. Per treatment, 12 pigs were euthanized (72 pigs total) and the right metacarpal, fibula, 2nd rib, and 10th ribs were collected to determine bone density, bone ash weight, and percentage bone ash utilizing the de-fatted processing method. Bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were dried at 105°C for 7 d in a drying oven and then ashed in a muffle furnace at 600°C for 24 h.

[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[3]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC (2012) requirement estimate treatment diet in phases 1, 2, and 3 respectively.

[4]Bone density was measured on each bone based on the Archimedes principle. Linear added 25(OH)D_3 × STTD P × bone interaction, P = 0.058. Added 25(OH)D_3 effect for all bones, P = 0.938. Linear STTD P effect across all bones, P < 0.001. Quadratic STTD P effect across all bones, P = 0.001. Main effect of bone, P < 0.001. SEM = 0.016. For the interactive means of 25(OH)D_3 × STTD P averaged across all 4 bones (fibula, 2nd and 10th ribs, and metacarpals), linear and quadratic STTD P, P < 0.001. Added 25(OH)D_3 effect for the interactive means, P = 0.938.

[5]Dry bone weight was measured for each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, P > 0.10. Added 25(OH)D_3 effect for all bones, P = 0.494. Linear and quadratic STTD P effect across all bones, $P \leq 0.001$. Main effect of bone, P < 0.001. SEM = 0.036. For the interactive means of 25(OH)D_3 × STTD P averaged across all 4 bones (fibula, 2nd and 10th ribs, and metacarpals), linear and quadratic STTD P, $P \leq 0.001$. Added 25(OH)D_3 effect for the interactive means, P = 0.494.

[6]Bone ash percentage was measured on each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, P > 0.10. Added 25(OH)D_3 effect for all bones, P = 0.183. Linear and quadratic STTD P effect for all bones, P < 0.001. Main effect of bone, P < 0.001. SEM = 0.67. For the interactive means of 25(OH)D_3 × STTD P averaged across all 4 bones (fibula, 2nd and 10th ribs, and metacarpals), linear added 25(OH)D_3 × STTD P interaction, P = 0.098. Linear and quadratic added STTD P effect for the interactive means, P < 0.001.

[7]Bone ash weight was measured on each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, P > 0.10. Added 25(OH)D_3 effect for all bones, P = 0.675. Linear STTD P effect for all bones, P < 0.001. Quadratic STTD P effect for all bones, P = 0.001. Main effect of bone, P < 0.001. SEM = 0.081. For the interactive means of 25(OH)D_3 × STTD P averaged across all 4 bones (fibula, 2nd and 10th ribs, and metacarpals), linear and quadratic added STTD P, P < 0.001. Added 25(OH)D_3 effect for the interactive means, P = 0.675.

Table 2.6. Effects of added 25(OH)D_3 and STTD P on nursery pig bone histopathology[1]

Added 25(OH)D_3[2]	No			Yes		
STTD P, % of NRC[3]	70%	100%	130%	70%	100%	130%
Physis score frequency, %[4]						
0: no abnormalities	0.0	16.7	50.0	0.0	50.0	50.0
1: mild abnormalities	0.0	66.7	41.7	0.0	33.3	33.3
2: moderate abnormalities	25.0	0.0	8.3	41.7	16.7	8.3
3: extensive abnormalities	75.0	16.7	0.0	58.3	0.0	8.3
Cortical metaphysis score frequency, %[5]						
0: long anastomosing trabeculae	0.0	41.7	83.3	0.0	50.0	75.0
1: mild loss of cortical trabeculae	50.0	41.7	16.7	33.3	50.0	25.0
2: moderate loss of cortical trabeculae	50.0	16.7	0.0	66.7	0.0	0.0
3: extensive loss of trabeculae	0.0	0.0	0.0	0.0	0.0	0.0
Infraction/fracture score frequency, %[6]						
0: no infractions	0.0	83.3	100.0	0.0	100.0	83.3
1: small infractions	25.0	8.3	0.0	33.3	0.0	8.3
2: large infractions	41.7	8.3	0.0	41.7	0.0	8.3
3: cortical fracture	33.3	0.0	0.0	25.0	0.0	0.0
Fibrosis score frequency, %[7]						
0: no fibrosis	16.7	83.3	100.0	16.7	100.0	91.7
1: fibrosis	83.3	16.7	0.0	83.3	0.0	8.3

[11]A total of 360 pigs (initially 5.8 ± 0.54 kg) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P. Per treatment, 12 pigs

were euthanized (72 pigs total) and 10th ribs were collected. Left 10th rib was processed for histological examination. Microscopic examination was performed by blinded assessment from a board-certified anatomic pathologist.

[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[3]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC (2012) requirement estimate treatment diet in phases 1, 2, and 3 respectively.

[4]Physis score consisted of: (0) no histologic findings of significance, (1) multifocal small tongues or islands of viable cartilage extend into the primary spongiosa, (2) moderate-sized tongues or islands of viable cartilage extend into the primary and secondary spongiosa, and (3) extensive areas of the zone of hypertrophy are expanded and extend down into the primary and secondary spongiosa. Linear and quadratic 25(OH)D_3 × STTD P interaction, $P > 0.10$. Added 25(OH)D_3 effect, $P = 0.274$. Linear STTD P effect, $P < 0.001$. Quadratic STTD P effect, $P = 0.002$.

[5]Cortical metaphysis score consisted of: (0) long anastomosing trabeculae, (1) mild loss of cortical trabeculae with resorption, remodeling, or fibrosis, (2) moderate loss of cortical trabeculae with resorption, remodeling, or fibrosis, and (3) extensive loss of trabeculae with resorption, remodeling, or fibrosis. Linear and quadratic 25(OH)D_3 × STTD P interaction, $P > 0.10$. Added 25(OH)D_3 effect, $P = 0.772$. Linear STTD P effect, $P < 0.001$. Quadratic STTD P effect, $P = 0.147$.

[6]Infractions/fracture scoring consisted of: (0) no evidence of infractions, (1) small infractions covering <50% of the diameter of the bone, (2) large infractions covering > 50% of the diameter of the bone, and (3) cortical fracture. Due to failure of satisfactory model fit because of all observations within a treatment scoring in a single category, data were reported using descriptive statistics.

[7]Fibrosis score consisted of: (0) no evidence of fibrosis, and (1) fibrosis in the medullary bone. Due to failure of satisfactory model fit because of all observations within a treatment scoring in a single category, data were reported using descriptive statistics.

Chapter 3 - Influence of added $1,25(OH)_2D_3$-glycoside on nursery pig growth performance, bone measurements, and cytokine concentrations

Abstract

A total of 2,268 crossbred pigs (L337 × 1050, PIC; initially 5.5 ± 0.18 kg) were used in a 42-d growth study to evaluate the effects of $1,25(OH)_2D_3$-glycoside provided from a plant extract on growth performance, bone characteristics, and serum criteria of nursery pigs. Pigs were weaned at approximately 21-d of age and randomly assigned to 1 of 3 dietary treatments in a randomized complete block design. A total of 84 pens were used with 27 pigs per pen and 28 replications per treatment with pens blocked by BW and weaning date. Treatment diets were corn-soybean meal-based and consisted of a control diet (1,653 IU/kg of vitamin D_3), or the control diet with 1.2 or 2.0 μg of $1,25(OH)_2D_3$-glycoside/kg. Blood samples were collected from 25 gilts/treatment on d 21 and 42 to assess $25(OH)D_3$, cytokine concentrations, and antibody titers. At the end of the study, 10 pigs per treatment were euthanized and the right fibula, metacarpal, 2nd and 10th ribs were collected to determine bone density, breaking strength, and percentage bone ash. Overall, there was a tendency ($P = 0.067$) for a reduction in G:F as added $1,25(OH)_2D_3$-glycoside increased, but no significant effects on final BW, ADG, ADFI, or mortality were observed. There were no treatment × bone interactions for bone breaking strength and bone ash. Percentage bone ash increased (linear, $P = 0.030$) across all bones as $1,25(OH)_2D_3$-glycoside increased. Treatment did not affect bone ash weight and breaking strength. Metacarpals and 10th ribs had the greatest bone ash weight followed by the fibula with 2nd ribs having the lowest ($P < 0.05$).

Metacarpals had greater breaking strength compared to all other bones, followed by the fibula and 10th rib, with the 2nd rib having the lowest ($P < 0.001$). There was a bone × treatment interaction for bone density, where increasing 1,25$(OH)_2D_3$-glycoside increased bone density for the 2nd rib ($P = 0.012$), but there was no treatment difference for other bones. There was no difference between treatments for antibody titers, 25$(OH)D_3$ status, or circulating cytokine concentrations except for IL-8 concentrations which decreased (linear, $P = 0.037$) as 1,25$(OH)_2D_3$-glycoside increased. In summary, adding 1.2 or 2.0 μg 1,25$(OH)_2D_3$-glycoside/kg provided from a plant extract to a diet already containing 1,653 IU/kg of vitamin D_3 had minimal effects on growth or the evaluated serum parameters; however, increasing 1,25$(OH)_2D_3$-glycoside increased percentage bone ash.

Key Words: bone characteristics, cytokine, growth, nursery pig, vitamin D

Introduction

Vitamin D is a lipophilic vitamin that is required for growth, bone development and mineralization, and immune function. The two major forms of vitamin D are ergocalciferol (vitamin D_2), which is synthesized in plants, and cholecalciferol (vitamin D_3), which can be synthesized in the skin of many animals and humans (Baeke et al., 2010). Vitamin D_3 must undergo a two-step hydroxylation process to become the bioactive form. After absorption in the small intestine, vitamin D_3 is stored in the liver where it is hydroxylated to produce 25-hydroxyvitamin D_3 [25$(OH)D_3$], which is the major circulating metabolite of vitamin D (DeLuca, 2008). After hydroxylation in the liver, 25$(OH)D_3$ is transported to the kidney and undergoes a second hydroxylation process in the proximal tubules to become 1,25-

dihydroxicholecalciferol [1,25$(OH)_2D_3$], which is the most bioactive form of vitamin D in the body (Norman, 2008). Under disease challenges, low feed intake situations, or situations where liver or kidney conversions are less than sufficient, direct supplementation of dietary 1,25$(OH)_2D_3$ may be beneficial.

Direct addition of 1,25$(OH)_2D_3$ will provide the bioactive form to the pig to be readily utilized. In recent years, experiments have been conducted with different concentrations of dietary vitamin D_3 and 25$(OH)D_3$ (Flohr et al., 2014; Duffy et al., 2018; Williams et al., 2023). The absorption rate of 25$(OH)D_3$ is approximately 20% higher than that of vitamin D_3 (Applegate and Angel, 2005; Garcia et al., 2013). Previous research has indicated that serum 25$(OH)D_3$ concentrations are not always maintained above recommendations (Lauridsen, 2014; Arnold et al., 2015) when supplementing with vitamin D_3 alone in the feed even when fed at levels well above the NRC (2012) requirements (Flohr et al., 2014). However, supplementation of 25$(OH)D_3$ allows pigs to elevate serum 25$(OH)D_3$ concentrations (Zhang et al., 2021; Williams et al., 2023) but still requires renal metabolism to be converted to the bioactive form. The 1,25$(OH)_2D_3$ metabolite does not require renal metabolism, but little research has been conducted on it in swine diets (Schlegel et al., 2017; Trautenmüller et al., 2021). An experiment conducted by Alves et al. (2018) in broilers observed a reduction in growth performance when 1,25$(OH)_2D_3$ completely replaced vitamin D_3 in the diet. Thus, this data may suggest that 1,25$(OH)_2D_3$ may need to be added to diets containing a basal level of vitamin D_3.

The 1,25$(OH)_2D_3$-glycoside used in this study is derived from a plant mixture of herbal origin. Bacteria with glycosidase activity in the colon metabolize 1,25$(OH)_2D_3$-glycoside before it can be absorbed (Zimmerman et al., 2015). This results in a slow release of 1,25$(OH)_2D_3$ and decreases the risk of toxicity due to a lower plasma peak concentration and a longer half-life

(Mathis et al., 2016). In addition, the pure form of $1,25(OH)_2D_3$ is not stable during thermal processing, specifically pelleting. The glycoside stabilizes the $1,25(OH)_2D_3$ and allows it to withstand the high temperatures associated with pelleting. Therefore, this study aimed to evaluate the effects of dietary $1,25(OH)_2D_3$-glycoside on growth performance, bone characteristics, and serum criteria of nursery pigs in diets containing standard industry vitamin D_3 concentrations (Faccin et al., 2023). We hypothesized that while $1,25(OH)_2D_3$-glycoside bypasses the hydroxylation steps in the liver and kidneys, it would be a more readily available metabolite of vitamin D and thus increase growth performance and bone mineralization.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at New Horizon Farms nursery research facility located in Pipestone, MN. The experiment utilized two identical nursery rooms that were completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 6-hole, dry self-feeder, and a pan waterer to provide *ad libitum* access to feed and water. Feed additions were accomplished using a robotic feeding system (FeedPro, FeedLogic Corp., Wilmar, MN).

Animals and housing

A total of 2,268 crossbred pigs (L337 × 1050, PIC; initially 5.5 ± 0.18 kg) were used in a 42-d growth study to evaluate the effects of $1,25(OH)_2D_3$-glycoside on growth performance, bone characteristics, and serum criteria of nursery pigs. Pigs were weaned at approximately 21-d of age and assigned to 1 of 3 dietary treatments in a randomized complete block design. A total of 84 pens were used with 27 pigs per pen and 28 replications per treatment across 2 rooms with

pens blocked by BW and weaning date. Treatment diets were corn-soybean meal-based and consisted of a control diet (1,653 IU/kg of added vitamin D_3 provided from vitamin premix), or the control diet with 1.2 or 2.0 μg of added 1,25$(OH)_2D_3$-glycoside/kg diet (Table 3.1). The 1,25$(OH)_2D_3$-glycoside was provided by a plant extract (Herbal Active D, Phytobiotics, Cary, NC) that contained 10 mg 1,25$(OH)_2D_3$ per kg.

During the experiment, pens of pigs were weighed and feed disappearance was recorded every 7 d to determine ADG, ADFI, and G:F. Pigs that died or were removed during this study because of sickness or injury were recorded. Removals were defined as any pig removed from a test pen and placed into a treatment specific off-test pen where they remained on treatment diets for the duration of the study. Any pig that died while in a test pen or a pig that died from an off-test pen was defined as a mortality.

Blood sampling

On d 21 and 42, individual gilts in 25 pens per treatment were bled via jugular venipuncture and circulating cytokine concentrations, antibody titers, and vitamin D_3 levels were determined. An average BW gilt in each pen was selected for both blood collection days. Blood was collected in tubes without anticoagulant to obtain serum. Blood was allowed to clot before centrifuging for 15 min at 1,500 × *g* and then serum was stored at -80°C until analyzed using a panel testing for 13 cytokines (GM-CSF, IFNγ, IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNFα; Eve Technologies, Calgary, AB Canada) via 13-Plex Discovery Assay (MilliporeSigma, Burlington, MA, USA). Serum samples also were sent to the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) to determine 25$(OH)D_3$ (d 42 samples only; via liquid chromatography with tandem mass spectrometry) and antibody titers of porcine circovirus type 2 (PCV2; INgezim Circovirus IgG/IgM, Ingenasa, Madrid, Spain), porcine

reproductive and respiratory syndrome virus (PRRSV; PRRS X3 Ab Test, IDEXX Laboratories, Westbrook, Maine), and *Mycoplasma hyopneumoniae* (*M. hyo* Ab Test, IDEXX Laboratories, Westbrook, Maine).

Bone characteristics

At the end of the study, 10 gilts per treatment (weighing closest to the average BW of the 10 pens) were euthanized and the right fibula, metacarpal, 2nd rib, and 10th rib were collected to determine bone density, bone breaking strength, and percentage bone ash by utilizing the de-fatted processing method (Wensley et al., 2020). After removal, bones were stored at -20°C until analysis. Extraneous soft tissues and cartilage were removed from the bones prior to assessment. Bone density was measured on each bone based on Archimedes principle (Williams et al., 2023). A dry bone weight was collected, then bones were submerged in ultra-purified water under a negative pressure vacuum with 1.06 kg per cubic centimeter for a minimum of 4 h. Bones were then weighed while suspended in a vessel of ultra-purified water, and the weight was used to calculate bone density. Bone breaking strength is reported as the maximum compressive load on each bone via an Instron (Instron 5569, NV Lab, Norwood, MA). Briefly, each bone was held by two supports spaced 30 mm apart and were broken by a wedge lowered on the center of the bone at a speed of 100 mm per min and a maximal pressure of 5,000 kg. The force was measured by a pressure-sensitive cell, and peaks of maximum force were recorded (Williams et al., 2023). For defatted bone ash, all bones were placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. Bones were dried at 105°C for 7 d in a drying oven and then ashed in a muffle furnace at 600°C for 24 h to determine total bone ash weight and percentage ash relative to dried bone weight (Wensley et al., 2020).

Additionally, the left 10th rib was processed for histopathology examination. Bones were fixed in 10% neutral buffered formalin for at least 24 h and decalcified using DeltaCAL (Delta Medical, Inc., Aurora, IL, USA) for at least 4 to 6 h. The costochondral junction and the body of the 10^{th} rib were embedded in paraffin blocks, sectioned at 4 μm thickness, and stained with hematoxylin and eosin. The microscopic examination was performed by blinded assessment from three pathologists at Iowa State University Veterinary Diagnostic Laboratory (Ames, IA). Histopathology grading of each bone consisted of physis grading, infractions/fracture lines, and fibrosis grading as described by Williams et al., 2023. A full description of the scoring system is presented in Supplemental Table 1.

Statistical analysis

Growth performance, vitamin D status, bone characteristics, and histopathology data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Pen was considered the experimental unit. Treatment was used as the fixed effect and block was used as the random effect. Initial pen average BW and date of entry into the facility were incorporated within the blocking structure. Linear and quadratic contrasts were evaluated within increasing $1,25(OH)_2D_3$-glycoside considering the control diet as no added $1,25(OH)_2D_3$-glycoside. For bone characteristics and histopathology, treatment, bone, and associated interactions were considered fixed effects, with block and pig serving as random effects. Antibody titers and cytokines were analyzed as repeated measures representing multiple observations on each pen over time. Treatment, day, and the associated interactions were considered fixed effects. A Log_2 transformation was used for PCV2 antibody titers. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Growth performance

From d 0 to 7, ADG, d 7 BW, and G:F increased (linear, $P \leq 0.048$) as 1,25$(OH)_2D_3$-glycoside increased (Table 3.2). Treatment diets had no effect on ADFI. From d 7 to 21, increasing 1,25$(OH)_2D_3$-glycoside decreased (linear, $P \leq 0.050$) d 21 BW, ADG, and G:F. No differences were observed in ADFI. Overall (d 0 to 42), a marginally significant decrease (linear, $P = 0.067$) in G:F was observed as 1,25$(OH)_2D_3$-glycoside increased. Overall, treatment diets had no significant effect on final BW, ADG, or ADFI. No statistical differences in mortality, removals, or mortality of the removed pigs were observed.

Bone characteristics

A linear 1,25$(OH)_2D_3$-glycoside × bone interaction ($P = 0.021$) was observed for bone density (Figure 1). The interaction was the result of a linear ($P = 0.012$) increase in bone density as 1,25$(OH)_2D_3$-glycoside increased in the 2nd rib, but no effects for the other bones. A main effect of bone was observed with 10th ribs having the greatest bone density followed by fibulas, 2nd ribs, and metacarpals having the least ($P < 0.001$). No 1,25$(OH)_2D_3$-glycoside × bone interactions were observed for bone breaking strength (Table 3.3). However, a main effect of bone ($P < 0.001$) was observed with metacarpals having the highest values for breaking strength and 2nd ribs having the lowest values (Table 3.4). Treatment diets had no effect on bone breaking strength.

For percentage bone ash, no 1,25$(OH)_2D_3$-glycoside × bone interactions were observed. Percentage bone ash increased (linear, $P = 0.030$) as 1,25$(OH)_2D_3$-glycoside increased. Additionally, a main effect of bone was observed where fibulas had the greatest percentage bone ash, followed by the 10th rib, with the 2nd rib and metacarpal having the lowest ($P < 0.05$)

percentage bone ash. For bone ash weight, no 1,25$(OH)_2D_3$-glycoside × bone interactions were observed. However, a main effect of bone ($P < 0.001$) was observed with metacarpals and 10th ribs having the greatest bone ash weight followed by the fibula with 2nd rib having the lowest ($P < 0.05$) bone ash weight. Treatment diets had no effect on bone ash weight.

For histopathologic evaluation of the physis of the 10th ribs, there tended to be a greater probability of having a higher score (indicating abnormal bone architecture) as 1,25$(OH)_2D_3$-glycoside increased (linear, $P < 0.052$; Table 3.5). For infraction and fibrosis scores, no effect of 1,25$(OH)_2D_3$-glycoside was observed.

Blood analysis

No treatment × day interactions were observed for any of the blood measurements collected on d 21 and 42 (Table 3.6). Treatment diets had no effect on PCV2, PRRS, and *Mycoplasma hyopneumoniae* antibody titers, vitamin D status, or most circulating cytokine concentrations. However, IL-8 concentrations decreased (linear, $P = 0.037$) as 1,25$(OH)_2D_3$-glycoside increased. Furthermore, a main effect of day was observed where pigs had increased ($P < 0.001$) PCV2, PRRS, and *Mycoplasma hyopneumoniae* antibody titers, and lower circulating cytokine concentrations on d 42 compared to d 21. However, a main effect of day was not observed for IL-1ra and IL-8 ($P \geq 0.473$).

Discussion

Vitamin D_3 requirement estimates set by the NRC (2012) are 220 IU/kg of complete diet for nursery pigs weighing 5 to 11 kg, and 200 IU/kg for those weighing 11 to 25 kg. Nevertheless, commercial swine diets typically contain concentrations of vitamin D_3 that are 5 to 7 times higher (Reese and Hill, 2010). Faccin et al. (2023) surveyed nutritionists in commercial production and found a wide range of vitamin D_3 levels in weanling pig diets from 1,389 to

10,494 IU/kg with a weighted average of 2,397 IU/kg. Our diets provided 1,653 IU/kg of vitamin D_3, falling within the 25th percentile according to the survey (Faccin et al., 2023). Additionally, the diets in the current experiment were formulated to contain levels of STTD P that exceed the requirement estimate for nursery pigs (NRC, 2012). The total Ca levels were established by utilizing a 1.1:1 total Ca:P ratio.

Dietary vitamin D_3 undergoes a two-step hydroxylation process for activation. The first hydroxylation step primarily occurs in the liver produce $25(OH)D_3$, the primary circulating metabolite of vitamin D (DeLuca, 2008). The enzyme responsible for catalyzing the initial hydroxylation step is 25-hydroxylase (Hewison et al., 2000). Vitamin D_3 must undergo further hydroxylation to form $1,25(OH)_2D_3$, the most biologically active form of vitamin D_3 (Norman, 2008). The second hydroxylation step takes place in the kidney, facilitated by the enzyme 1α-hydroxylase (Hewison et al., 2000). This active metabolite of vitamin D_3 plays an important role in the absorption of calcium and phosphorus, bone development and mineralization, and immune function (Hewison et al., 2000).

Weaning is a stressful period for pigs and can lead to increased hepatic oxidative stress that may result in liver abnormalities (Luo et al., 2016). Direct supplementation of $1,25(OH)_2D_3$ enables pigs to bypass the hydroxylation steps in the liver and kidney, providing them with a readily available source of the bioactive form of vitamin D. In the current study, $1,25(OH)_2D_3$-glycoside was added to a diet containing 1,653 IU/kg of vitamin D_3. Previous research in broilers indicated that $1,25(OH)_2D_3$ cannot replace basal levels of vitamin D_3 otherwise a decrease in performance occurs (Alves et al., 2018). It may be necessary to provide basal levels of vitamin D_3 in the diet because the metabolite after the first hydroxylation step, $25(OH)D_3$, is used for other metabolic processes in the body and is not completely hydroxylated to $1,25(OH)_2D_3$. The

$25(OH)D_3$ can also be converted to 24,25-dihydroxycholecalciferol [$24,25(OH)_2D_3$] which plays an important role in bone mineralization (Boyan et al., 2001). Given this need for circulating $25(OH)D_3$, the current study aimed to determine the effects of adding $1,25(OH)_2D_3$-glycoside into a diet that already contained 1,653 IU/kg of vitamin D_3.

Several studies have evaluated the effects of added $25(OH)D_3$, the vitamin D_3 metabolite produced after the first hydroxylation step, on growth performance and bone characteristics. When $25(OH)D_3$ was added to a diet that contained vitamin D_3, no differences were observed in growth performance or bone characteristics (O'Doherty et al., 2010; Sandoval et al., 2022; Williams et al., 2023). Although the addition of $25(OH)D_3$ bypasses the first hydroxylation step, addition of this metabolite is not translated into increased growth performance.

Serum or plasma $25(OH)D_3$ is considered the best biomarker of vitamin D status in mammals (Jones, 2012). The hydroxylation process from vitamin D_3 to $25(OH)_2D_3$ and $1,25(OH)_2D_3$ is not reversible. Therefore, we did not anticipate increased levels of serum $25(OH)D_3$ in pigs fed supplemental $1,25(OH)_2D_3$-glycoside. Additionally, serum concentrations of $25(OH)D_3$ below 10 to 15 ng/mL are speculated to be deficient in swine (Lauridsen, 2014); however, we are not aware of any data confirming this threshold. Arnold et al. (2015) reported reference values for serum $25(OH)D_3$ concentrations between 18 and 30 ng/mL in 2- to 4-week-old pigs. The serum concentrations of $25(OH)D_3$ were between 19 and 20 ng/mL in the current study indicating that these pigs were not deficient in vitamin D_3. These values were greater than observed by Williams et al. (2023) who observed serum $25(OH)D_3$ levels between 10.9 and 14.1 ng/mL in nursery pigs when fed diets containing the same level of added vitamin D_3 as in our study. This may be a result of the duration of the study and amount of vitamin D_3 consumption as

Williams et al. (2023) fed experimental diets for 28 d before measuring serum $25(OH)D_3$, whereas in our study status was measured after 42 d of feeding.

Calcium and P are essential for multiple physiological roles in the body including growth, development, and maintenance of the skeletal system. Vitamin D is also an important factor in the absorption and retention of Ca and P for bone mineralization (O'Doherty et al., 2010). Schlegel et al. (2017) observed no differences in bone density or bone ash when 10.0 or 20.0 μg/kg of plant-based $1,25(OH)_2D_3$ was added to a diet already containing 2,000 IU/kg of vitamin D_3 in pigs. In broilers, the addition of 0.5 μg/kg plant-based $1,25(OH)_2D_3$-glycoside had no effect on bone density, bone breaking strength, or percentage bone ash (Alves et al., 2018; Castro et al., 2018). In contrast, our study observed a linear increase in percentage bone ash as plant-based $1,25(OH)_2D_3$-glycoside/kg increased. The high concentrations of 10 or 20 μg/kg of added $1,25(OH)_2D_3$ fed by Schlegel et al. (2017) may have been in excess nearing toxicity levels. Evidence of toxicity appears with plasma Ca concentrations above 3.0 mmol/L which is slightly above the upper normal limit of 2.9 mmol/L (Kaneko et al., 2008). Excessive intake of $1,25(OH)_2D_3$ promotes intestinal absorption of Ca which can cause soft tissue calcification when chronically high plasma Ca concentrations are experienced. Conversely, providing 0.5 μg/kg of $1,25(OH)_2D_3$ in previous research may not have provided enough of the active metabolite to observe differences in bone characteristics (Alves et al., 2018; Castro et al., 2018) compared to the 1.2 or 2.0 μg $1,25(OH)_2D_3$-glycoside/kg used in the current experiment.

Histopathology can also be used to evaluate metabolic bone disease in pigs (Williams et al., 2023). Based on findings of Williams et al. (2023), tenth ribs were sampled for histopathology because they are more sensitive in response to P- or vitamin D-deficiency compared to 2nd ribs and have the potential to show failure of endochondral ossification and

infraction. In the current experiment, increasing 1,25$(OH)_2D_3$-glycoside increased the likelihood of having abnormal histopathology findings. This response was not expected because previous research observed no differences in physeal score or infraction score with the addition of a vitamin D_3 metabolite 25$(OH)D_3$ (Williams et al., 2023). Further research is needed on the effect of vitamin D_3 and its metabolites on histopathology because few data exist in literature.

Beyond being important for growth performance and bone characteristics, vitamin D_3 also plays an important role in immune function because vitamin D receptor signaling occurs in several immune cells (Yang and Ma, 2021). In the current experiment, addition of 1.2 or 2.0 μg 1,25$(OH)_2D_3$-glycoside/kg to a diet containing 1,635 IU/kg vitamin D_3 had no effect on antibody titers and circulating cytokines, with the exception of IL-8. Thirteen cytokines were measured to get an understanding of how vitamin D modulates the immune system and as a biomarker for inflammation. The addition of 1,25$(OH)_2D_3$-glycoside downregulated IL-8, which is a pro-inflammatory cytokine. Vitamin D inhibits the secretion of pro-inflammatory cytokines and promotes production of more anti-inflammatory cytokines. This was observed in humans where 1,25$(OH)_2D_3$ inhibited the production of type 1 pro-inflammatory cytokines including IL-8, and downregulated type 1 helper cells, part of the adaptive immune system (Bui et al., 2021). A downregulation of IL-8 can result in more severe diseases because IL-8 plays a key role in the control of bacteria translocation (Mahanty et al., 2001). However, this downregulation of IL-8 was not observed in pigs supplemented with 25$(OH)D_3$ (Madsen et al., 2023), although age at sampling differed between these experiments. The reasoning behind the observed reduction in IL-8 is not fully understood and requires further research to further elucidate.

In the current experiment, several cytokines (CM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-18, and TNFα) decreased between d 21 and 42 postweaning. A reduction in pro-

inflammatory cytokines concentrations indicates an improvement in immune status suggesting that these pigs can spend less energy on immune overexpression (Gessner et al., 2017). Weaning is a stressful period for pigs and can result in inflammation due to an increase in cytokine production (Gessner et al., 2017). Although there are no reference values for cytokine concentrations based on a pigs' age, we expected the cytokine concentrations to decrease as the pigs became older because feed intake increased and the weaning stressors are reduced. de Groot et al. (2021) also observed a decrease in cytokines (IFN-γ, IL-1α, and TNFα) between d 30 and 45 postweaning in pigs' jejunum, ileum, or colon tissues. However, Rao et al. (2023) observed an increase in cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, and IL-12) between d 10 and 42 post weaning in pigs. This variation in the literature suggests that the environment in which pigs are raised plays a role in cytokine concentrations due to different stressors or immune challenges.

In conclusion, although $1,25(OH)_2D_3$-glycoside bypasses the hydroxylation steps and provides a readily available metabolite of vitamin D_3, its addition to a diet already containing vitamin D_3 did not translate to changes in growth, bone density, or bone breaking strength. However, the addition of 1.2 or 2.0 μg $1,25(OH)_2D_3$-glycoside/kg to a diet containing 1,653 IU/kg vitamin D_3 increased percentage bone ash.

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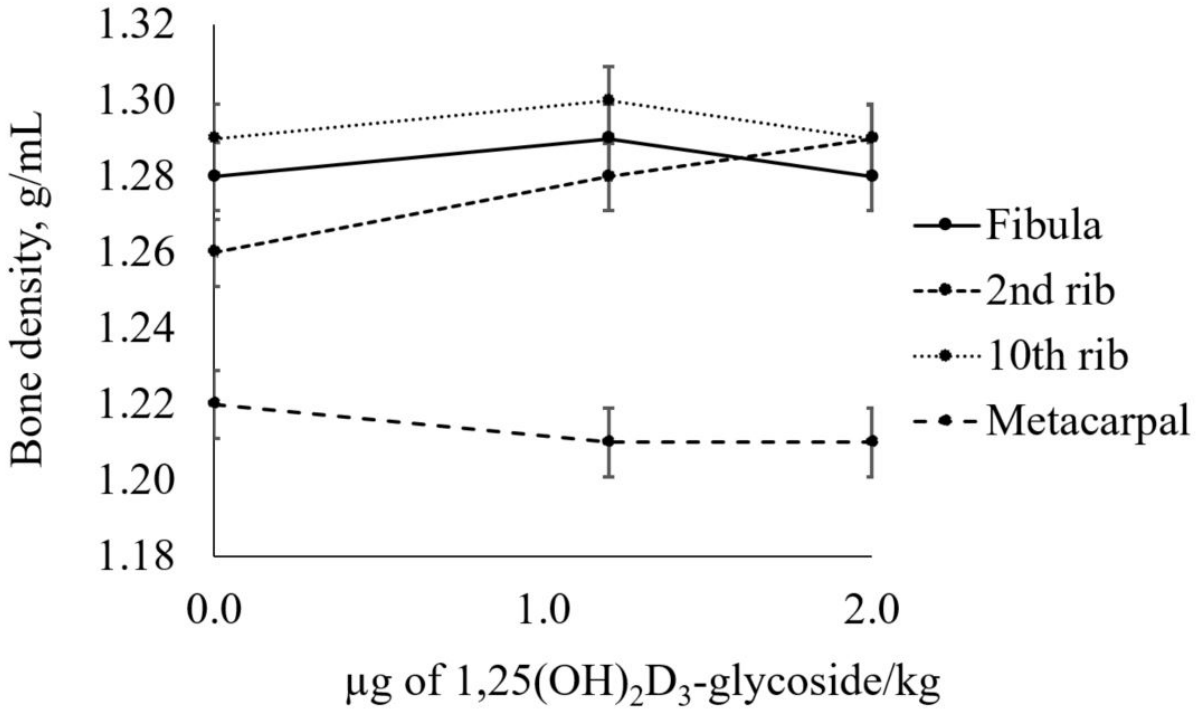

Figure 3.1. Influence of 1,25(OH)2D3-glycoside on nursery pig bone density. Density was measured on each bone based on Archimedes principle. Linear 1,25(OH)2D3-glycoside × bone interaction, P = 0.021. Linear effect of 1,25(OH)2D3-glycoside for 2nd rib, P = 0.012. Linear effect of 1,25(OH)2D3-glycoside for all other bones, P > 0.10. Error bars represent +/- 1 SEM.

Table 3.1. Diet composition (as-fed basis)

Ingredient, %	Phase 1[1]	Phase 2[2]	Phase 3[3]
Corn	38.28	54.07	57.10
Soybean meal, dehulled	25.14	26.13	29.57
Dried distillers grains with solubles	---	---	10.00
Whey powder	12.50	10.00	---
Whey permeate	11.25	---	---
Microbial-enhanced soy protein[4]	6.25	5.00	---
Choice white grease	---	1.00	---
Soybean oil	3.00	---	---
Calcium carbonate	0.62	0.70	0.90
Monocalcium P, (21% P)	0.88	0.95	0.65
Salt	0.35	0.60	0.50
L-Lys-HCl	0.40	0.38	0.45
DL-Met	0.25	0.20	0.12
L-Thr	0.18	0.19	0.19
L-Trp	0.02	0.03	0.03
L-Val	0.10	0.07	0.05
Vitamin and trace mineral premixes[5]	0.40	0.45	0.45
Zinc oxide	0.39	0.25	---
$1,25(OH)_2D_3$-glycoside[6]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
SID AA, %			
Lys	1.40	1.35	1.30
Ile:Lys	61	62	59
Leu:Lys	114	122	130
Met:Lys	38	37	33
Met and Cys:Lys	56	56	56
Thr:Lys	64	64	63
Trp:Lys	20.4	20.2	19.2
Val:Lys	77	71	70
Total Lys, %	1.55	1.50	1.47
ME, kcal/kg	3,485	3,338	3,258
NE, kcal/kg	2,612	2,471	2,388
SID Lys:ME, g/Mcal	5.36	5.46	5.44
CP, %	22.1	22.3	22.3
Ca, %	0.68	0.68	0.65
STTD P, %	0.54	0.52	0.45

[1]Phase 1 diets were fed from approximately d 0 to 7 (5.5 to 5.9 kg).
[2]Phase 2 diets were fed from approximately d 7 to 21 (5.9 to 10.2 kg).

[3]Phase 3 diets were fed from approximately d 21 to 42 (10.2 to 20.0 kg).

[4]Me-Pro, Prairie AquaTech, Brookings, SD.

[5]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin; 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite. Ronozyme HiPhos (DSM, Parsippany, NJ) included in phase 1 diets at 1,250 FTU/kg provided an estimated release of 0.13% STTD P. Optiphos 2,500 G (Huvepharma; Peachtree City, GA) included in phase 2 and 3 diets provided an estimated release of 0.13% STTD P with 1,251 FTU/kg.

[6]1,25$(OH)_2D_3$-glycoside derived from a plant extract (Herbal Active D, Phytobiotics, Cary, NC) was diluted with wheat middlings and added to provide 120 or 200 mg/kg of 1,25$(OH)_2D_3$-glycoside in the final diet.

Table 3.2. Influence of $1,25(OH)_2D_3$-glycoside on nursery pig growth performance[1]

	μg of $1,25(OH)_2D_3$-glycoside/kg[2]				*P* =	
	0	1.2	2.0	SEM	Linear	Quadratic
BW, kg						
d 0	5.5	5.5	5.5	0.18	0.924	0.639
d 7	5.8	5.9	5.9	0.18	0.056	0.855
d 21	10.2	10.2	10.1	0.27	0.050	0.615
d 42	20.1	20.1	19.7	0.42	0.115	0.300
d 0 to 7						
ADG, g	46	54	58	5.0	0.030	0.921
ADFI, g	113	116	120	5.0	0.224	0.731
G:F, g/kg	395	466	482	36.3	0.048	0.626
d 7 to 21						
ADG, g	296[a]	292[ab]	281[b]	8.2	0.015	0.361
ADFI, g	354	359	358	7.6	0.448	0.641
G:F, g/kg	835[a]	809[b]	783[c]	12.0	< 0.001	0.536
d 21 to 42						
ADG, g	459	459	447	9.9	0.304	0.420
ADFI, g	695	697	680	14.2	0.306	0.371
G:F, g/kg	660	660	658	6.4	0.825	0.912
d 0 to 42						
ADG, g	327	329	320	7.1	0.359	0.289
ADFI, g	472	477	470	8.9	0.891	0.352
G:F, g/kg	693	689	681	5.1	0.067	0.589
Removals, %	11.87	9.90	10.03	1.331	0.221	0.545
Mortality, %	3.69	2.90	3.29	0.705	0.613	0.493
Total mortality, %[3]	5.09	4.32	4.07	0.896	0.322	0.889
Total removals and mortality, %[4]	15.54	12.77	13.30	1.528	0.177	0.371

[1]A total of 2,268 pigs (initially 5.5 ± 0.18 kg) were used with 27 pigs per pen and 28 replications per treatment. Treatment diets were fed in all 3 phases.

[2]$1,25(OH)_2D_3$-glycoside derived from a plant extract (Herbal Active D, Phytobiotics, Cary, NC).

[3]Percentage of pigs that died in original pen or off-test pen after being removed.

[4]Percentage of pigs that were removed from original pen or died in original pen.

Table 3.3. Influence of 1,25$(OH)_2D_3$-glycoside on nursery pig bone characteristics[1]

	μg of 1,25$(OH)_2D_3$-glycoside/kg[2]			
Item	0	1.2	2.0	SEM
Bone breaking strength, kg[3]				
Fibula	15.5	14.9	17.7	1.50
2nd rib	5.9	6.7	6.2	
10th rib	13.3	13.8	15.0	
Metacarpal	40.1	38.9	38.9	
Bone ash, %[4]				
Fibula	63.1	63.7	63.5	0.43
2nd rib	58.3	59.1	59.1	
10th rib	59.0	60.5	60.5	
Metacarpal	58.4	58.6	59.4	
Bone ash, g[5]				
Fibula	1.02	1.03	1.14	0.078
2nd rib	0.77	0.83	0.82	
10th rib	1.45	1.50	1.54	
Metacarpal	1.50	1.51	1.55	

[1]A total of 2,268 pigs (initially 5.5 ± 0.18 kg) were used with 27 pigs per pen and 28 replications per treatment. Ten pigs per treatment were euthanized and the right metacarpal, fibula, 2nd rib, and 10th rib were collected. Values reported are the averages within each bone.

[2]1,25$(OH)_2D_3$-glycoside derived from a plant extract (Herbal Active D, Phytobiotics, Cary, NC).

[3]Bone breaking strength is reported as the maximum compressive load on each bone via an Instron (Instron 5569, NV Lab, Norwood, MA). Linear and quadratic 1,25$(OH)_2D_3$-glycoside × bone interaction, $P > 0.10$. Main effect of 1,25$(OH)_2D_3$-glycoside, $P > 0.10$. Main effect of bone, $P < 0.0001$.

[4]Bone ash was measured on each bone based on utilizing the de-fatted processing method. Bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. Bones were dried at 105°C for 7 d in a drying oven and then ashed in a muffle furnace at 600°C for 24 h. Linear and quadratic 1,25$(OH)_2D_3$-glycoside × bone interaction, $P > 0.10$. Linear effect of 1,25$(OH)_2D_3$-glycoside, linear $P = 0.030$. Main effect of bone, $P < 0.0001$.

[5]Bone ash weight was measured on each bone based. Linear and quadratic effect of 1,25$(OH)_2D_3$-glycoside, $P > 0.10$. Main effect of bone, $P < 0.0001$.

Table 3.4. Main effects of $1,25(OH)_2D_3$-glycoside and bone on nursery pig bone characteristics[1]

	μg of $1,25(OH)_2D_3$-glycoside/kg[2]				Bone[3]				
Item	0	1.2	2.0	SEM	Fibula	2nd rib	10th rib	Metacarpal	SEM
Bone density, g/mL[4]	1.262	1.270	1.270	0.0066	1.286^{a,b}	1.276^{b}	1.292^{a}	1.216^{c}	0.0056
Bone breaking strength, kg[5]	18.7	18.6	19.4	1.03	16.0^{b}	6.3^{c}	14.0^{b}	39.3^{a}	1.07
Bone ash, %[6]	59.7	60.5	60.6	0.30	63.4^{a}	58.8^{c}	60.0^{b}	58.8^{c}	0.26
Bone ash, g[7]	1.19	1.22	1.26	0.071	1.06^{b}	0.81^{c}	1.50^{a}	1.52^{a}	0.066

[1]A total of 2,268 pigs (initially 5.5 ± 0.18 kg) were used with 27 pigs per pen and 28 replications per treatment. Ten pigs per treatment were euthanized and the right metacarpal, fibula, 2nd rib, and 10th ribs were collected.

[2]$1,25(OH)_2D_3$-glycoside derived from a plant extract (Herbal Active D, Phytobiotics, Cary, NC). Values reported represent the main effect of treatment averaged across all 4 bones (fibula, 2nd rib, 10th rib, and metacarpal).

[3]Values reported represent the main effect of bone averaged across all treatments (0, 1.2, and 2.0 μg of $1,25(OH)_2D_3$-glycoside/kg)

[4]Bone density was measured on each bone based on Archimedes principle. Linear and quadratic effect of $1,25(OH)_2D_3$-glycoside, $P > 0.10$. Main effect of bone, $P < 0.0001$. Linear 1,25(OH)2D3-glycoside × bone interaction, $P = 0.021$. Linear effect of 1,25(OH)2D3-glycoside for 2nd rib, $P = 0.012$. Linear effect of 1,25(OH)2D3-glycoside for all other bones, $P > 0.10$.

[5]Bone breaking strength is reported as the maximum compressive load on each bone via the Instron machine (Instron 5569, NV Lab, Norwood, MA). Linear and quadratic effect of $1,25(OH)_2D_3$-glycoside, $P > 0.10$. Main effect of bone, $P < 0.0001$.

[6]Bone ash was measured on each bone based on utilizing the de-fatted processing method. Bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. Bones were dried at 105°C for 7 d in a drying oven and then ashed in a muffle furnace at 600°C for 24 h. Linear effect of $1,25(OH)_2D_3$-glycoside, $P = 0.030$. Main effect of bone, $P < 0.0001$.

[7]Bone ash weight was measured on each bone based on utilizing the de-fatted processing method. Linear and quadratic effect of $1,25(OH)_2D_3$-glycoside, $P > 0.10$. Main effect of bone, $P < 0.0001$.

Table 3.5. Influence of $1,25(OH)_2D_3$-glycoside on nursery pig bone histopathology measurements[1]

	μg of $1,25(OH)_2D_3$-glycoside/kg[2]		
Item	0	1.2	2.0
Physis score frequency, %[3]			
0: no abnormalities	50.0	23.3	30.0
1: mild abnormalities	36.7	36.7	30.0
2: moderate abnormalities	13.3	33.3	20.0
3: extensive abnormalities	0.0	6.7	20.0
Infraction score frequency, %[4]			
0: no infractions	93.3	60.0	73.3
1: small infractions	6.7	23.3	16.7
2: large infractions	0.0	16.7	10.0
3: cortical fracture	0.0	0.0	0.0
Fibrosis score frequency, %[5]			
0: no fibrosis	90.0	90.0	93.3
1: fibrosis	10.0	10.0	6.7

[1]A total of 2,268 pigs (initially 5.5 ± 0.18 kg) were used with 27 pigs per pen and 28 replications per treatment. Left 10th rib was processed for histopathology examination. Microscopic examination was performed by blinded assessment from three pathologists at Iowa State University Veterinary Diagnostic Laboratory (Ames, IA).

[2]$1,25(OH)_2D_3$-glycoside derived from a plant extract (Herbal Active D, Phytobiotics, Cary, NC).

[3]Physis score consisted of: (0) no histologic findings of significance, (1) multifocal small tongues or islands of viable cartilage extend into the primary spongiosa, (2) moderate-sized tongues or islands of viable cartilage extend into the primary and secondary spongiosa, and (3) extensive areas of the zone of hypertrophy are expanded and extend down into the primary and secondary spongiosa. Linear $1,25(OH)_2D_3$-glycoside effect, $P = 0.052$.

[4]Infractions scoring consisted of: (0) no evidence of infractions, (1) small infractions covering <50% of the diameter of the bone, (2) large infractions covering > 50% of the diameter of the bone, and (3) cortical fracture. No effect of $1,25(OH)_2D_3$-glycoside was observed, $P > 0.10$.

[5]Fibrosis score consisted of: (0) no evidence of fibrosis, and (1) fibrosis in the medullary bone. No effect of $1,25(OH)_2D_3$-glycoside was observed, $P > 0.10$.

Table 3.6. Main effects of 1,25(OH)$_2$D$_3$-glycoside on nursery pig serum performance[1]

	μg of 1,25(OH)$_2$D$_3$-glycoside/kg[2]				*P* =		Day			
Item	0	1.2	2.0	SEM	Linear	Quadratic	21	42	SEM	*P* =
Porcine circovirus type 2										
S/P ratio	0.47	0.44	0.42	0.035	0.373	0.997	0.31	0.58	0.031	< 0.001
Porcine reproductive and respiratory syndrome										
S/P ratio	0.78	0.77	0.78	0.050	0.972	0.870	0.08	1.48	0.057	< 0.001
Mycoplasma hyopneumoniae										
S/P ratio	0.20	0.19	0.20	0.039	0.949	0.825	0.08	0.31	0.034	< 0.001
25-hydroxyvitamin D$_3$, ng/mL[3]	20.0	20.1	19.1	0.93	0.514	0.600	---	---	---	---
Cytokine										
GM-CSF, pg/mL	40	43	72	27.6	0.447	0.633	86	17	27.7	0.006
IFNγ, pg/mL	8,945	9,984	9,048	1,806.5	0.925	0.651	15,211	3,441	1,841.7	< 0.001
IL-1α, pg/mL	93	95	96	14.1	0.873	0.960	124	66	13.2	0.001
IL-1β, pg/mL	599	554	579	79.2	0.824	0.736	737	417	73.5	0.001
IL-1ra, pg/mL	1,201	1,356	1,213	102.7	0.824	0.247	1,253	1,261	97.0	0.957
IL-2, pg/mL	657	621	622	98.5	0.774	0.895	872.7	394	93.7	< 0.001
IL-4, pg/mL	3,239	2,929	2,939	543.6	0.677	0.845	4,542	1,529	540.6	< 0.001
IL-6, pg/mL	294	265	266	56.7	0.699	0.853	390	160	56.8	< 0.001
IL-8, pg/mL	365	329	189	64.1	0.037	0.315	311	278	54.2	0.473
IL-10, pg/mL	1,648	1,644	1,516	243.5	0.707	0.793	2,207	998	238.7	< 0.001
IL-12, pg/mL	1,127	1,192	1,124	68.5	0.954	0.430	934	1,361	70.3	< 0.001
IL-18, pg/mL	4,155	3,920	3,416	625.5	0.407	0.782	4,997	2,664	603.5	0.001
TNFα, pg/mL	159	211	178	58.5	0.759	0.572	317	48.5	64.2	< 0.001

[1]A total of 2,268 pigs (initially 5.5 ± 0.18 kg) were used with 27 pigs per pen and 28 replications per treatment. Serum samples were collected from 1 average weight gilt from 25 pens per treatment. Blood was collected from the same gilts on d d21 and 42. Antibody titers and

vitamin D concentration were analyzed at Iowa State Veterinary Diagnostic Lab (Ames, IA). Cytokine analysis was conducted at Eve Technologies (Calgary, AB Canada). No treatment × day interactions were observed, $P > 0.10$.

[2]1,25$(OH)_2D_3$-glycoside derived from a plant extract (Herbal Active D, Phytobiotics, Cary, NC).

[3]Samples were only analyzed on d 42.

Chapter 4 - Effects of added folic acid on growth performance, short chain fatty acid concentrations, and serum homocysteine concentrations in nursery pigs

Abstract

Recent research reported an increase in growth performance when feeding supra-nutritional (up to 18 mg/kg) levels of folic acid to weanling pigs. Therefore, two experiments were conducted to validate these responses to folic acid in U.S. nursery pig diets. In Exp. 1, 360 barrows (DNA 600 × 241; initially 5.5 ± 0.03 kg) were used in a 38-d study to evaluate the effects of added folic acid (Rovimix Folic Acid, dsm-firmenich, Plainsboro, NJ) with or without pharmacological levels of Zn on growth performance and fecal characteristics. Treatments were arranged in a 3 × 2 factorial with main effects of folic acid (0, 20, or 40 mg/kg) and Zn (3,000 mg/kg of Zn in phase 1 and 2,000 mg/kg in phase 2 or no Zn other than 110 mg/kg from the trace mineral premix). Diets were corn-soybean meal-based and fed in 2 phases. A common phase 3 diet (110 mg/kg added Zn) was fed to all pigs. Increasing folic acid decreased (quadratic, $P < 0.003$) final BW, ADG, and ADFI with pigs fed 20 mg/kg having the poorest performance. From d 0 to 24, pigs fed diets with pharmacological levels of Zn had increased ($P \leq 0.030$) BW, ADG, and ADFI compared to pigs fed 110 mg/kg added Zn; however, no differences were observed in overall performance. No statistical differences were observed for mortality; however, pigs fed no added folic acid had no mortalities compared to 10 and 7.5% mortality in pigs fed 20 or 40 mg/kg of folic acid, respectively, regardless of Zn addition. Fecal total short chain fatty acid concentrations were greater ($P = 0.053$) on d 24 compared to d 9. Additionally, isobutyrate increased (linear, $P = 0.030$) as folic acid increased; however, most samples had nondetectable

levels. In Exp. 2, 350 barrows (DNA 200 × 400; initially 6.0 ± 0.06 kg) were used in a 38-d study. Treatments were corn-soybean meal-based, fed in 3 phases, and consisted of increasing folic acid: 0, 5, 10, 20, or 40 mg/kg. Overall, final BW, ADG, ADFI, and G:F were reduced (quadratic, $P \leq 0.049$) as folic acid increased with the poorest performance observed when pigs were fed 20 mg/kg of folic acid. On d 10 and 23, blood samples were collected from 70 pigs to determine serum homocysteine concentration, and a marginally significant linear folic acid × day interaction was observed ($P = 0.069$). Homocysteine concentrations increased (linear, $P = 0.037$) as folic acid increased from 0 to 40 mg/kg on d 10; however, no differences were observed on d 23. In conclusion, added folic acid reduced growth performance and increased serum homocysteine concentrations.

Key Words: folic acid, growth, homocysteine, nursery pig, Zn

Introduction

Folic acid (vitamin B9) is a water-soluble vitamin that plays an important role in growth, health, and nutrient metabolism. Folic acid is necessary for one-carbon transfer metabolic reactions, including nucleic acid synthesis, amino acid metabolism, and remethylation (conversion of homocysteine to methionine; Hayashi et al., 2007). Therefore, folic acid plays an important role in protein deposition and tissue synthesis. However, before folic acid can serve as a coenzyme and be used for methionine metabolism and protein synthesis, it first needs to be reduced and biochemically activated. This occurs in the liver and requires dihydrofolate reductase (DHFR) and other B vitamins (Raghubeer and Matsha, 2021).

Additionally, folic acid is necessary for proper brain function. It is also needed for the production of DNA and RNA. This is especially important during periods of rapid cell and tissue growth, such as pregnancy, infancy, and adolescence in humans. In swine, folic acid is commonly supplemented in sow diets to improve sow reproductive performance by positively impacting embryo development and increasing the number of pigs born alive (Matte et al., 1984; Thaler et al., 1989; Guay et al., 2002). An industry survey conducted by Faccin et al. (2023) found a range of 0.44 to 20.6 mg/kg of added folic acid in diets from weaning to 7 kg. While the NRC (2012) suggests nursery pigs from 5 to 25 kg BW require 0.30 mg/kg of dietary folic acid, recent research with weanling pigs supplemented up to 18 mg/kg folic acid in diets without antibiotics and pharmacological levels of Zn observed a shift in short chain fatty acid (SCFA) metabolism (Wang et al., 2021a). The SCFA shifted towards improved energy efficiency by increasing the isobutyrate concentration which can be used as an energy source for colonocytes (Jaskiewicz et al., 1996). The shift in SCFA metabolism and increased cecum crypt depth might have been responsible for increased ADG found with increased dietary folic acid (Wang et al., 2021a, b). Other research evaluated levels up to 12.5 mg/kg of folic acid in nursery pigs and observed no improvement in growth performance in diets containing 150 mg/kg Zn (Wang et al., 2020). Zinc is known to have antibacterial properties (Sirelkhatim et al., 2015). Pharmacological levels of Zn from zinc oxide (ZnO) in nursery pig diets has been observed to reduce the incidence of diarrhea and improve growth performance (Carlson et al., 1999; Hill et al., 2001; Laskoski et al., 2021). Because of the increased ADG and altered SCFA metabolism observed by Wang et al. (2021a, b) with supra-nutritional additions of folic acid in nursery pig diets without pharmacological levels of Zn and antibiotics, our objective for Exp. 1 was to determine the effects of including folic acid with or without pharmacological levels of Zn on nursery pig

growth performance and fecal characteristics. We hypothesized that growth performance would increase with the addition of folic acid but may be dependent on Zn level of the diet.

Based on the results of Exp. 1, a second experiment was conducted to further understand the effect of folic acid on growth performance and its role in protein synthesis, specifically the remethylation of homocysteine to methionine. Our objective was to validate results of Exp. 1 and determine if serum homocysteine played a role in this response. Based on the results of Exp. 1, we hypothesized that excess folic acid and increased serum homocysteine concentrations may result in poorer growth performance.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (1.52 × 1.52 m) had metal tri-bar flooring, one 4-hole self-feeder, and a nipple waterer to provide *ad libitum* access to feed and water. Experiment 2 was conducted at the Kansas State University Segregated Early Weaning Research Facility in Manhattan, KS. Each pen (1.22 × 1.22 m) had metal tri-bar floors, one 4-hole self-feeder, and nipple waterer for *ad libitum* access to feed and water. The facilities are completely enclosed, environmentally controlled, and mechanically ventilated.

Experiment 1

A total of 360 barrows (DNA 600 × 241; initially 5.5 ± 0.03 kg) were used in a 38-d growth study to evaluate the effects of including folic acid with or without pharmacological levels of Zn (from ZnO) on growth performance and fecal characteristics in nursery pigs. Diets did not contain any feed-grade medications. Pigs were weaned at approximately 19 d of age and

randomly allotted to 1 of 6 dietary treatments. A total of 72 pens were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 3 × 2 factorial with main effects of folic acid (0, 20, or 40 mg/kg; Rovimix Folic Acid, dsm-firmenich, Plainsboro, NJ; Table 4.1) and Zn provided by ZnO (3,000 mg/kg of Zn from d 0 to 9 and 2,000 mg/kg from d 9 to 24, or no additional Zn above that provided in the trace mineral premix). Experimental diets were corn-soybean meal-based with added specialty lactose and protein sources and fed in 2 phases in meal form. A common phase 3 diet was fed to all pigs in meal form. All diets contained 110 mg/kg of Zn provided by the trace mineral premix, but the premixes did not contain folic acid. At the time of feed manufacturing, a single base diet was manufactured at Hubbard Feeds in Beloit, KS, packaged in 22.7 kg bags and then transported to O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS, where treatment specific ingredients were mixed with the base diet to form experimental treatments. Pigs were weighed on d 0, 9, 17, 24, 31, and 38 to determine ADG, ADFI, and G:F.

Fecal characteristics

On d 9 and 24 of the experiment, fecal samples were collected from 3 pigs per pen. Samples were collected into sealable plastic bags for each individual pig. Fecal scores were assigned to each sample based on a 0 to 2 scale, with 0 indicating firm feces, 1 as soft feces, and 2 as diarrhea. Fecal dry matter (DM) was analyzed independently on all 3 samples per pen for both collection days. After collection, fecal samples were dried at 55°C for 48 h in a forced air oven, and the ratio of dried to wet fecal weight determined the percentage fecal DM.

The SCFA profiles were measured using a gas chromatographic method as described by Zhou et al. (2017) with minor modifications. Briefly, 1 g of sample was suspended in 3 mL of distilled water and settled for 60 min. Afterward, the sample was centrifuged (15,000 × g) at 4°C

for 10 min. The 1 mL supernatant was transferred and mixed with 0.25 mL metaphosphoric acid. The sample rested overnight and then centrifuged (3,000 × g) for 10 min. The supernatant (0.4 mL) was transferred to gas chromatography vials and analyzed using an Agilent 7890 Gas Chromatography (Agilent Technologies, Santa Clara, CA).

Experiment 2

A total of 350 barrows (DNA 200 × 400; initially 6.0 ± 0.06 kg) were used in a 38-d growth trial. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 5 dietary treatments in a completely randomized design. A total of 70 pens were used with 5 pigs per pen and 14 replications per treatment. Dietary treatments were corn-soybean meal-based with added specialty lactose and protein sources, and consisted of increasing folic acid: 0, 5, 10, 20, or 40 mg/kg (Rovimix Folic Acid, dsm-firmenich, Plainsboro, NJ; Table 4.2). The vitamin premix used in these diets did not contain folic acid. Treatment diets were fed in three phases from d 0 to 10 (phase 1), d 10 to 23 (phase 2), and d 23 to 38 (phase 3) after weaning. Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Phase 1 diets were fed in pelleted form. Phases 2 and 3 diets were fed in meal form. Complete diet samples were taken using a grain probe during bagging from every fourth bag and pooled into one homogenized sample per dietary treatment, then stored at -20°C. Pigs were weighed on d 0, 10, 17, 23, 31, and 38 to determine ADG, ADFI, and G:F.

Blood characteristics

On d 10 and 23, blood samples were collected from one average weight barrow from each pen (14 per treatment) for analysis of serum homocysteine concentration. A blood sample was taken from the jugular vein using a red-top, no anti-coagulant blood tube (Mansfield, MA). Ten mL of blood was centrifuged (3000 × g, 30 min) within 2 h after collection, and serum was

stored at −20°C until analysis. Serum homocysteine concentrations were determined using ELISA kits per the instructions of the manufacturer (Aspira Chemical, Oakland, CA).

Statistical analysis

Growth performance and SCFA data were analyzed as a completely randomized design for one-way ANOVA using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Pen was considered the experimental unit and treatment was used as the fixed effect when evaluating growth performance. Linear and quadratic contrasts were evaluated within increasing folic acid. Main effects of Zn and the interaction between Zn and added folic acid were evaluated in Exp 1. Additionally in Exp. 1, fecal DM, fecal score, and SCFA were analyzed as repeated measures representing multiple observations on each pen over time, and pen nested within treatment was included in the model as a random intercept to account for subsampling attributed to the multiple observations for each experimental unit on each day. Treatment, day, and the associated interactions were considered fixed effects. For fecal score analysis, ordinal data were analyzed using a generalized linear mixed model using a multinomial response distribution using a cumulative logit link function. Data were summarized using the FREQ procedure of SAS and reported as percentage of observations within each fecal score category by treatment and day. For Exp. 2, homocysteine concentrations were analyzed as repeated measures with pen included in the model as a random intercept to account for each sample being analyzed in duplicate, and microplate was used as a random effect. Treatment, day, and the associated interaction were considered fixed effects. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Experiment 1

No folic acid × pharmacological levels of Zn interactions were observed throughout the study (Table 4.3). For the experimental period (d 0 to 24), pigs fed diets with pharmacological levels of Zn had increased ($P \leq 0.030$) d 24 BW, ADG, and ADFI compared with pigs fed 110 mg/kg added Zn. During the common period (d 24 to 38), pigs previously fed pharmacological levels of Zn tended to have decreased ADG ($P = 0.096$) and had poorer G:F ($P = 0.028$) compared to pigs not previously fed pharmacological levels of Zn. Overall (d 0 to 38), no differences were observed when pigs were fed diets with or without pharmacological levels of Zn from d 0 to 24.

For the experimental period (d 0 to 24), common period (d 24 to 38) and overall (d 0 to 38), BW, ADG, and ADFI decreased then returned to control values (quadratic, $P \leq 0.019$) as folic acid increased. No statistical differences were observed in G:F or mortality based on folic acid supplementation. However, numerical differences in mortality were observed with pigs fed no added folic acid having 0% mortality compared to pigs fed 20 or 40 mg/kg having 10 or 7.5% mortality, respectively.

For fecal DM or scores, no treatment × day or folic acid × Zn interactions were observed. Fecal DM was greater ($P = 0.007$; Table 4.4) on d 24 compared to d 9. A marginally significant main effect of pharmacological levels of Zn was observed where pigs fed diets with added Zn had increased ($P = 0.080$) fecal DM averaged across d 9 and 24. There was a marginally significant pharmacological Zn × day interaction ($P = 0.064$) where pigs fed 110 mg/kg added Zn had a greater frequency of diarrhea on d 9 compared to d 24 (Figure 4.1). Pigs fed

pharmacological levels of Zn had reduced ($P < 0.001$) frequency of diarrhea compared to pigs fed diets with 110 mg/kg added Zn (Figure 4.2).

A total of 432 samples (72 samples per treatment) were analyzed for SCFA concentrations. No folic acid × Zn × day, folic acid × day, or folic acid × Zn interactions were observed. A Zn × day interaction ($P = 0.035$) was observed for isovalerate concentration from a total of 63 samples with detectable levels across all treatments. On d 9, isovalerate concentrations were greater in fecal samples of pigs fed 110 mg/kg added Zn compared to fecal samples from pigs fed pharmacological Zn (0.84 vs. 0.33 μmol/g, respectively; $P = 0.008$). On d 24, there was no difference in isovalerate concentrations between Zn treatments (0.44 vs. 0.50 μmol/g, respectively). A tendency for a Zn × day interaction ($P = 0.087$) was also observed for isobutyrate concentration from a total of 6 samples with detectable levels across all treatments. On d 9, isobutyrate concentration was greater in fecal samples from pigs fed 110 mg/kg added Zn compared to fecal samples from pigs fed pharmacological Zn (0.08 vs. 0.0003 μmol/g, respectively). On d 24, there was no difference in isobutyrate concentrations between Zn treatments (0.02 vs. 0.04 μmol/g, respectively). Additionally, increasing folic acid increased (linear, $P = 0.030$) isobutyrate concentrations. Pigs fed pharmacological Zn had increased ($P = 0.044$) valerate concentrations compared to those fed 110 mg/kg added Zn from a total of 84 samples with detectable levels across all treatments. Acetate, valerate, and total SCFA concentrations were greater ($P \leq 0.053$) on d 24 compared with values on d 9.

Experiment 2

For phase 1 (d 0 to 10), there were no differences in d 10 BW, ADG, or ADFI with increasing folic acid; however, increasing folic acid decreased (linear, $P = 0.032$; Table 4.5) G:F. For phase 2 (d 10 to 23), d 23 BW, ADG, and ADFI decreased then increased (quadratic, $P \leq$

0.079) as folic acid increased, with pigs fed 20 mg/kg having the poorest performance. No treatment differences were observed for G:F. For phase 3 (d 23 to 38) and overall (d 0 to 38), BW, ADG, ADFI, and G:F decreased then increased (quadratic, $P \leq 0.047$) as folic acid increased, but similar to d 10 to 23, pigs fed 20 mg/kg of folic acid had the poorest performance.

A marginally significant linear treatment × day interaction was observed ($P = 0.069$; Table 4.6) for serum homocysteine concentrations. On d 10, an increase (linear, $P = 0.037$) in homocysteine concentration was observed as folic acid increased. However, no differences were observed among treatments on d 23. Pigs had greater ($P < 0.001$) serum homocysteine concentrations on d 10 compared to d 23.

Discussion

Folic acid is necessary for one-carbon transfer metabolic reactions, including nucleic acid synthesis, amino acid metabolism, and remethylation (Hayashi et al., 2007). Folic acid must be provided in the diet either from the ingredients or added in a vitamin premix. For folic acid to be used in one-carbon transfer reactions, including methionine metabolism, and act as a coenzyme, it needs to be reduced and biochemically activated. This involves a 2-step reduction with the conversion of folic acid to dihydrofolate (DHF) which is then converted to tetrahydrofolate (THF). Both conversions are facilitated by the enzyme DHFR and occur in the liver (Raghubeer and Matsha, 2021; Scaglione and Panzavolta, 2014). Folic acid must then be biochemically activated involving THF to be converted to 5,10-methyleneTHF and then converted to 5-methylTHF via 5,10-methylenetetrahydrofolate reductase (MTHFR; Raghubeer and Matsha, 2021). 5-methylTHF is used as a methyl donor in pyrimidine and purine synthesis and can donate a methyl group to convert homocysteine to methionine. The conversion reaction of

homocysteine to methionine is known as remethylation and is catalyzed by methionine synthase with vitamin B12 as a cofactor (Raghubeer and Matsha, 2021). The reduction and activation steps for folic acid metabolism are essential for its utilization in methionine metabolism and protein synthesis.

In the current experiment, we evaluated supra-nutritional additions of folic acid up to 40 mg/kg. The NRC (2012) requirement estimate for folic acid in 5 to 25 kg pigs is 0.30 mg/kg. However, the requirement estimate was established based on research in the 1980's and may not be representative of today's genetics (Easter et al., 1983; Lindemann and Kornegay, 1986). Recently, an industry survey by Faccin et al. (2023) found a range of 0.44 to 20.6 mg/kg of added folic acid in diets from weaning to 7 kg. Generally, vitamin toxicity is low for water-soluble B-vitamins compared with fat soluble vitamins (Rafeeq et al., 2020). Wang et al. (2020) found no improvement in ADG when adding up to 12.5 mg/kg of folic acid for nursery pigs. A different research group, Wang et al. (2021a, b), fed weanling pigs up to 18 mg/kg of added folic acid and observed a linear increase in ADG associated with increased cecum crypt depth, increased organ weight of liver, spleen, and kidney, and altered SCFA concentrations in the colon observed via increased isobutyrate concentration. The shift in SCFA metabolism results in increased energy efficiency because isobutyrate can be used as an energy source for colonocytes (Jaskiewicz et al., 1996). No other reports of this high of folic acid supplementation to weanling pigs are in the literature and therefore, Exp. 1 was designed to investigate folic acid additions of 0, 20, or 40 mg/kg with or without pharmacological additions of Zn. Because results of Wang et al. (2021 a, b) were linear, we fed up to 40 mg/kg folic acid to determine if there would be further improvements in growth performance beyond the levels previously evaluated. Pharmacological levels of Zn are known to reduce diarrhea and increase growth performance

(Carlson et al., 1999; Hill et al., 2001; Laskoski et al., 2021). The study by Wang et al. (2021 a,b) was conducted in diets without pharmacological levels of zinc. Consequently, by including added zinc in the factorial arrangement, we attempted to determine if the response to folic acid was dependent on the Zn level in the diet.

Based on results of Exp. 1, we decided to feed graded levels of folic acid (0, 5, 10, 20, and 40 mg/kg) to determine a dose-response curve. Results of both experiments reported herein observed decreased growth performance contradicting the linear increase observed by Wang et al. (2021b). The experimental diets used in the current study had a similar formulation strategy to Wang et al. (2021a, b) being based on corn and soybean meal with added lactose sources, and similar vitamin premixes, with the exception of Wang et al. (2021a, b) including pyridoxine, thiamine and biotin in their premix. However, our diets were not deficient in pyridoxine, thiamine, or biotin according to the NRC (2012) requirement estimates. Furthermore, our diets were fed for 38-d during both experiments, whereas studies by Wang et al. (2021a, b) fed experimental diets for 14 d. However, the negative effects on growth performance were observed by d 9 of our initial study and continued until the end of the experiment.

Folic acid is primarily absorbed in the duodenum and jejunum (Qiu et al., 2006; 2007). Weaning stress negatively impacts intestinal morphology and function by causing villus atrophy, crypt hyperplasia, and impaired barrier function and cell renewal (Yang et al., 2016). Folic acid supplementation to sows during gestation and lactation (Zhang et al., 2023), as well as during the early nursery period (Wang et al., 2021a) has been shown to improve intestinal morphology by enhancing proliferation and differentiation of intestinal cells through increasing the villus height-to-crypt depth ratio. Furthermore, folic acid has been reported to promote favorable conditions for the proliferation of beneficial bacteria by altering the intestinal microbial population

(Gurwara et al., 2019; Steinert et al., 2020; Mjaaseth et al., 2021). Folic acid supplementation modulates the gut microbiota by increasing the abundance of *Lactobacillus*, *Pediococcus*, and *Bifidobacterium*, while decreasing the abundance of *Bacteroides* in humans (Zheng et al., 2024). *Lactobacillus* and *Bifidobacterium* can utilize folic acid to positively regulate the gut microbiota (Degnan et al., 2014). This may be explained by the fact that *Lactobacillus* and *Bifidobacterium* can synthesize folic acid (Rossi et al., 2011). Previous research has reported a connection between SCFA concentrations and gut microbiota (Peng et al., 2013; Zheng et al., 2024).

An increase in SCFA concentrations can promote the intestinal health of weaned pigs and help mitigate the negative effects of weaning on the intestinal tract. The SCFAs play a crucial role in maintaining the morphology and function of intestinal epithelial cells, gut microbiota balance, and intestinal immune response (Liu et al., 2018). The SCFAs provide 60 to 70% of the energy for the colonic epithelium, with butyrate being the most energy-efficient SCFA, followed by propionate and acetate (Roediger, 1982). Additionally, SCFAs can inhibit the colonization and growth of *Salmonella* and *Escherichia coli* by lowering the pH of the large intestine (Shibata et al., 2017). Wang et al. (2021a) observed an increase in isobutyrate, isovalerate, and valerate concentrations in the colon when folic acid levels increased from 0 to 18 mg/kg. We observed a similar increase in isobutyrate concentration. The shift in SCFA metabolism may result in increased energy efficiency because isobutyrate can be used as an energy source for colonocytes (Jaskiewicz et al., 1996). However, in the current experiment, most pigs had nondetectable levels of isobutyrate. Within the samples that had detectable levels, the concentrations were low. In the current study, the small increase in isobutyrate concentration did not translate to increased growth performance.

In both experiments, a quadratic response to folic acid was observed for growth performance with a reduction observed between 0 and 20 mg/kg of folic acid followed by an increase from 20 to 40 mg/kg of folic acid. Wang et al. (2020) also observed a similar quadratic response to folic acid for feed efficiency with a reduction observed between 0 and 2 mg/kg of folic acid followed by an increase from 2 to 12.5 mg/kg of folic acid. A possible explanation for the reduction in G:F at 20 mg/kg in our study may be due to the oversupply of folic acid resulting in a downregulation of the enzymes necessary for folic acid and methionine metabolism. However, the slight increase in performance from 20 to 40 mg/kg of folic acid may be due to the body excreting more folic acid at 40 mg/kg compared to 20 mg/kg. Because folic acid is a water-soluble vitamin, excess is excreted in urine. Folic acid is filtered in the glomerulus and is reabsorbed in the proximal renal tubule (Bailey and Ayling, 2009). When high folic acid doses are given, more folic acid is excreted in the urine due to exceeding the capacity of the liver and kidney. However, this threshold is not clearly defined, and variation exists in literature (Bailey et al., 2015). According to our data, growth performance was reduced when folic acid was fed up to 20 mg/kg, but at 40 mg/kg growth was comparable to lower folic acid feeding levels. This may partially be explained by this theory of excess folic acid being excreted via urinary excretion and thus reducing the downregulation of the enzymes involved with folic acid metabolism.

Homocysteine is an indicator of folic acid status (Bailey et al., 2015). Serum homocysteine concentrations are controlled by two metabolic pathways: (1) remethylation of homocysteine to methionine, which requires the presence of folic acid and vitamin B12 as coenzymes and occurs within the cell and in all body tissues or (2) transsulfuration of homocysteine to cysteine, which requires vitamin B6 (Vezzoli et al., 2020). In the current

experiment, serum homocysteine concentrations increased with increasing folic acid from 0 to 40 mg/kg on d 10 post-weaning suggesting that remethylation of homocysteine to methionine did not take place. This may be explained by a downregulation of 5-methylTHF and MTHFR resulting in a decrease in methyl groups to donate. However, cysteine concentrations were not measured in the current experiment. Another explanation may be that pigs had adequate levels of methionine from the diet and therefore didn't need to remethylate homocysteine.

The enzyme DHFR is needed to reduce folic acid in the liver and convert it to a form that can be utilized for one-carbon transfer reactions. However, the liver has low activity of DHFR and therefore, limited ability to reduce folic acid (Scaglione and Panzavolta, 2014). This limitation results in the inability to metabolize high doses of folic acid and leads to unmetabolized folic acid in circulation in the form of DHF. High levels of DHF can inhibit MTHFR (Smith et al., 2008). Without MTHFR and 5-methylTHF, no methyl groups are available for donation to facilitate the remethylation of homocysteine to methionine. Thus, high concentrations of homocysteine were likely observed in serum because of this limitation. High concentrations of homocysteine, or hyperhomocysteinemia, a major risk factor for the development of cardiovascular disease in humans because it can lead to endoethlial cell damage and reduced flexibility of vessels (Baszczuk and Kopczyński, 2014; Ganguly and Alam, 2015). Hyperhomocysteinemia also alters the metabolism and mobilization of the trace minerals iron, copper, and nickel resulting in elevated iron stores in pigs (Ambrosi et al., 1999).

During Exp. 1, numerically higher mortality was observed when pigs were fed 20 or 40 mg/kg of folic acid. Folic acid is not regarded as toxic because it is a water-soluble vitamin. Further research is needed to understand if the increased mortality is related to hyperhomocysteinemia; however, speculation for the mortality observed in Exp. 1 could be that

when high amounts of folic acid are fed and surpass the liver's capacity to reduce folic acid, high amounts of unmetabolized folic acid occur in circulation. The activity of natural killer cells has been reported to be decreased in older women having higher plasma levels of unmetabolized folic acid (Troen et al., 2006). Natural killer cells are large lymphocytes that play a role in the innate immune response mainly against viruses (Lemire et al., 2017). Natural killer cells respond via the production of pro-inflammatory cytokines. In the current study, all deceased pigs were transported to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS) for necropsy. The necropsy reports provided evidence that *Streptococcus suis* was the leading cause of death for the pigs and is a pathogen commonly identified in this research herd. Therefore, this leads us to speculate that high folic acid supplementation decreased the activity of natural killer cells and reduced the pig's ability to combat *Streptococcus suis* infection. However, the high mortality was not observed in Exp. 2, which was conducted in a different facility and with a different population of pigs. Additional research is warranted to further understand the possible connection between high dietary folic acid levels and increased mortality.

It is not known whether the addition of supra-nutritional levels of folic acid resulted in overgrowth of pathogenic bacteria resulting in the numerical increase in mortality in Exp. 1. However, it is important to note there are two antibiotics available for inhibiting folic acid synthesis and therefore hindering bacterial growth. Specifically, trimethoprim inhibits the enzyme DHFR which prevents the production of THF and ultimately reduces DNA synthesis and suppresses bacterial growth (Scocchera and Wright, 2017). Another antibiotic class, sulfonamides, inhibit the enzyme dihydropteroate synthase which is exclusive to bacteria (Kompis et al., 2005). Dihydropteroate synthase catalyzes the synthesis reaction of dihyropteroate, the immediate precursor to DHF, which is then reduced to THF (Kompis et al.,

2005). The end result is decreased DNA synthesis for bacterial growth. While not a specific objective of this experiment, it is important to note that the antimicrobial properties of these antibiotic classes are involved with inhibiting folic acid metabolism.

Pharmacological levels of Zn from ZnO were evaluated in Exp. 1 because previous researchers have observed reduced incidence of diarrhea and improved growth performance with Zn supplementation in nursery pigs (Carlson et al., 1999; Hill et al., 2001; Laskoski et al., 2021). In the current experiment, we observed increased growth performance immediately after weaning with pharmacological levels of Zn. However, the response was mostly lost during the common period when pigs were fed diets without Zn resulting in no overall benefit to pharmacological levels of Zn. Other researchers have also observed a benefit to Zn during the experimental period but it was not sustained throughout the overall nursery period (Batson et al., 2021; Stas et al., 2024). Additionally, pharmacological levels of Zn improved fecal consistency in the current experiment. This may be explained by the antibacterial properties associated with zinc and improved gut health (Sirelkhatim et al., 2015; Luise et al., 2024). Laskoksi et al. (2021) and Batson et al. (2021) also observed increased fecal consistency with the addition of pharmacological levels of Zn to nursery pig diets. Furthermore, the inclusion of pharmacological levels of Zn did not impact the growth or fecal consistency response to added folic acid.

In conclusion, supplementing folic acid in nursery pig diets and exceeding NRC (2012) requirement estimates resulted in no improvements in growth performance and increased serum homocysteine concentrations. The greatest negative impact was observed when pigs were fed 20 mg/kg of folic acid, regardless of Zn inclusion in the diet. Additionally, pigs fed pharmacological levels of Zn had improved growth performance during the experimental period, but the benefit was not sustained for the overall nursery period.

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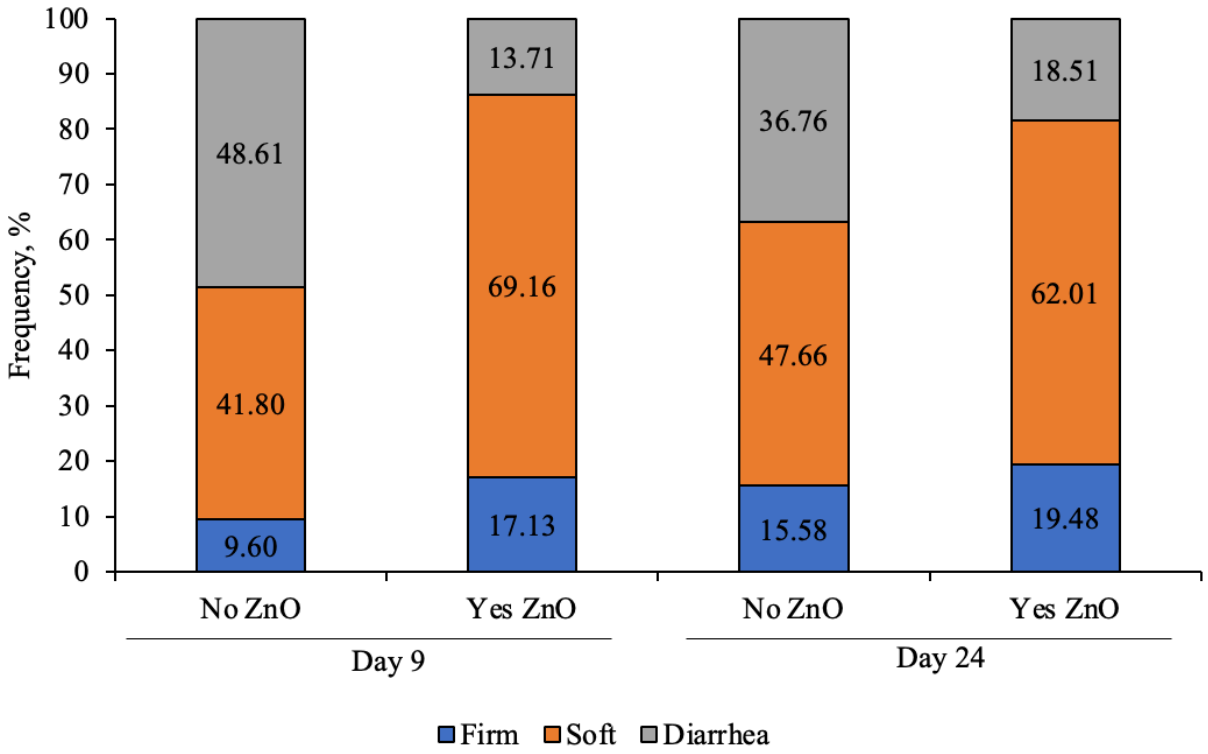

Figure 4.1. Fecal score frequency on d 9 and 24 by main effect of pharmacological levels of Zn. Zn × day, $P = 0.064$. Zinc Oxide was included in the diet to provide 3,000 mg/kg of Zn in phase 1 (d 0 to 9) and 2,000 mg/kg in phase 2 (d 9 to 24).

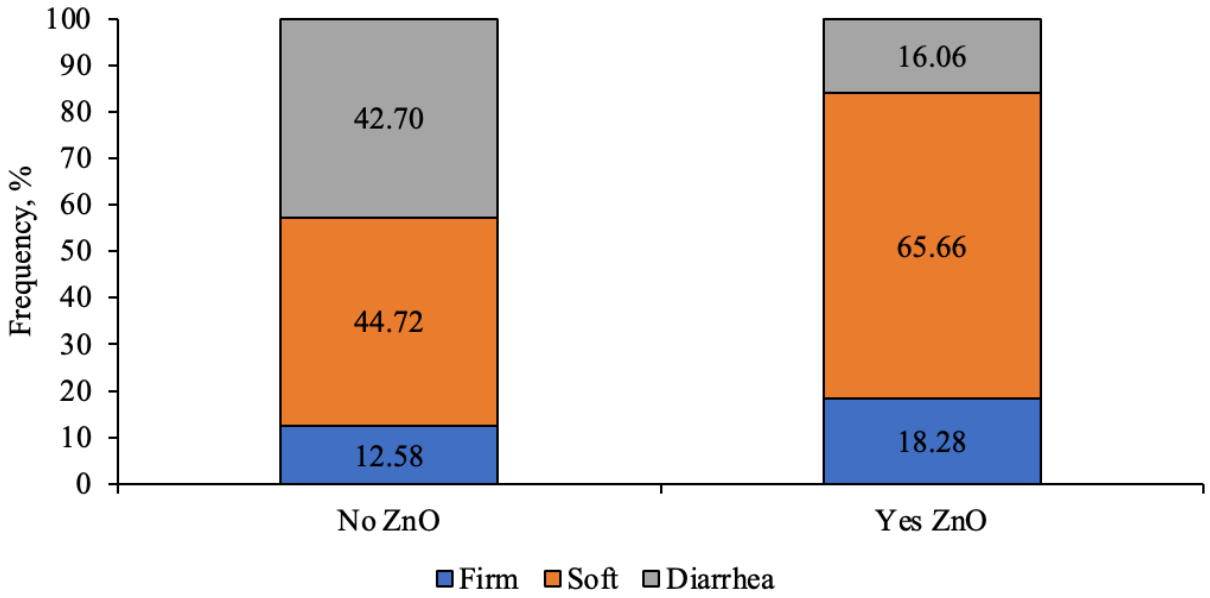

Figure 4.2. Fecal score frequency by main effect of pharmacological levels of Zn for d 9 and 24. Zn, $P < 0.001$. Zinc Oxide was included in the diet to provide 3,000 mg/kg of Zn in phase 1 (d 0 to 9) and 2,000 mg/kg in phase 2 (d 9 to 24).

Table 4.1. Diet composition (as-fed basis), Exp. 1[1]

Ingredient, %	Phase 1[2]	Phase 2[3]	Phase 3[4]
Corn	44.97	49.90	64.24
Soybean meal (47.7% CP)	17.34	23.55	31.78
Bovine blood plasma	2.00	---	---
DDGS	5.00	7.50	---
Fish meal	2.50	---	---
Whey powder	10.00	---	---
Whey permeate	10.00	10.00	---
Microbial-enhanced soy protein[5]	4.00	4.00	---
Choice white grease	1.00	1.00	---
Calcium carbonate	0.45	0.75	0.90
Monocalcium P (21% P)	0.80	1.00	1.00
Salt	0.30	0.50	0.60
L-Lys-HCl	0.40	0.55	0.50
DL-Met	0.18	0.22	0.21
L-Thr	0.17	0.22	0.21
L-Trp	0.02	0.04	0.04
L-Val	0.08	0.12	0.13
Vitamin and trace mineral premixes[6]	0.40	0.40	0.40
Zinc oxide[7]	+/-	+/-	---
Folic acid[8]	+/-	+/-	---
Total	100	100	100
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.35
Ile:Lys	57	57	56
Leu:Lys	120	118	115
Met:Lys	35	37	36
Met and cys:Lys	58	58	58
Thr:Lys	64	63	63
Trp:Lys	19.2	19.3	19.4
Val:Lys	70	70	70
Total Lys, %	1.51	1.51	1.50
NE, kcal/kg	2,553	2,500	2,418
SID Lys:NE, g/Mcal	5.83	6.15	5.68
CP, %	21.5	21.5	21.3
Ca, %	0.66	0.66	0.71
STTD P, %	0.58	0.51	0.47

[1]Dietary treatments were arranged in a 3 × 2 factorial with main effects of folic acid and pharmacological levels of Zn.

[2]Phase 1 diets were fed from d 0 to 9 (6 to 7 kg).

[3]Phase 2 diets were fed from d 9 to 24 (7 to 12 kg).

[4]A common phase 3 diet was fed to all pigs from d 24 to 38 (12 to 20 kg).

[5]ME-PRO, Prairie AquaTech, Brookings SD.

[6]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin; 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite. Ronozyme HiPhos (dsm-firmenich, Plainsboro, NJ) included at 1,250 FYT/kg provided an estimated release of 0.13% STTD P.

[7]Zinc oxide was included in the diet at the expense of corn to provide 3,000 mg/kg of Zn in phase 1 and 2,000 mg/kg in phase 2 in the treatment groups containing pharmacological levels of Zn from ZnO.

[8]Rovimix Folic Acid (dsm-firmenich, Plainsboro, NJ) included at the expense of corn at 0.0025% or 0.005% of the diet for the respective treatments to provide 0, 20, or 40 mg/kg folic acid.

Table 4.2. Diet composition (as-fed basis), Exp. 2[1]

Ingredient, %	Phase 1[2]	Phase 2[3]	Phase 3[4]
Corn	43.08	53.55	65.73
Soybean meal (47.7% CP)	17.25	23.36	30.52
Bovine blood plasma	2.00	---	---
DDGS	5.00	7.50	---
Fish meal	2.50	---	---
Whey powder	10.00	---	---
Whey permeate	10.00	5.50	---
Microbial-enhanced soy protein[5]	5.00	5.00	---
Soybean oil	2.00	1.00	---
Calcium carbonate	0.40	0.70	0.75
Monocalcium P (21% P)	0.75	1.00	0.90
Salt	0.30	0.50	0.60
L-Lys-HCl	0.40	0.55	0.48
DL-Met	0.18	0.21	0.19
L-Thr	0.17	0.23	0.21
L-Trp	0.03	0.04	0.04
L-Val	0.08	0.12	0.11
Vitamin and trace mineral premixes[6]	0.40	0.40	0.40
Zinc oxide	0.40	0.26	---
Folic acid[7]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.30
Ile:Lys	56	56	56
Leu:Lys	118	119	117
Met:Lys	35	37	36
Met and Cys:Lys	57	57	57
Thr:Lys	64	64	64
Trp:Lys	19.3	19.0	19.2
Val:Lys	69	70	69
Total Lys, %	1.51	1.51	1.44
NE, kcal/kg	2,595	2,485	2,429
SID Lys:NE, g/Mcal	5.20	5.43	5.35
CP, %	21.2	21.5	20.8
Ca, %	0.66	0.66	0.65
STTD P, %	0.58	0.51	0.46

[1]Dietary treatments consisted of increasing added folic acid.
[2]Phase 1 diets were fed from d 0 to 10 (6 to 7 kg).

[3]Phase 2 diets were fed from d 10 to 23 (7 to 13 kg).

[4]Phase 3 diets were fed from d 23 to 38 (13 to 22 kg).

[5]HP 300, Hamlet Protein, Findlay, OH.

[6]Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin; 110 mg Zn from zinc sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30mg I from calcium iodate; 0.30 mg Se from sodium selenite. Ronozyme HiPhos (dsm-firmenich, Plainsboro, NJ) included at 1,250 FYT/kg provided an estimated release of 0.13% STTD P.

[7]Rovimix Folic Acid (dsm-firmenich, Plainsboro, NJ) included at the expense of corn at 0.0006, 0.0013, 0.0025, or 0.005% of the diet for the respective treatments to provide 5, 10, 20, or 40 mg/kg folic acid.

Table 4.3. The effects of folic acid and pharmacological levels of Zn on nursery pig growth performance, Exp. 1[1]

	Pharmacological levels of Zn[2]							*P* =		
	No			Yes					Folic acid	
Folic acid, mg/kg[3]	0	20	40	0	20	40	SEM	Zn	Linear	Quadratic
BW, kg										
d 0	5.5	5.5	5.5	5.5	5.5	5.5	0.03	0.771	0.878	0.837
d 24	12.0	11.4	12.0	12.3	12.0	12.4	0.24	0.030	0.825	0.019
d 38	20.2	18.7	20.2	20.1	19.4	20.0	0.41	0.812	0.878	0.003
d 0 to 24 (experimental period)										
ADG, g	270	242	267	285	269	284	9.8	0.016	0.808	0.015
ADFI, g	353	318	348	369	348	371	11.8	0.019	0.896	0.009
G:F, g/kg	766	762	768	772	773	766	10.4	0.568	0.803	0.942
d 24 to 38 (common period)										
ADG, g	590	521	588	555	529	540	18.1	0.096	0.649	0.008
ADFI, g	835	748	830	808	781	793	22.1	0.573	0.647	0.008
G:F, g/kg	705	698	708	686	677	682	12.0	0.028	0.953	0.452
d 0 to 38										
ADG, g	388	342	382	385	360	375	11.2	0.761	0.497	0.002
ADFI, g	531	471	521	531	499	522	15.0	0.439	0.522	0.002
G:F, g/kg	732	726	733	724	721	721	7.0	0.154	0.867	0.522
Mortality, %	0.0	10.0	5.0	0.0	10.0	10.0	3.87	0.999	0.969	0.967

[1]A total of 360 pigs (initially 5.5 ± 0.03 kg) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 3 × 2 factorial with main effects of folic acid and pharmacological Zn. No folic acid × Zn interactions were observed ($P > 0.10$).

[2]Zinc oxide was included in the diet to provide 3,000 mg/kg of Zn from d 0 to 9 and 2,000 mg/kg from d 9 to 24.

[3]Rovimix Folic Acid (dsm-firmenich, Plainsboro, NJ).

Table 4.4. The effects of folic acid and pharmacological levels of Zn on nursery pig fecal characteristics, Exp. 1[1]

	Pharmacological levels of Zn[2]										
	No			Yes				Day			
Folic acid, mg/kg[3]	0	20	40	0	20	40	SEM	9	24	SEM	*P* =
DM, %[4]	19.7	20.5	21.3	21.9	22.0	21.0	0.80	20.3	21.8	0.42	0.007
SCFA[5]											
Acetate, µmol/g	55.2	51.3	51.0	52.0	53.6	51.1	3.76	51.0	54.7	1.91	0.042
N[6]	67	63	64	63	66	61	---	190	194	---	---
Butyrate, µmol/g	7.1	5.9	6.9	6.8	7.0	6.0	1.01	6.3	6.9	0.508	0.280
N[6]	48	41	47	44	46	39	---	122	143	---	---
Propionate, µmol/g	19.8	18.4	18.8	18.2	19.5	18.0	1.25	18.3	19.3	0.64	0.231
N[6]	65	61	63	63	66	59	---	183	194	---	---
Isovalerate, µmol/g[7]	0.54	0.77	0.62	0.27	0.38	0.59	0.172	0.58	0.47	0.096	0.388
N[6]	10	13	11	6	10	13	---	33	30	---	---
Valerate, µmol/g[8]	0.75	1.04	1.18	0.43	0.38	0.75	0.283	0.55	0.97	0.133	0.003
N[6]	15	19	19	8	9	14	---	26	58	---	---
Isobutyrate, µmol/g[9]	0	0.03	0.12	0	0.03	0.03	0.036	0.04	0.03	0.021	0.697
N[6]	0	1	3	0	1	1	---	3	3	---	---
Total SCFA, µmol/g	83.6	77.4	78.7	77.8	80.8	76.5	5.36	75.9	82.4	2.74	0.053

[1]A total of 360 pigs (initially 5.5 ± 0.03 kg) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 3 × 2 factorial with main effects of folic acid and zinc oxide. Fecal samples were collected individually from 3 pigs per pen (216 pigs total) on d 9 and 24. No folic acid × Zn × day, folic acid × day, Zn × day, or folic acid × Zn interactions were observed ($P > 0.10$) unless otherwise noted.

[2]Zinc oxide was included in the diet to provide 3,000 mg/kg of Zn from d 0 to 9 and 2,000 mg/kg from d 9 to 24.

[3]Rovimix Folic Acid (dsm-firmenich, Plainsboro, NJ).

[4]A marginally significant main effect of pharmacological levels of Zn was observed where pigs fed diets with added Zn had increased ($P = 0.080$) fecal DM.

[5]SCFA = short chain fatty acids. Concentrations were measured using a gas chromatographic method as described by Zhou et al. (2017) with minor modifications.

[6]A total of 72 samples per treatment were analyzed. Values represent the number of samples with detectable SCFA. Samples without detectable SCFA were considered as 0.00 concentration for analysis. There were no samples with detectable levels of isocaproate, caproate, or heptanoate.

[7]Zn × day, $P = 0.035$. On d 9, isovalerate was greater in fecal samples from pigs not fed pharmacological Zn compared to fecal samples from pigs fed pharmacological Zn (0.84 vs. 0.33 μmol/g, respectively; $P = 0.008$). On d 24, there was no difference in isovalerate between Zn treatments (0.44 vs. 0.50 μmol/g, respectively; $P = 0.781$).

[8]Main effect of ZnO, $P = 0.044$.

[9]Zn × day, $P = 0.087$. On d 9, isobutyrate was greater in fecal samples from pigs not fed pharmacological Zn compared to fecal samples from pigs fed pharmacological Zn (0.08 vs. 0.0003 μmol/g, respectively; $P = 0.052$). On d 24, there was no difference in isobutyrate between Zn treatments (0.02 vs. 0.04 μmol/g, respectively; $P = 0.577$). Linear effect of folic acid, $P = 0.030$.

Table 4.5. Effects of folic acid on nursery pig growth performance, Exp. 2[1]

	Folic acid, mg/kg[2]						*P* =	
Item	0	5	10	20	40	SEM	Linear	Quadratic
BW, kg								
d 0	6.0	6.0	6.0	6.0	6.0	0.06	0.759	0.482
d 10	7.4	7.4	7.3	7.1	7.2	0.20	0.115	0.255
d 23	12.8	12.5	12.7	12.1	12.4	0.21	0.068	0.069
d 38	22.5	21.8	21.9	20.9	21.6	0.54	0.023	0.002
d 0 to 10 (phase 1)								
ADG, g	138	139	139	115	125	15.4	0.127	0.315
ADFI, g	142	142	142	131	139	9.4	0.494	0.375
G:F, g/kg	959	974	968	868	886	57.1	0.032	0.458
d 10 to 23 (phase 2)								
ADG, g	421	392	406	384	397	10.2	0.203	0.079
ADFI, g	547	515	535	494	507	11.0	0.015	0.055
G:F, g/kg	770	761	757	779	783	13.0	0.148	0.780
d 23 to 38 (phase 3)								
ADG, g	642	624	616	587	618	24.9	0.059	0.001
ADFI, g	911	892	890	851	879	38.0	0.025	0.005
G:F, g/kg	706	699	693	689	703	8.9	0.948	0.049
d 0 to 38								
ADG, g	434	417	417	393	413	12.7	0.031	0.002
ADFI, g	584	566	570	539	557	17.6	0.014	0.008
G:F, g/kg	742	737	732	729	741	5.6	0.966	0.047

[1]A total of 350 barrows (initially 6.0 ± 0.06 kg) were used with 5 pigs per pen and 14 replications per treatment.
[2]Rovimix Folic Acid (dsm-firmenich, Plainsboro, NJ).

Table 4.6. Effects of folic acid on nursery pig serum homocysteine concentrations, Exp. 2[1]

	Folic acid, mg/kg[2]						*P* =	
Homocysteine, umol/L[3]	0	5	10	20	40	SEM	Linear treatment × day[3]	Folic acid, linear
d 10	19.2	20.9	22.2	26.9	29.8	4.70	0.069	0.037
d 23	14.5	17.9	15.2	24.8	17.7			0.450

[1]A total of 350 barrows (initially 6.0 ± 0.06 kg) were used with 5 pigs per pen and 14 replications per treatment. Blood samples were collected from one average weight barrow from each pen on d 10 and 23.

[2]Rovimix Folic Acid (dsm-firmenich, Plainsboro, NJ).

[3]Quadratic folic acid × day interaction also tested, $P = 0.193$. Main effect of day, $P < 0.001$.

Chapter 5 - A meta-regression analysis to evaluate the effects of narasin on grow-finish pig performance

Abstract[2]

Ionophores are feed additives that decrease gram-positive microbial populations by disrupting the ion transfer across cell membranes resulting in improved growth performance. Narasin (Skycis; Elanco Animal Health, Greenfield, IN) is an FDA-approved ionophore utilized for increased rate of weight gain and improved feed efficiency in growing-finishing pigs. A meta-regression analysis was conducted to evaluate the effects of added narasin in growing-finishing pig diets to predict its influence on average daily gain (ADG), feed efficiency (G:F), and carcass yield. A database was developed containing 21 technical reports, abstracts, and refereed papers from 2012 to 2021 representing 35 observations for growth performance data in studies ranging from 35 to 116 days in length (overall data). In addition, within these 35 observations, individual period data was evaluated (143 observations) using weekly, bi-weekly or monthly performance intervals (period data). Regression model equations were developed, and predictor variables were assessed with a stepwise manual forward selection procedure. The ADG model using the overall data included ADG, ADFI, and G:F of the control group, added narasin dose and narasin feeding duration categorized as longer or shorter than 65 days. Predictor variables included in the G:F model using overall data were ADG, ADFI, and G:F of the control

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[2] This work has been published in *Translational Animal Science*: Becker, L. L., J. T. Gebhardt, M. D. Tokach, R. A Arentson, M. Shields, J. C. Woodworth, R. D. Goodband, J. M. DeRouchey, J. A. Seltzer, and Chris. L. Puls. 2024. A meta-regression analysis to evaluate the effects of narasin on grow-finish pig performance. Transl. Anim. Sci. 8: txae099. doi:10.1093/tas/txae099.

group and added narasin dose. For carcass yield, the final model included ADFI and G:F of the control group, added narasin dose and narasin feeding duration of longer than 65 days. In the period model for ADG, the predictors included ADG, ADFI, and G:F of the control group, added narasin dose and average BW of the control group categorized into greater than or less than 105 kg. For period data for G:F, the model selected ADG, ADFI, and G:F of the control group and added narasin dose. Based on the results, the overall response to added narasin for ADG and G:F was quadratic and tended to decrease as ADG and G:F increased. A similar quadratic response was observed for the individual period data. In summary, using median values from the database for predictor variables, this meta-analysis demonstrated narasin would be expected to improve ADG between 1.06 to 1.65%, G:F between 0.71 to 1.71%, and carcass yield by 0.31% when fed continuously for longer than 65 days.

Introduction

Narasin is a polyether ionophore produced by the fermentation of *Streptomyces aureofaciens* (Berg and Hamill, 1978). Polyether ionophores have a hydrophobic region that allows them to interact with a cell's lipid bilayer and the hydrophilic region that forms binding sites for ions. The hydrophobic region allows the ionophore to pass through the lipid bilayers and once inside the cell membrane, the hydrophilic region of narasin interacts with the aqueous environment allowing it to selectively bind to the target ions, H+, K+, and Na+ (Wuethrich et al., 1998).

Narasin has a high affinity for cations such as Na+ and K+ and facilitates their transport across lipid bilayers (Caughey et al., 1985). Once narasin is bound to the K+ or Na+ ions, it shuttles them across the bacterial cell membrane, resulting in an increase in H+ concentration on the inside of gram-positive cells (Russell and Strobel, 1989). As a result, the Na+ K+ ATPase is

activated to transport excess H+ out of the cell. This activation depletes the gram-positive bacteria of energy and reduces fermentative functions and cell division. The ultimate result is a reduction in the number of gram-positive bacteria (Russell and Strobel, 1989).

As a result of a shift towards fewer gram-positive bacteria relative to gram-negative bacteria in the digestive tract, short chain fatty acid (SCFA) profiles are changed (Dawson and Boling, 1983). Gram-positive bacteria produce more acetate and butyrate whereas gram-negative bacteria produce more propionate. The reduction of gram-positive bacteria increases the relative proportion of propionate to acetate and butyrate (Richardson et al., 1976; Nagaraja et al., 1987). Propionate is an end product of carbohydrate fermentation and is more desirable than other SCFAs because it is gluconeogenic (Wolin, 1981). In ruminants, increased propionate production results in increased growth and feed efficiency (Ruan et al., 1976; Bergen and Bates, 1984; Barreras et al., 2013).

Ionophores have been widely used in diets for ruminants to improve growth performance (Ruan et al., 1976). Ionophores are also used in poultry diets to aid in control of parasitic diseases (Chapman et al., 2010) and now can be used to improve growth performance in pigs (Arkfeld et al., 2015). However, there is no summary of all available data indicating the expected magnitude of response when including ionophores, specifically narasin, in swine diets. Therefore, our objective of this meta-regression analysis was to evaluate the effects of added narasin in growing-finishing pig diets to predict the response in ADG, G:F, and carcass yield.

Materials and Methods

Database

A literature search was conducted utilizing CAB Direct to evaluate the effects of added narasin in diets for growing-finishing pigs. Key search terms included one of the following

terms: narasin, ionophore, Monteban, or Skycis (Elanco Animal Health, Greenfield, IN). In addition, Elanco Animal Health (Greenfield, IN) provided internal reports for trials with narasin fed to grow-finish pigs. Response criteria of interest from each trial were recorded in a spreadsheet template to compare performance of pigs fed diets without narasin (control pigs) and pigs fed diets with narasin. The percentage change in response was calculated for pigs fed narasin diets relative to the control pigs. Commonly reported data included year the study was completed, length of the trial, narasin dosage, diet composition, gender, narasin feeding duration, initial and final body weight, ADG, ADFI, G:F, hot carcass weight (HCW), carcass yield, backfat, percentage lean, and loin depth.

Results of the initial search yielded 24 papers. However, 3 such papers were excluded: 1 paper for observing treatment diet × gender interactions, and 2 papers for initial BW of less than 21 kg. The final database contained data from 21 papers from 2012 to 2021 representing 35 observations for growth performance data in studies ranging from 35 to 116 days in length (overall data). In addition, within these 35 observations, individual period data was evaluated (143 observations) using weekly, bi-weekly or monthly performance intervals (period data). Of the 21 papers included, 9 were internal reports (Elanco Animal Health, Greenfield, IN), 10 were peer reviewed publications, and 2 were technical reports (JBS United technical bulletin and Kent Nutrition Notes).

In the final database, studies ranged from initial BW of 23 kg to a final BW of 139 kg and the narasin feeding duration ranged from 35 to 116 days (Table 5.1). The database consisted of research conducted using 15, 20 or 30 mg/kg of narasin. For the overall trial data, there were a total of 35 observations with 21 observations comparing dosages of 0 to 15 mg/kg of narasin, 4 observations comparing 0 to 20 mg/kg of narasin, and 4 observations comparing 0 to 30 mg/kg

of narasin. Additionally, there were 4 studies where narasin dosage increased during the study (Table 5.2). Dosages increased from 0 to 15 mg/kg (4 observations) or increased from 15 to 30 mg/kg (2 observations). For the carcass yield model, there were a total of 24 observations for the overall data. There were 15 observations comparing 0 to 15 mg/kg of narasin, 2 observations comparing 0 to 20 mg/kg, and 3 observations comparing 0 to 30 mg/kg. Additionally, there were 3 observations where narasin dosage increased from 0 to 15 mg/kg during the study, and 1 observation where narasin increased from 15 to 30 mg/kg during the study.

In the period database, there were a total of 143 observations. Ninety-nine of those observations were comparing 0 to 15 mg/kg, 14 observations compared 0 to 20 mg/kg, and 30 observations compared 0 to 30 mg/kg of narasin.

Statistical analysis

Models were created with the relative change in response criteria between the control pigs and pigs fed narasin as the outcome. The period data model considered data with single continuous narasin feeding periods (weekly, bi-weekly or monthly periods) while the overall model considered complete data sets representing periods with and without narasin feeding.

Regression equations were developed using the GLIMMIX procedure of SAS (Version 9.4, SAS institute, Cary, NC). Study was included as a random intercept when fitting models using period data due to multiple weigh periods occurring in each experiment. To begin model building, the single-variable model with the lowest Bayesian Information Criterion (BIC) was selected, and then additional predictor variables were assessed through a stepwise manual forward selection for final model inclusion (Holen et al., 2022). Predictor variables were required to provide an improvement of at least 2 BIC units to be included in the final model. When the

model with the lowest BIC was obtained, visual assessment of studentized residual plots was performed to assess model assumptions for ADG, G:F, and carcass yield.

Regression models were developed to predict the percentage change in response for ADG, G:F, and carcass yield by comparing pigs fed diets with or without narasin. The predictor variables evaluated in the statistical model to predict the change in ADG and G:F included added narasin, feeding duration category (longer or shorter than 65 days), average BW category (less than or greater than 105 kg), days of narasin feeding, initial and final BW of the control pigs, and ADG, ADFI and G:F of the control pigs. The same predictor variables were evaluated in the carcass yield model as the ADG and G:F models, with the addition of HCW, backfat, percentage lean, and loin depth of the control pigs. The significant predictors used in the models included ADG, ADFI, and G:F of the control group, feeding duration, added narasin dose, and average BW. The regression equations can be used to estimate the expected percentage change for ADG, G:F, and carcass yield when feeding narasin to growing-finishing pigs (Table 5.3).

Categories were created for feeding duration and average BW within the database. To determine ranges for each category, a scatter plot was created to display the distribution and variation for each response. A natural break in the data was observed at 65 days for feeding duration and 105 kg for average BW. The feeding duration category was created with the goal of determining the effect of longer or shorter feeding durations of narasin on improving growth performance in pigs. The average BW category was created with the goal of determining the effect of feeding narasin to heavy or light weight pigs.

Results

Overall data

Results from the overall database for improvements in ADG ranged from 0 to 5.79% with an average response of 1.61% for all narasin dosages. For the ADG model, significant predictor variables were ADG of the control pigs (quadratic), ADFI of the control pigs (quadratic), G:F of the control pigs (quadratic), added narasin dose (quadratic), and feeding duration. The observed predicted improvements in ADG were influenced by control pigs' ADG for overall data when narasin is fed for longer than 65 days. The regression curve fit based on predicted performance shows that the response to narasin is quadratic and tends to decrease as control ADG increases (Figure 5.1).

The range of ADG improvement for observations comparing pigs fed a control diet versus a diet with 15 mg/kg of narasin was 0 to 5.26% with an average improvement of 1.71%. Results from the meta-regression analysis indicated that feeding diets with 15 mg/kg of narasin increased ADG by 1.21% when fed for longer than 65 days when using median values for control pigs (G:F, 0.379; ADG, 0.960 kg; ADFI, 2.533 kg calculated by dividing ADG and G:F). However, when feeding 15 mg/kg of narasin for less than 65 days, the meta-analysis found that ADG improved by 0.33% when using median values for control pigs (G:F, 0.413; ADG, 0.990 kg; ADFI, 2.397 kg). Thus, indicating that a longer feeding duration of narasin results in a greater improvement of ADG.

When comparing pigs fed a control diet versus a diet with 20 mg/kg of narasin, the range of ADG improvement was 0 to 1.71% with an average of 1.03%. The model predicts an average of 1.10% when using median values for control pigs (G:F, 0.379; ADG, 0.960 kg; ADFI, 2.533 kg) and fed for longer than 65 days. When feeding 20 mg/kg of narasin for less than 65 days, the

regression predicts an improvement in ADG of 0.23%. However, within the database, there was only 1 observation where 20 mg/kg was fed for less than 65 days. Thus, further research is warranted to determine the effect of feeding 20 mg/kg of narasin for less than 65 days.

Within the database for the comparison of pigs fed a control diet versus a diet with 30 mg/kg of narasin, the range of ADG improvement was 1.39 to 5.79% with an average of 3.02%. The model predicts an average of 1.65% when using median values for control pigs (G:F, 0.379; ADG, 0.960 kg; ADFI, 2.533 kg), and fed for longer than 65 days. When feeding 30 mg/kg of narasin for less than 65 days, the regression predicts an improvement in ADG of 0.77%. Similar to the lower dosages of narasin, the response in ADG is greater when narasin is fed for a longer feeding duration.

In summary for ADG, the regression analysis predicts an improvement in ADG of 1.21% at 15 mg/kg of added narasin, 1.10% at 20 mg/kg, and 1.65% at 30 mg/kg when fed for longer than 65 days. The raw data in the database indicated a greater improvement in ADG when 30 mg/kg of narasin is fed compared to 15 mg/kg which is in agreement with the predicted improvements from the model. However, it's important to consider the low number of observations for 30 mg/kg as compared with 15 mg/kg of narasin.

For G:F, results from the overall database ranged from -1.41 to 3.16% with an average response of 0.96% for all narasin dosages. There were two competing models with a similar fit. Model 1 had a BIC of 59.81 and included the following predictor variables: control ADG (quadratic), control ADFI (quadratic), control G:F (quadratic), and added narasin dose (quadratic). Model 2 had a BIC of 56.24 and included the same predictor variables as model 1 with the addition of narasin feeding duration. Within the database, there are only 9 observations where narasin is fed for less than 65 days. For these 9 observations, the range was from -1.17 to

2.77% with the average response being a 0.30% increase in G:F. Due to the low number of observations used in this model for short feeding duration, the regression equation predicted a negative improvement in G:F when including narasin in the diet. With only 9 observations for the short feeding duration, model 2 was not able to accurately predict improvements in G:F. Therefore, model 1 was selected as the final model (Figure 5.2).

When comparing pigs fed a control diet versus a diet with 15 mg/kg of narasin, the range of G:F improvement was -1.41 to 3.16% with an average of 1.10%. The model predicted an average of 0.99% when using median values for control pigs (G:F, 0.379; ADG, 0.966 kg; ADFI, 2.549 kg). Additionally, when comparing pigs fed a control diet versus a diet with 20 mg/kg of narasin, the range of G:F improvement was -1.17 to 1.94% with an average of 0.82%. The model predicted an average of 1.10% when using median values for control pigs (G:F, 0.379; ADG, 0.966 kg; ADFI, 2.549 kg). In comparison to the average response of 0.82% in the database, the G:F model may slightly over predict the response in G:F from feeding 20 mg/kg. However, it is important to consider there are only 4 observations in the database with doses of 20 mg/kg.

Within the database comparing pigs fed a control diet versus a diet with 30 mg/kg of narasin, the range of G:F improvement was -0.79 to 1.98% with an average of 1.01%. The model predicted an average of 0.71% when using median values for control pigs (G:F, 0.379; ADG, 0.966 kg; ADFI, 5.549 kg). Unlike ADG, feeding 30 mg/kg of added narasin did not further improve G:F compared to 15 mg/kg of added narasin (0.71% vs 0.99% for 30 and 15 mg/kg of added narasin respectively).

In summary for G:F, the regression analysis predicted an improvement in G:F of 0.99% at 15 mg/kg of added narasin, 1.10% at 20 mg/kg, and 0.71% at 30 mg/kg. With the exception of

the narasin dose of 20 mg/kg, the predicted improvements align with the observed raw data in the database.

Results from the overall database for improvements in carcass yield ranged from -0.40 to 1.18% with an average response of 0.22% for all narasin dosages. The predictor variables that were included in the carcass yield model were control ADFI (quadratic), control G:F (quadratic), added narasin dose (quadratic), and narasin feeding duration. The observed and predicted improvements in carcass yield as influenced by control G:F for overall data are relatively consistent as G:F increased (Figure 5.3). For comparing pigs fed a control diet versus 15 mg/kg, the range of yield improvement was -0.40 to 1.18% with an average of 0.37%. The model predicted an average of 0.31% when using median values for control pigs (G:F, 0.367; ADFI, 2.549 kg) and fed for longer than 65 days. With only 2 observations comparing a control versus 20 mg/kg, the range of values were -0.40 and 0.13%. Thus, results from the meta-regression analysis suggest that feeding 20 mg/kg resulted in no benefit in carcass yield (-0.02%) when using median values for G:F (0.367), ADFI (2.549 kg), and narasin fed for longer than 65 days. Furthermore, when comparing a control diet versus diet with 30 mg/kg of narasin, the range of yield improvement was -0.13 to 0.53% with an average of 0.18%. The model predicted an average of 0.13% when using median values for control pigs (G:F, 0.367; ADFI, 2.549 kg), and fed for longer than 65 days. When narasin was fed for less than 65 days at 15, 20, or 30 mg/kg, no benefit in carcass yield was predicted using the regression equations.

In summary for carcass yield, the regression analysis predicted an improvement in carcass yield of 0.31% at 15 mg/kg narasin and 0.13% at 30 mg/kg. No benefit in carcass yield was predicted when feeding 20 mg/kg of narasin.

Period data

For results from the database consisting of period data, the improvements in ADG ranged from -9.06 to 12.86% with an average response of 1.49% for all narasin dosages. The significant predictor variables in the ADG regression equation were control ADG (quadratic), control ADFI (quadratic), control G:F (quadratic), added narasin dose (quadratic), and average body weight category. The observed and predicted improvements in ADG for period data models have less variation compared to the overall data models because there are more observations included in the period data (Figure 5.4).

The range of ADG improvement when comparing a control diet versus a diet with 15 mg/kg was the same as the overall database (-9.06 to 12.86%) with an average of 1.43%. The model predicted an average of 1.53% when fed to pigs with an average BW of less than 105 kg and using median values for control pigs (G:F, 0.400; ADG, 0.930 kg; ADFI, 2.325 kg). When feeding 15 mg/kg of narasin to pigs with an average BW of greater than 105 kg, the meta-analysis suggested an improvement in ADG of 0.57% when using median values for control pigs (G:F, 0.322; ADG, 0.953 kg; ADFI, 2.960 kg). Thus, feeding narasin to lighter BW pigs resulted in a greater improvement of ADG than heavier BW pigs when using the period data model.

Within the database comparing pigs fed a control diet versus a diet with 20 mg/kg, the range of ADG improvement was -0.87 to 6.82% with an average of 1.46%. The model predicted an average of 1.06% when using median values for control pigs (G:F, 0.400; ADG, 0.930 kg; ADFI, 2.325 kg) with an average BW of less than 105 kg. When 20 mg/kg of narasin was fed to pigs with an average BW heavier than 105 kg, the predicted improvement in ADG was 0.10% when using median values for control pigs (G:F, 0.322; ADG, 0.953 kg; ADFI, 2.960 kg). Additionally, the range of ADG improvement for comparing a control diet versus a diet with 30

mg/kg of narasin was -8.45 to 11.25% with an average of 1.68%. The model predicted an average of 1.59% when using median values for control pigs (G:F, 0.400; ADG, 0.930 kg; ADFI, 2.325 kg) with an average BW of less than 105 kg. When 30 mg/kg of narasin was fed to pigs with an average BW greater than 105 kg, the predicted improvement in ADG was 0.63% when using median values for control pigs (G:F, 0.322; ADG, 0.953 kg; ADFI, 2.960 kg).

In summary for ADG, the regression analysis predicted an improvement in ADG of 1.53% at 15 mg/kg of added narasin, 1.06% at 20 mg/kg, and 1.59% at 30 mg/kg when fed to pigs weighing less than 105 kg. For pigs weighing greater than 105 kg, an improvement of 0.57% at 15 mg/kg of added narasin, 0.10% at 20 mg/kg, and 0.63% at 30 mg/kg was predicted. The predicted improvements for the period ADG model aligned with the predicted changes for the overall ADG model where a slightly lower improvement was observed for 20 mg/kg of narasin compared to 15 or 30 mg/kg.

A regression equation was also developed for G:F for period data. Results from the database for improvements in G:F ranged from -4.26 to 6.80% with an average response of 1.04% for all narasin dosages. The predictor variables included in the G:F regression equation were control ADG (quadratic), control ADFI (quadratic), and control G:F (quadratic), and added narasin dose (quadratic). The observed and predicted improvement in G:F that was influenced by control G:F for period data had a quadratic response to narasin and tended to decrease as G:F increased (Figure 5.5). Within the database, the range of G:F improvement for comparing pigs fed a control versus 15 mg/kg of narasin was -4.26 to 6.80% with an average of 0.95%. The model predicted an average of 1.20% when using median values for control pigs (G:F, 0.363; ADG, 0.939 kg; ADFI, 2.587 kg).

When comparing a control diet versus a diet with 20 mg/kg of narasin, the range of G:F improvement was -0.64 to 5.94% with an average of 1.89%. The model predicted an average of 1.71% when using median values for control pigs (G:F, 0.363; ADG, 0.939 kg; ADFI, 2.587 kg). Furthermore, for the comparison of a control versus 30 mg/kg of narasin, the range of G:F improvement for pigs fed 30 mg/kg of narasin was -2.85 to 6.34% with an average of 0.92%. The model predicted an average of 0.80% when using median values for control pigs (G:F, 0.363; ADG, 0.939 kg; ADFI, 2.587 kg).

In summary for G:F, the regression model predicted an improvement in G:F of 1.20% at 15 mg/kg inclusion of narasin, 1.71% at 20 mg/kg, and 0.80% at 30 mg/kg. The predicted improvements for the period G:F model align with the predicted improvements for the overall G:F model where a slightly greater improvement was observed for 20 mg/kg of narasin compared to 15 mg/kg.

Discussion

Narasin improves the efficiency of microbial fermentation in the gut leading to increased production of SCFAs, specifically propionate, which are used as energy sources by the pig (Wuethrich et al., 1998). Propionate production is more desirable because it is energy efficient as the liver converts propionate to glucose which can be utilized as an energy source (Wolin, 1981; Wuethrich et al., 1998). Research conducted in ruminants has shown that increased propionate production resulted in increased growth and feed efficiency (Ruan et al., 1976). The same result can be expected in swine because of the similar microbial environment (Salanitro et al., 1977). Therefore, ADG and feed efficiency are included as predictor variables in the regression models because of the effect narasin has on improving nutrient utilization, resulting in enhanced feed conversion and growth performance.

The overall ADG model resulted in a similar predicted response to narasin compared to the raw data in the database (1.21 vs 1.71% for model prediction and raw data, respectively for 15 mg/kg of added narasin). The period model for ADG also resulted in a similar predicted response to narasin compared to the raw data in the database (1.53 vs 1.43% for model prediction and raw data, respectively for 15 mg/kg of added narasin).

The ADG response to narasin is quadratic and gradually decreased as ADG increased. This response was observed in both the overall model and period model (Figures 1 and 4, respectively). Narasin provides a greater benefit in terms of improving ADG in poorer performing pigs indicating that the SCFA shift toward propionate production is translated to improvements in growth more readily in poor performing pigs which are in a high demand for energy. However, when pigs have high ADG, they are already efficiently converting propionate and dietary contents to energy resulting in a lesser benefit to narasin.

The overall G:F model resulted in a similar predicted response to narasin compared to the raw data in the database (0.99 vs 1.10% for model prediction and raw data, respectively for 15 mg/kg narasin of added). For the ADG period model when comparing to the raw data, the model may be over predicting the response to narasin (1.20 vs 0.95% for model prediction and raw data, respectively for 15 mg/kg narasin dose). This may be due to the variation that occurs during individual growth periods consisting of weekly, bi-weekly or monthly performance intervals.

The G:F response to narasin is quadratic and decreased then increased as G:F improved for both the overall and period model (Figures 2 and 5, respectively). This could be due to limited observations at low or high G:F of control pigs. For example, in Figure 2 the overall database consisted of 2 observations with low G:F of 0.31 and 2 observations with high G:F of 0.47. With limited data at the extremes for G:F, the response to narasin is driven to be quadratic

because of the influence of these 4 observations. Furthermore, there is more variation in the response to narasin for the period data compared to the overall data (Figure 5.5).

Narasin also has the ability to influence carcass yield because of the effect on growth performance and energy utilization. When pigs have improved growth rates and high lean muscle development, it can contribute to increased carcass yield. In the database, there were 24 total observations reported for carcass yield. Sixteen observations reported increased carcass yield when narasin was added to the diet with 13 of those observations reporting improvements in feed efficiency and ADG. The improved growth performance is due to the effect narasin has on microbial fermentation resulting in increased energy production. The pig can utilize the energy towards increasing carcass weight rather than viscera weight. An increase in carcass weight results in increased carcass yield. Furthermore, research suggests that improved growth performance due to increased energy utilization increased HCW and positively benefited carcass yield (Smith et al., 1999; De la Llata et al., 2007; Bromm et al., 2023).

However, in the regression models, the benefit in carcass yield was only observed when narasin was fed for longer than 65 days. Narasin may provide cumulative growth benefits when supplemented over extended periods of time. The prolonged duration allows for continued inhibition of gram-positive bacteria and increased proportions of propionate production allowing for continued improvements in energy utilization and improved growth (Russell and Strobel, 1989).

In conclusion, this meta-regression analysis used available data to develop regression equations for predicting the percentage change in ADG, G:F, and carcass yield when feeding narasin to growing-finishing pigs. Important predictors used in the different models included ADG, ADFI, and G:F of the control group in the studies, feeding duration, added narasin dose,

and average BW. These regression equations can be used to estimate the expected percent change for ADG, G:F, and carcass yield when feeding narasin to finishing pigs using production system-specific performance estimates.

Implications

By using median values from the database for predictor variables, this meta-analysis demonstrated narasin would be expected to improve ADG between 1.06 to 1.65%, G:F between 0.71 to 1.71%, and carcass yield by 0.31% when fed for longer than 65 days.

Acknowledgements

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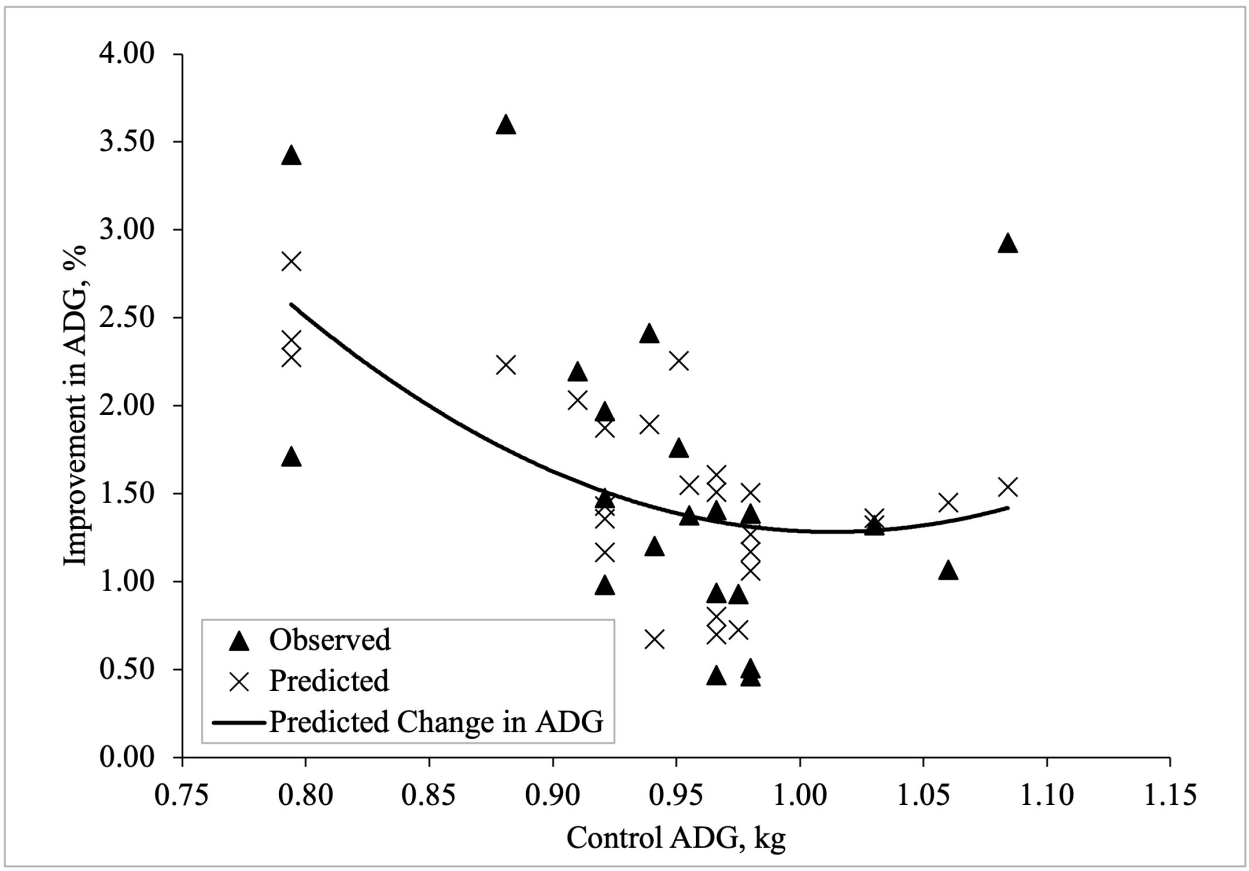

Figure 5.1. Observed and predicted improvements in ADG as influenced by control ADG (pigs fed 0 mg/kg narasin) for overall data when 15 mg/kg of narasin is fed for longer than 65 days. A regression curve was fitted from the predicted performance. There were a total of 21 observations comparing 0 to 15 mg/kg of narasin in the database.

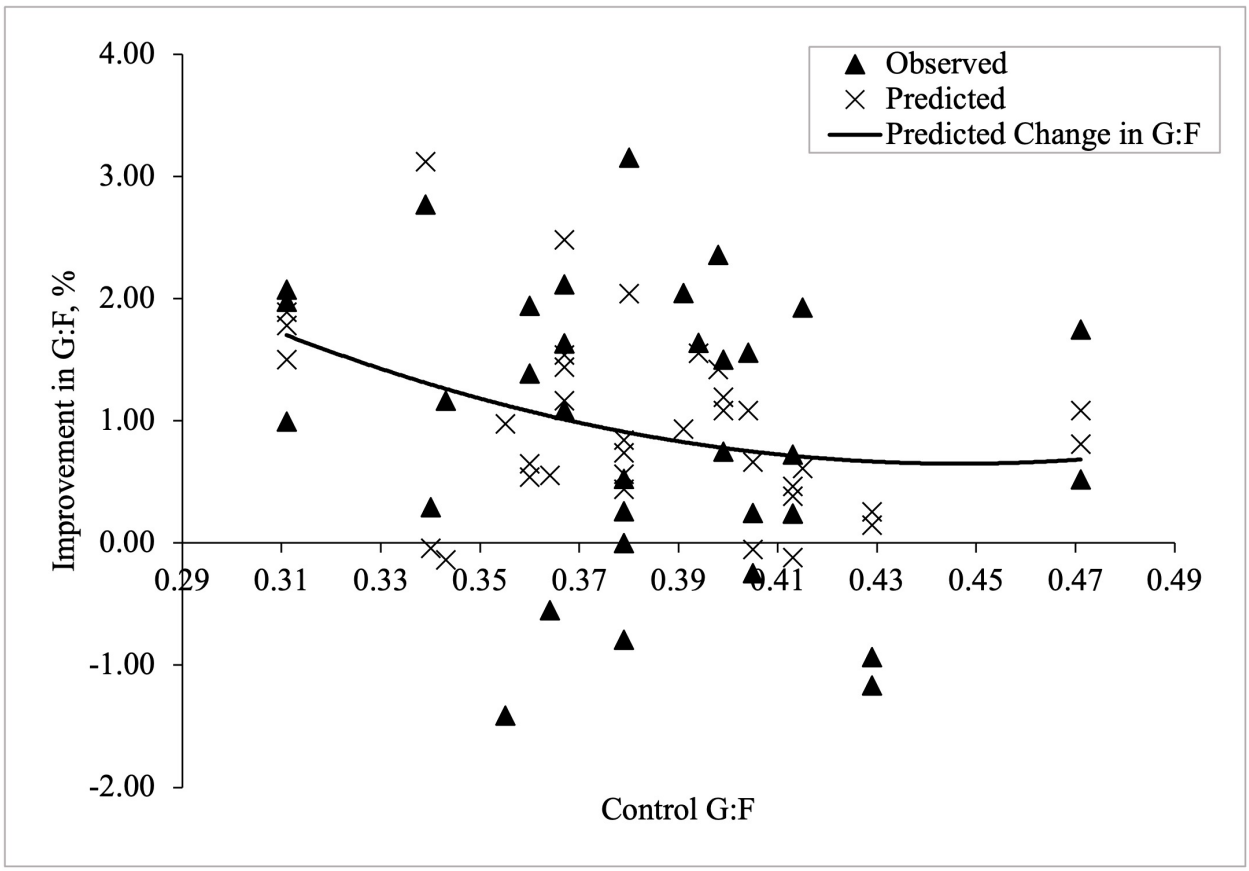

Figure 5.2. Observed and predicted improvements in G:F as influenced by control G:F (pigs fed 0 mg/kg of narasin) for overall data when 15 mg/kg of narasin is fed. A regression curve was fitted from the predicted performance. There were a total of 21 observations comparing 0 to 15 mg/kg of narasin in the database.

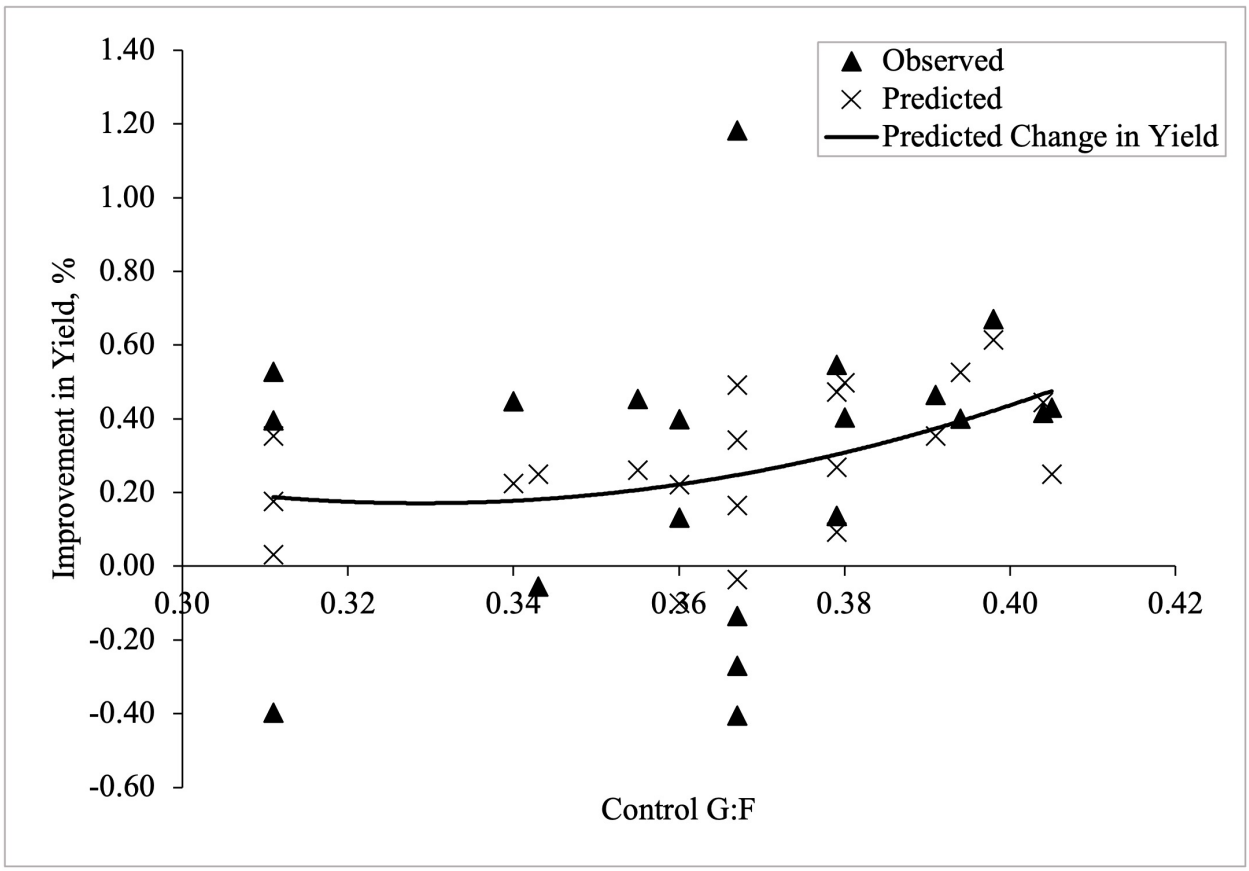

Figure 5.3. Observed and predicted improvements in carcass yield as influenced by control G:F (pigs fed 0 mg/kg of narasin) for overall data when 15 mg/kg of narasin is fed for longer than 65 days. A regression curve was fitted from the predicted performance. There were a total of 15 observations comparing 0 to 15 mg/kg of narasin in the database.

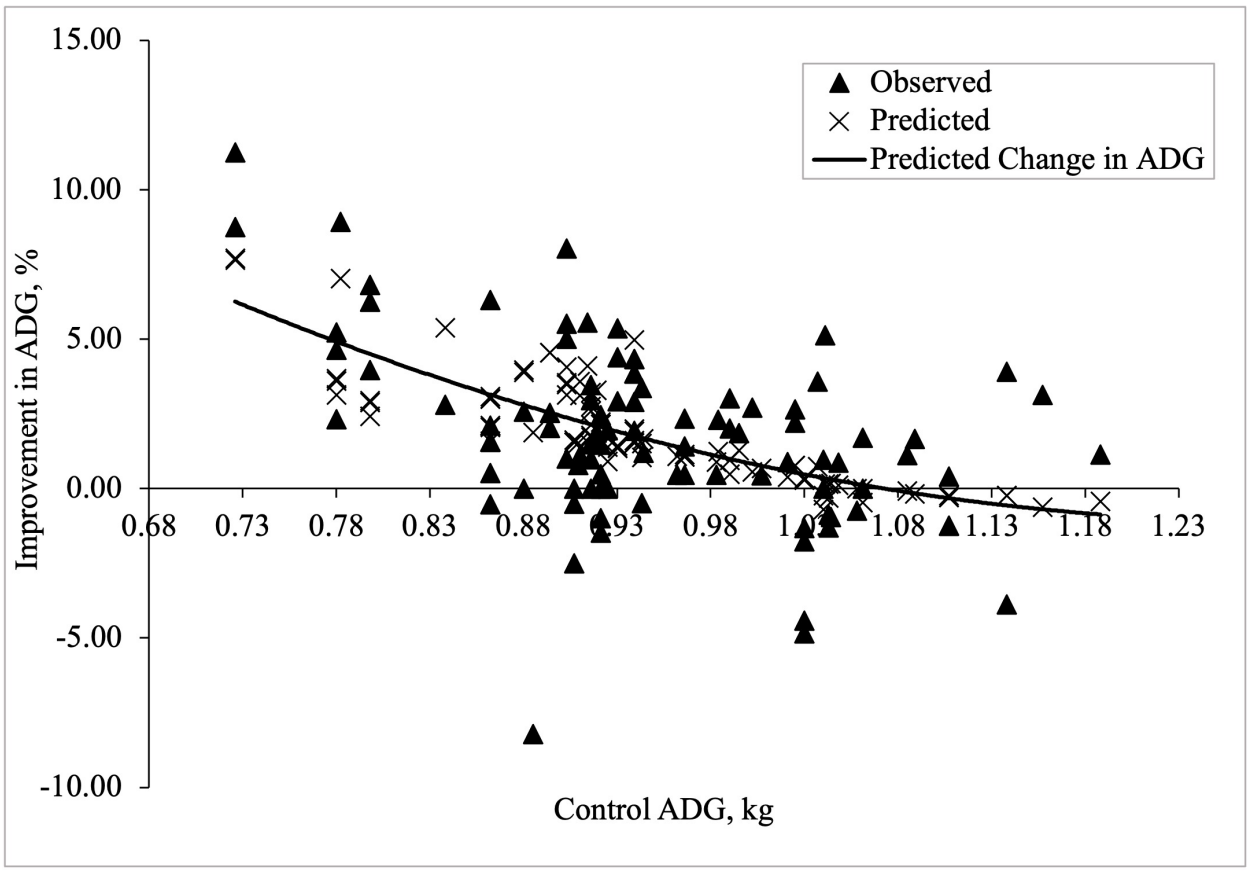

Figure 5.4. Observed and predicted improvements in ADG as influenced by control ADG (pigs fed 0 mg/kg of narasin) for period data when 15 mg/kg of narasin is fed to pigs lighter than 105 kg. A regression curve was fitted from the predicted performance. The individual period data considered data with single continuous narasin feeding periods (weekly, bi-weekly or monthly periods). There were a total of 99 observations comparing 0 to 15 mg/kg of narasin in the database.

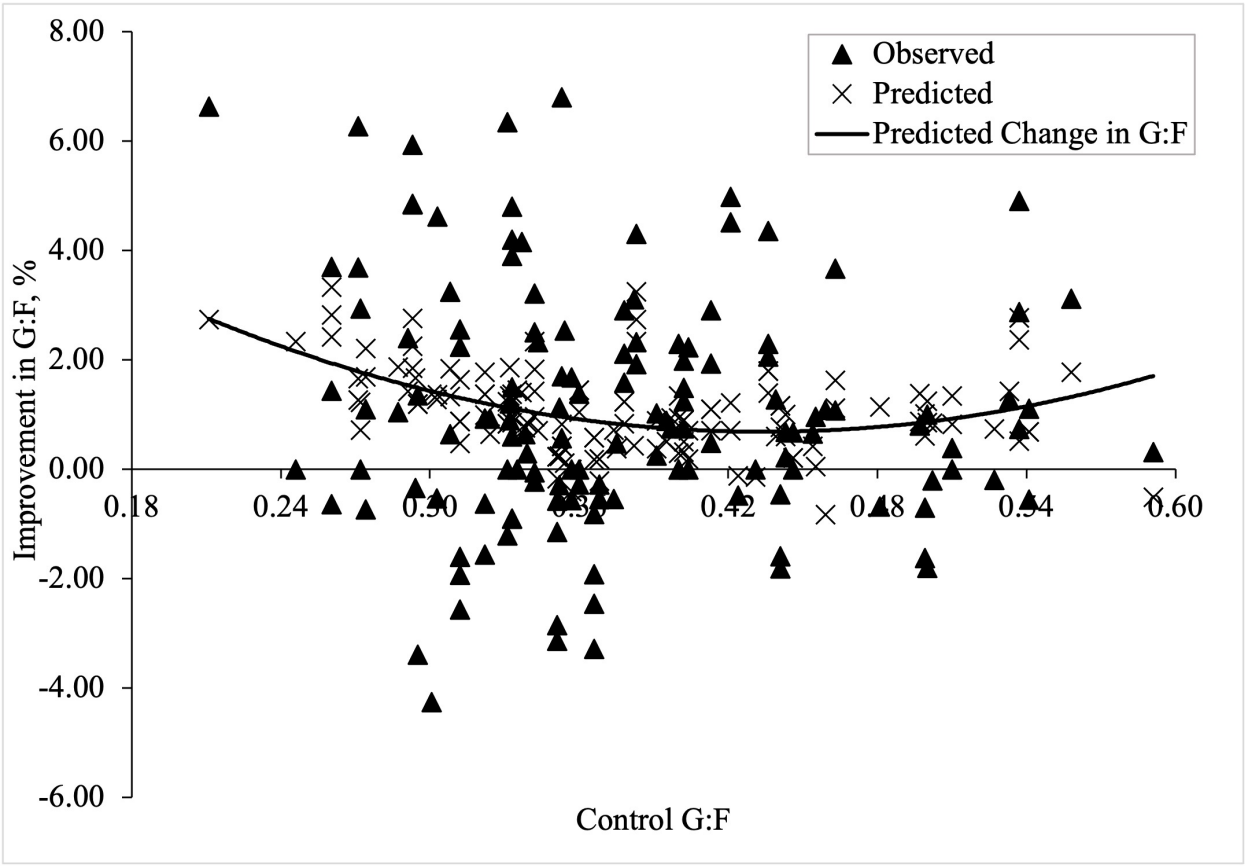

Figure 5.5. Observed and predicted improvements in G:F as influenced by control G:F (pigs fed 0 mg/kg of narasin) for period data when pigs were fed 15 mg/kg of narasin. A regression curve was fitted from the predicted performance. The individual period data model considered data with single continuous narasin feeding periods (weekly, bi-weekly or monthly periods). There were a total of 99 observations comparing 0 to 15 mg/kg of narasin in the database.

Table 5.1. Effect of narasin on growth performance of growing-finishing pigs for overall data

Publication	Feeding duration, d	Initial BW, kg	Final BW, kg	Change, %				
				BW	ADG	ADFI	G:F	Carcass yield
0 vs 15 mg/kg of narasin								
Report T2NUS120004, 2012	85	48.19	121.53	0.649	0.930	-0.476	1.166	-0.054
Report 12S03, 2013	69	29.48	95.16	1.239	2.415	0.760	1.643	0.401
JBS United Report, 2013	75	61.19	128.28	0.670	1.714	-0.356	2.078	0.397
Arentson et al., 2013	91	24.99	100.50	0.452	0.509	-1.630	2.046	0.466
Report ELAUS140179, 2014	102	39.60	124.10	3.947	1.970	3.304	-1.408	0.454
Report T2NUS130009, 2014	56	47.22	102.92	0.309	0.000	0.335	-0.549	---
Greiner et. al, 2014	63	27.00	89.37	0.000	0.000	0.000	-0.932	---
Report ELAUS150089, 2015	98	26.26	130.14	0.697	1.070	1.031	0.247	0.432
Report ELAUS150321, 2015	35	84.41	124.78	0.582	2.926	0.402	2.772	-0.208
Knauer et al., 2015	90	22.95	120.57	2.295	2.929	0.868	1.928	---
Arentson et al., 2016	50	26.72	69.49	3.525	5.263	4.715	0.524	---
Edmonds, 2016	110	22.91	119.85	2.914	3.603	1.453	2.119	1.183
Rickard et al., 2017	105	43.89	133.63	1.514	1.205	0.932	0.294	0.449
Report ELA210244, 2021	111	39.05	139.12	1.109	1.389	1.226	0.000	0.546
Report ELA210431, 2021	116	28.76	134.40	1.147	1.322	1.457	0.242	---
Report ELAVV200324, 2021	111	42.68	131.95	0.997	0.985	-0.542	1.635	-0.404
Ewing et al., 2021	89	40.50	121.60	0.822	2.198	-1.667	3.158	0.404
Linneen et al., 2021 (Exp. 1)	109	23.28	126.38	1.256	1.378	0.233	2.361	0.671
Linneen et al., 2021 (Exp. 2)	110	26.39	129.42	1.391	1.765	0.650	1.560	0.417
Puls et al., 2021 (Exp. 1)	85	33.38	116.26	1.014	1.408	0.749	0.752	---
Puls et al., 2021 (Exp. 2)	113	28.03	123.79	1.136	0.939	-0.169	1.389	0.400
0 vs. 20 mg/kg of narasin								
JBS United Report, 2013	75	61.19	128.28	0.702	1.714	0.712	0.995	-0.397
Greiner et. al, 2014	63	27.00	89.37	0.705	1.010	-3.930	-1.166	---
Puls et al., 2021 (Exp. 1)	85	33.38	116.26	0.819	0.939	-0.187	1.504	---
Puls et al., 2021 (Exp. 2)	113	28.03	123.79	0.623	0.469	-1.351	1.944	0.133
0 vs. 30 mg/kg of narasin								
JBS United Report, 2013	75	61.19	128.28	1.380	3.429	1.423	1.977	0.529
Arentson et al., 2016	50	26.72	89.37	3.982	5.789	3.970	1.750	---
Report ELA210244, 2021	111	39.05	139.12	0.946	1.389	1.926	-0.792	0.137
Report ELAVV200324 , 2021	111	42.68	131.95	1.272	1.478	0.181	1.090	-0.135

[1]Database contained data from 21 studies for growth performance data. The percentage change in response was calculated for pigs fed narasin diets relative to the control pigs.

Table 5.2. Effects of narasin on growth performance when narasin inclusion rate changed over time of grow-finish pigs for overall data[1]

				Initial[2]		Final[3]		Change, %				
Publication	Trial duration, d	Initial BW, kg	Final BW, kg	Dose, mg/kg	Duration, d	Dose, mg/kg	Duration, d	BW	ADG	ADFI	G:F	Carcass yield
Report ELAUS150089, 2015	98	26.26	130.14	0	56	15	42	0.732	1.027	1.203	-0.247	-0.054
Report ELA210244, 2021	111	39.05	139.12	0	48	15	63	0.196	0.463	-0.175	0.264	-0.273
Report ELA210244, 2021	111	39.05	139.12	0	14	15	97	0.326	0.463	-0.175	0.528	0.137
Report ELA210431, 2021	116	28.76	134.40	0	57	15	59	-0.236	0.000	-0.182	0.242	---
Report ELA210431, 2021	116	28.76	134.40	15	57	30	116	0.810	1.322	0.729	0.726	---
Report ELAVV200324, 2021	111	42.68	131.95	15	56	30	111	0.859	0.985	-0.361	1.635	-0.270

[1]The percentage change in response was calculated for pigs fed narasin diets relative to the control pigs.
[2]Represents the narasin dose at the start of the research trial and how many days it was fed.
[3]Represents the narasin dose at the end of the research trial and how many days it was fed.

Table 5.3. Regression coefficients to predict percent change in ADG, G:F and carcass yield for overall and period data[1]

	Overall data models[2]			Period data models[3]	
Predictor variable:	ADG, kg	G:F	Carcass yield	ADG, kg	G:F
Added narasin, g/kg					
Linear term	-194.0400	165.3000	-248.7500	-438.6600	553.2900
Quadratic term	4972.6400	-4083.2400	5265.2100	9844.2300	-12889.0000
ADG of control group, kg					
Linear term	-155.5300	-189.2000	---	-20.3343	-11.7242
Quadratic term	69.1111	93.3874	---	3.9187	3.0086
ADFI of control group, kg					
Linear term	-20.1232	-22.5132	-13.0956	-7.3680	-5.9921
Quadratic term	4.9058	4.1902	2.3828	1.5496	1.0498
G:F of control group					
Linear term	204.6900	498.2900	-9.3526	-12.8490	23.2097
Quadratic term	-198.5700	-648.0100	12.5142	26.7571	-39.5517
Feeding duration category, d	1.3140	---	1.4097	---	---
Average BW category, kg	---	---	---	-0.6774	---
Intercept	57.7410	29.4398	21.0875	31.0257	9.4187

[1] Overall data represents growth performance data in studies ranging from 35 to 116 days in length. Within the overall data, period data was evaluated using weekly, bi-weekly or monthly performance intervals.
[2] A total of 35 observations using 15, 20, or 30 mg/kg of narasin. For the carcass yield model, there were a total of 24 observations.
[3] A total of 143 observations using 15, 20, or 30 mg/kg of narasin.

Chapter 6 - Effects of a pre-weaning socialization system on piglet livability, lifetime growth performance, and subsequent sow performance

Abstract

A total of 3,307 (PIC L 42) mixed parity sows and 55,160 (PIC 337 × L 42) piglets were used to determine the effects of different farrowing systems on piglet livability, lifetime growth performance, and subsequent sow performance. Treatments were assigned to farrowing rooms and consisted of a conventional farrowing system (sows and piglets housed in individual farrowing stalls) or a pre-weaning socialization system (stall dividers removed between farrowing stalls and walkways within 6 to 24 h post-farrowing such that 12 to 32 litters of piglets were co-mingled). A total of 40 farrowing rooms with 80 stalls each were used with 20 rooms per treatment. Pigs were weaned at approximately 23 d of age. No differences were observed in lactation length, total born, born alive, stillborn, mummies, or number of pigs weaned. Pre-wean mortality was increased ($P < 0.001$) for pigs from the pre-weaning socialization system compared to conventional farrowing system (14.7 vs 12.6%, respectively). At weaning, a subset of offspring (4,313 pigs initially 5.4 ± 0.15 kg) were transported to a commercial research facility to evaluate lifetime performance. Weaning weights were heavier ($P < 0.001$) for pigs in the conventional farrowing system compared to pre-weaning socialization system. Pigs were housed in pens according to sow treatment with 44 to 46 pigs per pen and 48 pens per treatment. During the nursery and finishing periods, pigs from the conventional farrowing system had increased ($P < 0.001$) BW, ADG, and ADFI, but decreased G:F compared to pre-weaning socialization system. In the nursery phase, removals, mortality, and total removals and mortality

were greater ($P \leq 0.059$) for pigs raised in the pre-weaning socialization system than the conventional farrowing system, but no differences were observed in the finishing phase. Overall (d 23 to 183), pigs from the conventional farrowing system had increased ($P \leq 0.001$) BW, ADG, and ADFI, but decreased ($P = 0.010$) G:F compared to pre-weaning socialization system. No differences were observed for overall removals and mortality after weaning. Pigs from the conventional farrowing system had increased ($P \leq 0.094$) live BW, HCW, carcass yield, loin depth, and percentage lean compared to the pre-weaning socialization system. No differences were observed in backfat. In summary, pigs raised in a conventional farrowing system had increased livability, lifetime growth performance, and improved carcass characteristics compared to the pre-weaning socialization system.

Key Words: Lactation, pre-weaning mortality, pre-weaning socialization system, sow

Introduction

Weaning is a stressful event in a pig's life due to maternal and littermate separation, diet and environmental changes, and social hierarchy establishment (Moeser et al., 2007; Wensley et al., 2021). Historically, there has been a focus on pre- and post-weaning nutritional strategies and post-weaning housing conditions to improve production and reduce morbidity and mortality after weaning (Pluske and Williams, 1996; Bruininx et al., 2002; Dividich, 2005). Recent research efforts have been made on alternative pre-weaning housing and management systems to promote the development of natural social behaviors in piglets before weaning because of their potential to ease the weaning transition (Van Kerschaver et al., 2021; Gainey, 2023; Schwartz, 2023).

A pre-weaning socialization system is described as the co-mingling of non-littermate piglets before weaning as a management strategy in which 2 or more unfamiliar litters can socialize during the lactation period (Kanaan et al., 2012). This system involves removal of dividers between farrowing stalls and allows piglets to freely socialize while the sow remains in her stall. Piglets are allowed to have their choice of sow to nurse and are exposed to larger social environments. By allowing piglets to nurse from multiple sows, it allows for the opportunity to increase milk intake leading to a reduction in piglet starvation and a potential reduction in pre-weaning mortality (Tang et al., 2023). Another potential route of reducing pre-weaning mortality with a socialization system is by allowing more space for the piglets to move away from the sow and avoid being laid on. By allowing piglets to co-mingle in walkways between farrowing stalls, more space is created encouraging piglets to move away from their mothers. Heat lamps, mats, and creep bowls can be placed in the walkways to further encourage piglets to co-mingle and avoid being laid on (Schwartz, 2023).

Pre-weaning socialization systems can also reduce aggressive behaviors when new groups of pigs are mixed after weaning due to the social skills they develop during lactation (Li and Wang, 2011). Socialized pigs develop the ability to differentiate between littermates and non-littermates and are more tolerant of unfamiliar pigs (Li and Wang, 2011). Pigs will fight to establish dominance (Van Kerschaver et al., 2021); however, the duration of these fights is shorter for younger, smaller pigs and results in less severe injuries compared to fighting in older pigs (Pitts et al., 2000).

We hypothesized that the pre-weaning socialization system would reduce pre-weaning mortality and increase weaning weights compared to a conventional farrowing system. The objective of this experiment was to determine the effects of a pre-weaning socialization system

during the lactation period on piglet growth and livability, pig growth performance from birth to market, and subsequent sow performance.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The pre-weaning portion of the study was conducted at Schwartz Farms, Inc. in Herington, KS. Sows were housed in individual farrowing stalls that measured 1.83 × 3.66 m including sow and litter area and were equipped with a dry self-feeder and a bowl waterer. The post-weaning portion of the study was conducted at Hubbard Feeds, Sleepy Eye, MN.

Animals and Housing

Lactation

A total of 3,307 (PIC L 42) mixed parity sows were used. On approximately d 112 of gestation, sows were moved from gestation to farrowing rooms. Treatment was assigned to an entire farrowing room in consecutive order such that each treatment was assigned to every other room. After 1 cycle through the farrowing rooms, the order was switched so that each treatment was represented in all rooms. A total of 40 farrowing rooms were used with 20 rooms (replications) per treatment. The sow farm consisted of 20 farrowing rooms. Therefore, the study occurred over 2 cycles through the farrowing rooms at the farm from August to October 2023. Farrowing rooms consisted of 80 stalls with 5 rows of 16 stalls. The farrowing rooms were managed in an all-in, all-out system. Sows were allowed *ad libitum* access to feed after parturition. Sows remained on their assigned management treatment until weaning.

All sows were fed a common diet and assigned to a management treatment of a conventional farrowing system (sows and piglets housed in individual farrowing stalls) or a pre-weaning socialization system (stall dividers removed between farrowing stalls and walkway within 6 to 24 h post-farrowing such that 12 to 32 litters of piglets were co-mingled; Figure 6.1). Thus, farrowing rooms were considered experimental units. Sub-populations were created within the pre-weaning socialization system and categorized as general, small, or critical populations (Figure 6.2). Small pigs were visually identified at birth and allowed to co-mingle in a designated area of approximately 4 to 6 farrowing stalls, depending on the number of small pigs born in each farrowing room. The critical population consisted of pigs that were very small at birth and needed intensive care. The designated area for this population was 2 farrowing stalls that remained closed and did not allow other pigs to co-mingle throughout the whole lactation period. Therefore, within the pre-weaning socialization system, all rooms had 1 group of 32 litters co-mingled, 1 group of 16 litters co-mingled, and 2 groups of 8 to 12 litters co-mingled depending on the placement of the sub-populations. All piglets had their needle teeth clipped and were given an antibiotic (ceftiofur crystalline free acid; Excede, Zoetis, Kalamazoo, MI) within 24 h after birth. Approximately 3 d after birth, all piglets received an iron supplement via intramuscular injection, tails were docked, and males were castrated.

Creep feed was offered to all piglets at approximately 18 d of age. For pigs raised in the conventional farrowing system, creep feed was provided on a mat in the farrowing stall. For pigs raised in the pre-weaning socialization system, creep feed was provided in rotary feeders with a hopper placed in the walkways between farrowing stalls. Feeders were monitored daily and new feed was added as needed. Creep feed was provided once daily to pigs raised in the conventional system.

Within the conventional farrowing system, litter size was standardized through cross-fostering of pigs within 24 h of parturition. The number of pigs born alive, stillborn, and mummified were recorded for each sow. Total pigs born per litter was calculated as the sum of pigs born alive, stillborn, and mummified. All data were managed on a farrowing room basis by adding the responses for all sows in a farrowing room and dividing by the total number of sows in the room.

Pre-weaning mortality and reason for death or euthanasia were recorded. Total pre-weaning mortality was calculated as: (number of pigs born alive – number of pigs weaned). Percentage pre-wean mortality was calculated as: (number of pre-weaning mortalities / number of pigs born alive).

On the day of weaning, sows were moved to gestation pens with free access to stalls and checked daily for signs of estrus. Wean-to-first service interval and the percentage of sows bred by d 4, 7, and 34 were recorded for the sows that remained in the herd after culling. Farrowing rate and subsequent liveborn were also evaluated. During this subsequent performance period, all sows consumed a common gestation and lactation diet, and farrowed in a conventional farrowing system.

Nursery

Pigs were weaned at approximately 23 d of age. A subset of the offspring (4,313 pigs; PIC 337 × L 42; initially 5.4 ± 0.15 kg) from 4 farrowing rooms (2 rooms per treatment) were transported to a commercial wean-to-finish research facility (Sleepy Eye, MN). Pigs were tagged with an RFID ear tag (LeeO, Merck Animal Health, Rahway, NJ) prior to weaning to identify the offspring from sow treatments by using 2 different color ear tags. All pigs weaned from the 4 farrowing rooms were used for subsequent performance. The rooms were selected such that both

treatments had the same weaning age. During the nursery period (d 23 to 64 of age), pigs were housed in pens according to sow treatment (conventional or pre-weaning socialization) with 44 to 46 mixed sex pigs per pen and 48 replications per treatment across 2 rooms. The nursery was "double-stocked" with approximately 0.37 m^2 per pig. The barn was filtered with totally slatted floors. Each pen was equipped with a 3-hole stainless steel self-feeder and a bowl waterer for *ad libitum* access to feed and water. Daily feed additions to each pen were made and recorded by an electronic feeding system (DryExact Pro; Big Dutchman North America, Holland, MI).

Cortisol concentrations in serum were used as an indicator to determine if farrowing system influenced the stress response of pigs post-weaning. Upon arrival at the wean-to-finish facility, pigs were randomly placed into holding pens and allowed to acclimate for 2 h. Then, 96 gilts per treatment (192 gilts total) were randomly selected for blood sample collection. Approximately 10 mL of blood was collected from the jugular vein using a red-top, no anti-coagulant blood tube. The blood was centrifuged (3000 × g, 30 min) within 2 h after collection, and serum was stored at −20°C until analysis. Serum was analyzed for cortisol concentrations using a commercial ELISA kit (Cortisol Parameter Assay Kit, R&D Systems, Minneapolis, MN).

Pens of pigs were weighed periodically throughout the nursery period to calculate ADG, ADFI, and G:F. Feed disappearance was measured by using a volumetric regression equation which estimated the quantity of feed remaining in the feeder subtracted from the quantity of feed added to the feeder.

Pigs that died or were removed during this study due to sickness or injury were recorded. Any pig that was removed from a test pen was considered a removal and placed into an off-test pen where they remained for the duration of the study. Mortality is defined as a pig that died while in a test pen.

Finishing

At the end of the nursery period, one-half of the pigs (one room) were moved to a different finishing facility where no further data capture occurred. The half of the pigs remaining at the research facility were separated into split-sex pens for the remainder of the growth period. This provided all remaining animals a minimum of 0.70 m^2 per pig. Thus, there were a total of 2,073 pigs (initially 24.9 ± 0.97 kg) with 18 to 24 pigs per pen and 48 pens per treatment. Data from the 2 pens (1 barrow pen and 1 gilt pen) were combined for finishing and wean-to-finish analysis (24 observations per finishing treatment). Pens of pigs were weighed periodically to calculate ADG, ADFI, and G:F.

Prior to marketing, all pigs were tattooed with a pen identification number to provide additional identification to the RFID ear tags received at weaning. During the first marketing event, the 6 heaviest pigs in each pen were visually selected and transported to a U. S. Department of Agriculture-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. The first marketing event occurred on d 154 for barrows and d 165 for gilts. The remaining pigs within pens were marketed to the same packing plant on d 171 for barrows and d 183 for gilts. Any pigs that were too light for marketing (less than 113 kg) were counted, weighed, and removed from the facility. For marketed pigs, carcass measurements were recorded using a RFID scanner (Merck Animal Health, Rahway, NJ). Data collected included HCW, carcass yield, backfat, loin depth, and percentage lean. Carcass yield was calculated by dividing the pen average HCW by the pen average live weight obtained at the farm. Percentage lean was calculated from a plant proprietary equation.

Statistical analysis

Experimental data were analyzed with farrowing room serving as the experimental unit for the pre-weaning portion of the study. Mortality within room was recorded as the count of mortalities and data were analyzed using a binomial distribution using a logit link function with total room inventory as the denominator to determine pre-weaning mortality. Treatment was used as the fixed effect. Sow farm cycle was used as a random effect because the research occurred during 2 cycles. For the percentage of sows that were bred back, data were analyzed using a binomial distribution. The count of sows bred back by the respective date (numerator) was compared to the number of sows eligible to be bred back following weaning (denominator). For the wean-to-finish period, pen served as the experimental unit and wean group was used as a random intercept. Mortality data were analyzed using a binomial distribution. All statistical models were fit using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC) and using a one-way ANOVA. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results

Sow performance

For sow and litter performance, no differences were observed in lactation length, total born, born alive, stillborn, mummies, or the number of pigs weaned (Table 6.1). Preweaning mortality was greater ($P < 0.001$) for pigs from the pre-weaning socialization farrowing system compared to pigs from the conventional farrowing system. An increased ($P \leq 0.015$) percentage of pigs died in the pre-weaning socialization system compared to pigs from the conventional farrowing system due to being laid on, starving out, *streptococcus suis*, and either umbilical or

scrotal ruptures. However, an increased ($P = 0.014$) percentage of pigs died from scours in the conventional farrowing system compared to the pre-weaning socialization system. No differences were observed for the percentage of pigs dying from unknown reasons, low viability, deformity, or greasy pig disease.

For subsequent sow performance, no differences were observed in wean-service interval, percentage of sows bred by d 34 post-farrow, farrowing rate, or number of liveborn piglets in the subsequent farrowing. A marginally significant ($P \leq 0.082$) increase in the percentage of sows bred by d 4 and 7 was observed for sows previously housed in the pre-weaning socialization system.

Wean-to-market performance

Pigs weaned from the conventional farrowing system were heavier ($P < 0.001$; Table 6.2) at weaning (23 d of age) compared to pigs from the pre-weaning socialization system. No differences were observed in serum cortisol concentrations between farrowing systems after placement in the wean-to-finish facility. However, visually, the pre-weaning socialization piglets were easier to move during weaning and arrival at the wean-to-finish facility.

During the nursery period (d 23 to 64), pigs from the conventional farrowing system had increased ($P < 0.001$) BW, ADG, and ADFI, but decreased G:F compared to pigs from the pre-weaning socialization system. Removals, mortality, and total removals and mortality were increased ($P \leq 0.059$) in pigs from the pre-weaning socialization system compared with those from the conventional farrowing system.

During the finishing period (d 64 to 183), pigs from the conventional farrowing system had increased ($P \leq 0.046$) BW, ADG, and ADFI, but decreased G:F compared to pigs from the pre-weaning socialization system. No differences were observed in total removals and mortality.

Additionally, the percentage of lightweight pigs (average BW of 95.8 kg) at the end of the grow-finish period was greater ($P < 0.001$) for pigs farrowed in the pre-weaning socialization system compared to pigs from the conventional farrowing system.

For the overall period from weaning to market (d 23 to 183), pigs from the conventional farrowing system had increased ($P \leq 0.010$) BW, ADG, and ADFI, but decreased G:F compared to pigs from the pre-weaning socialization system. No differences were observed for removals and mortality.

Carcass characteristics

For the first marketing event, pigs from the conventional farrowing system had increased ($P \leq 0.016$) live BW, HCW, loin depth, and percentage lean compared to pigs from the pre-weaning socialization system (Table 6.3). No differences were observed for carcass yield or backfat depth.

For the final marketing event, pigs from the conventional farrowing system had increased ($P \leq 0.001$) live BW, HCW, and loin depth compared to pigs from the pre-weaning socialization system. No differences were observed for carcass yield, backfat depth, or percentage lean.

Overall, pigs from the conventional farrowing system had increased ($P \leq 0.094$) live BW, HCW, carcass yield, loin depth, and percentage lean compared to pigs from the pre-weaning socialization system. No differences were observed for backfat depth.

Discussion

Different variations of pre-weaning socialization systems exist in terms of individual versus group housing of the sow, size of the co-mingled group, and timing of when piglets are co-mingled during lactation (Gainey, 2023; Van Kerschaver et al., 2023). When sows are able to

co-mingle, it can lead to a reduction in nursing time due to aggression between sows and consequently lead to a reduction in weight gain of piglets during lactation (Weary et al., 2002; Verdon et al., 2020; Tang et al., 2023). Therefore, in the current experiment, sows remained in their stall and weren't allowed to co-mingle with other sows throughout lactation. This practice has been used by other researchers as well (D'Eath, 2005; Hessel et al., 2006; Schwartz, 2023).

Within the pre-weaning socialization system, all rooms had 1 group of 32 litters co-mingled, 1 group of 16 litters co-mingled, and 2 groups of 8 to 12 litters co-mingled depending on the placement of the sub-populations. To our knowledge, no research has been conducted on this large of socialization groups. Previous research has allowed the socialization of between 2 (D'Eath, 2005) and 8 (Schwartz, 2023) litters, with a majority of studies socializing 2 to 4 litters of piglets. Limited data are available to determine the appropriate group size for socialization. However, studies indicate that pigs socialized pre-weaning from large groups were less aggressive than pigs from small groups when co-mingled at 11 weeks of age and re-grouped 8 weeks later (Samarakone and Gonyou, 2009). Pigs raised in small socialization groups fight aggressively to maintain their social status when mixed into a new group. Whereas pigs raised in large socialization groups adopt less aggressive and more tolerant social practices when mixed into a new group (Samarakone and Gonyou, 2009).

Our piglets were allowed to co-mingle and socialize as early as 6 h after birth but the appropriate piglet age for initiating co-mingling has not been defined (Salazar et al., 2018; Verdon et al., 2020; Van Kerschaver et al., 2021). Previous studies have implemented pre-weaning socialization from 3 (Van Kerschaaver et al., 2021) to 21 d of age (Greenwood et al., 2019). Our study began co-mingling shortly after birth in an effort to reduce pre-weaning mortality. Most of the pre-weaning mortality occurs within the first 3 to 5 d after birth (Weary et

al., 1996) and the leading causes are being laid on by sows and starvation (Svendsen et al., 1986; Varley, 1995).

We hypothesized that by providing piglets more space in the pre-weaning socialization system, piglets would be able to move away from sows and avoid being laid on. More space was created for the piglets by allowing them to co-mingle in the walkways between the farrowing stalls. Heat lamps, mats, and creep bowls were placed in the center walkways to encourage piglets to avoid being laid on by their mothers. However, we observed increased mortality caused by starvation and being laid on in the pre-weaning socialization system compared to pigs raised in a conventional farrowing system. Naturally, newborn piglets are attracted to the underline of the sow and generally choose to lay close to the sow (Lay et al., 2002). Starvation is a leading factor in the number of piglets being laid on (Lay et al., 2002). This is because starved piglets are less coordinated and have reduced ability to move away from the sow (Weary et al., 1996). Verdon et al. (2020) also observed an increase in pre-weaning mortality (d 6 post-farrowing until weaning) when piglets were allowed to co-mingle at 7 or 10 d of age but did not report the cause of mortality. It is important to note that Verdon et al. (2020) co-mingled 5 to 7 sows and their litters which may explain the increase in mortality due to being laid on or crushed by active sows. Gainey (2023) also observed a numerical increase in pre-weaning mortality when piglets were co-mingled at 5 d of age caused by starvation of piglets and being laid on. D'Eath (2005) and Schwartz (2023) did not observe any differences in pre-weaning mortality which may be due to limited replications with a total of 198 and 192 litters, respectively. The effect of co-mingling and socializing piglets during lactation on pre-weaning mortality is inconclusive because of limited replications and the lack of statistical power within most research trials.

A leading factor for mortality in the pre-weaning socialization system was *Streptococcus suis*. *Streptococcus suis* is normally found in the tonsil, nasal passages, and upper respiratory tract in pigs (Dutkiewicz et al., 2017). Therefore, the transmission of *Streptococcus suis* can be from the sow during the birthing process or by nose-to-nose contact between infected and uninfected pigs (Dutkiewicz et al., 2017). The increased mortality due to *Streptococcus suis* in the pre-weaning socialization system may have been a result of piglets co-mingling and socializing with 12 to 32 different litters of pigs. When piglets first interact with non-littermate pigs, it results in aggression involving nose-to-nose contact between piglets providing a route for *Streptococcus suis* transmission (Merlot et al., 2004). However, neither Gainey (2023) nor Schwartz (2023) observed an increase in *Streptococcus suis* presence when piglets were co-mingled during lactation in their studies. This may be due to the sows herein being younger with a lower parity structure and less innate immunity compared to Schwartz (2023) and Gainey (2023). At the time of the current experiment, the sow farm had been operational for approximately 11 months and the average parity of the herd was 2.1. With this young herd, the level of immunity may have been lower compared to a farm with an older parity structure, which may reduce the mortality attributed to health reasons such as *Streptococcus suis*.

Another potential reason for the increased mortality in the pre-weaning socialization system observed in our study is because of disrupted teat order as observed by increased mortality due to starvation (Pedersen et al., 1998). In pre-weaning socialization systems, cross-suckling occurs where piglets nurse different sows which can result in disturbed teat order (Dybjær et al., 2001). A stable teat order is developed within the first week after birth among piglets (Fraser, 1975; De Passillé et al., 1988). Afterward, piglets tend to suckle at the same teat for the rest of lactation (Rosillon-Warnier and Paquay, 1984). However, if a stable teat order is

not developed, piglets will compete for teats and fight to establish dominance (Van Kerschaver et al., 2021). When piglets fight instead of nurse during milk letdown, it results in decreased milk intake, increased pre-weaning mortality, and decreased growth (De Passillé et al., 1988; Pedersen et al., 1998). Furthermore, an increase in the percentage of sows bred by d 4 and d 7 post-weaning was observed for the pre-weaning socialization system compared to sows from the conventional farrowing system. Piglets from the conventional farrowing system had increased weaning weights which may be a result of increased milk supply. High milk production coupled with low sow feed intake may result in a negative energy balance and may affect wean-to-estrus interval (Valros, 2003). However, the number of sows bred by d 34 was not different among treatments.

Fighting is the main form of aggression in pigs and the degree of aggression can vary based on age (Pitts et al, 2000; D'Eath, 2005). The duration of these fights is shorter for younger, smaller pigs and results in less severe injuries compared to fighting in older pigs (Pitts et al., 2000). Previous research reported that 5-d old piglets have a shorter fight length compared to 26-d old piglets (101 vs 621 s) when mixed (Pitts et al., 2000). Short duration of fights indicates that fights are more decisive and lead to a clear dominance relationship (Jensen, 1994). This is because young pigs learn to accept the presence of non-littermate pigs more quickly (Wattanakul et al., 1997). The social skills that young piglets develop during early lactation in the pre-weaning socialization system result in less aggressive behaviors when mixed in new groups (Li and Wang, 2011). Pigs exposed to socialization early in life are less aggressive and more tolerant of unfamiliar pigs compared to pigs raised in conventional farrowing systems without socialization (Li and Wang, 2011). In the study herein, aggression or behavior scores were not assessed post-weaning. However, anecdotally, it was our observation that pigs were easier to

move during transport at weaning and upon arrival at the wean-to-finish facility. Co-mingled pigs have been observed to have greater exploratory and play behavior described as pigs investigating their surroundings and running with or without a gentle nudge (Pluske and Williams, 1996; Bolhuis et al., 2005; Turpin et al., 2017).

Aggression during the weaning process activates the hypothalamic-pituitary-adrenal axis which regulates stress responses and increases cortisol concentrations immediately after mixing pigs (Merlot et al., 2004; Colson et al., 2012; Salazar et al., 2018). At weaning, pigs in our study were also transported approximately 8 h to the wean-to-finish facility with 44 to 46 pigs placed in each pen according to sow treatment (control and pre-weaning socialization). We hypothesized that pigs raised in the pre-weaning socialization system would have decreased serum cortisol concentrations because of the social skills they developed during lactation compared to pigs in the conventional farrowing system. However, we did not observe any differences in serum cortisol, similar to results of Salazar et al. (2018) who observed no differences in salivary cortisol concentrations 1 d post-weaning when piglets were co-mingled during lactation at 7 or 14 d of age.

As a result of decreased weaning weight for pigs raised in the pre-weaning socialization system, lifetime growth performance was decreased in the current experiment. Low BW pigs have a decreased probability of living until market, decreased ADG, and increased time to reach market weight (Quiniou et al., 2002). This was observed in the current study with pre-weaning socialized pigs having decreased weaning weight, increased mortality during the nursery period, decreased lifetime ADG, and increased time to reach market weight. At the conclusion of the current experiment, approximately 7% of the pigs from the pre-weaning socialization strategy

did not reach market weight and needed more time to continue growing compared to 3% of pigs from the conventional farrowing system.

Despite decreased ADG and ADFI, pigs raised in the pre-weaning socialization system had improved feed efficiency compared to pigs raised in the conventional farrowing system. A low prevalence of aggressive behavior is associated with better feed efficiency in pigs (Pierozan et al., 2021). A possible explanation may be that socialized pigs do not fight as much and can cope better with stress at weaning. Van Nieuwamerongen et al. (2018) and Van Kerschaver et al. (2021) observed improved feed efficiency during the first-week post-weaning when pigs were co-mingled prior to weaning. On the contrary, Pluske and Williams (1996) and Turpin et al. (2017) did not observe improved feed efficiency during the immediate post-weaning period when piglets were co-mingled during lactation. Differences in co-mingling systems including the number of litters co-mingled may play a role in post-weaning performance. Another possible explanation for the difference in feed efficiency is the difference in market weight. Feed efficiency becomes poorer as pigs become heavier. Because pigs from the conventional system were 3.7 kg heavier at market than pigs from the pre-weaning socialization system, they would be expected to have poorer feed efficiency. In the current experiment pigs from the conventional system had a G:F of 463 g/kg. If feed efficiency was adjusted to the same market weight as pre-weaning socialized pigs, using equations from Gaines et al. (2012), pigs would have been expected to have G:F of 474 g/kg, which is an 11 g/kg difference. The actual difference was only 5 g/kg between the conventional and pre-weaning socialization systems, suggesting that the difference in market weight was likely the main reason for the difference in feed efficiency.

The increase in carcass characteristics (HCW, carcass yield, and loin depth) for pigs raised in the conventional farrowing system observed in the current experiment reflects the

increased ADG and final BW during the wean-to-finish period. Pigs with increased live weight at time of market have increased HCW, carcass yield, and loin depth (Ellis et al., 1996; Latorre et al., 2004; Shull, 2013).

In summary, pigs raised in the conventional farrowing system had increased livability, lifetime growth performance, and improved carcass characteristics compared to pigs raised in the pre-weaning socialization system. Further research is warranted on pre-weaning socialization farrowing systems with emphasis on timing of co-mingling to ensure piglets consume colostrum, fewer than 12 to 32 litters co-mingling, and an older parity structure to reduce *Streptococcus suis* risk.

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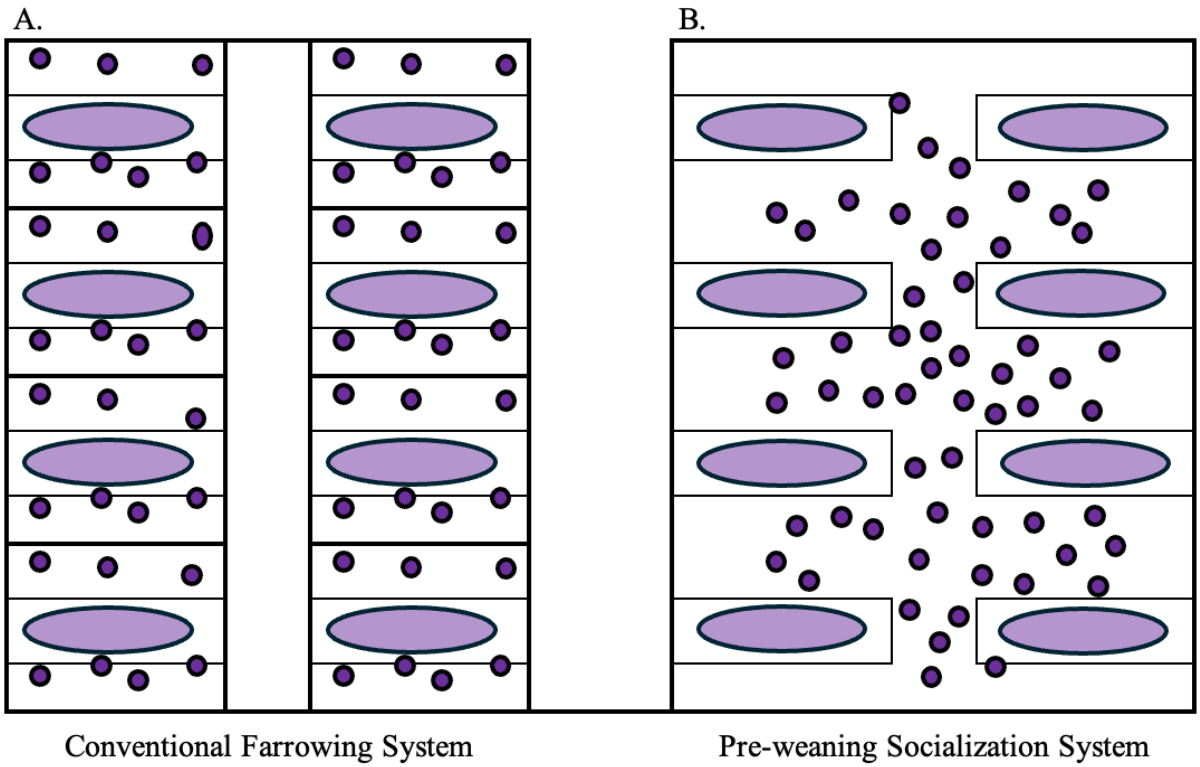

Figure 6.1. Treatments were assigned on a farrowing room basis. A total of 40 farrowing rooms were used with 20 replications per treatment. Farrowing rooms consisted of 80 stalls with 5 rows of 16 stalls each. Each oval represents a sow and each dark circle represents a piglet. For the conventional farrowing system A), sows and their corresponding piglets were kept separate in individual farrowing stalls (thin solid lines represent sow area and thick solid lines represent lay area for piglets within a farrowing stall). For the pre-weaning socialization system B), sows were housed in individual farrowing stalls (thin solid lines) and piglets were able to move between farrowing stalls and walkway within 6 to 24 h after farrowing was completed. Progeny from 12 to 32 farrowing stalls were allowed to co-mingle.

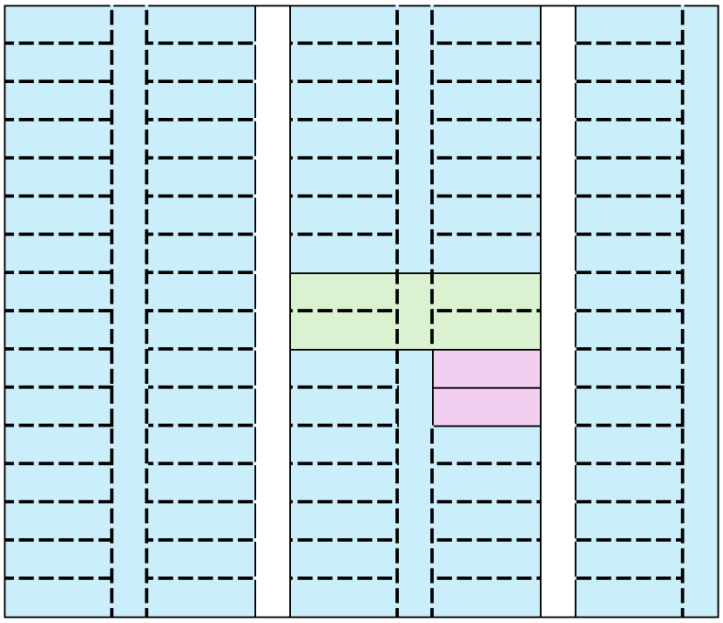

Figure 6.2. Sub-populations were created within the pre-weaning socialization system and categorized as general, small, or critical populations. Visibly small pigs were identified at birth and allowed to co-mingle in a designated area of approximately 4 to 6 stalls (green area with dashed lines representing stall dividers that were opened and solid lines representing stall dividers that were maintained to separate populations). The critical population consisted of pigs that were very small at birth and needed intensive care. The designated area for this population was 2 farrowing stalls that remained closed and did not allow pigs to co-mingle throughout the whole lactation period (pink area with solid lines representing closed stalls). The general population was allowed to co-mingle together within 12 to 32 farrowing stalls (blue area).

Table 6.1. Effects of farrowing strategy on sow and litter performance[1]

Item	Conventional[2]	Pre-weaning socialization[3]	SEM	*P* =
Farrowing rooms, n	20	20	---	---
Sow count, n	1,655	1,652	---	---
Sow parity	2.1	2.1	0.18	0.772
Lactation length, d	22.7	22.5	0.22	0.517
Liveborn, n	25,612	25,886	---	---
Litter characteristics				
Total born, n	16.6	16.8	0.30	0.153
Born alive, n	15.5	15.7	0.32	0.223
Stillborn, n	0.68	0.69	0.037	0.910
Mummies, n	0.41	0.44	0.028	0.434
Weaned, n	13.5	13.4	0.14	0.311
Born alive per room, n	1,281	1,294	21.8	0.273
Pigs weaned per room, n	1,118	1,103	8.2	0.193
Total mortality, n	3,245	3,827	---	---
Preweaning mortality, %	12.6	14.7	0.74	< 0.001
Reason, %[4]				
Laid on	4.93	6.13	0.178	< 0.001
Starve out	2.17	2.50	0.387	0.015
Streptococcus suis	0.47	0.85	0.854	< 0.001
Rupture	0.27	0.48	0.088	0.001
Scour	0.28	0.17	0.049	0.014
Unknown	0.82	0.90	0.097	0.310
Low viability, dead	0.86	0.97	0.075	0.182
Low viability, euthanized	2.48	2.39	0.328	0.389
Deformity	0.25	0.25	0.031	0.952
Greasy pig disease	0.01	0.01	0.007	0.651
Subsequent performance				
Wean-service interval, d	9.1	8.4	1.39	0.545
Sows stayed in herd, %	86.5	87.0	1.56	0.680
Bred by d 4, %	49.1	52.7	2.83	0.062
Bred by d 7, %	79.4	82.0	3.85	0.082
Bred by d 34%	98.7	98.9	0.37	0.602
Farrowing rate, %	84.2	85.2	0.99	0.381
Liveborn, n	15.6	15.9	0.14	0.215

[1]A total of 3,307 mixed parity sows and 55,160 piglets were used. Treatments were randomly assigned to farrowing rooms with 80 stalls per room. There were 20 replications (rooms) per treatment.

[2]Conventional farrowing strategy with sows and piglets housed in individual farrowing stalls.

[3]Pre-weaning socialization strategy defined as stall dividers removed between farrowing stalls and walkway within 6 to 24 h post-farrowing such that 12 to 32 litters of piglets were co-mingled.

[4]Represents percentage of piglets born alive for each mortality reason.

Table 6.2. Effects of farrowing strategy on wean-to-finish pig growth performance

Item	Conventional[1]	Pre-weaning socialization[2]	SEM	*P* =
Serum cortisol, ng/mL[3]	57.1	54.4	5.05	0.398
BW, kg				
Weaning, d 23	5.9	4.8	0.15	< 0.001
End of nursery, d 64	25.8	23.9	0.97	< 0.001
End of finisher, d 183[4]	129.2	125.5	2.71	0.001
Nursery period (d 23 to 64)[5]				
ADG, g	488	468	15.1	< 0.001
ADFI, g	614	573	22.2	< 0.001
G:F, g/kg	796	817	4.6	< 0.001
Removals, %	1.02	2.13	0.330	0.005
Mortality, %	0.35	0.78	0.281	0.059
Total removals and mortality, %	1.35	2.89	0.570	0.001
Finishing period (d 64 to 183)[6]				
ADG, g	948	920	14.5	0.001
ADFI, g	2,222	2,134	57.4	0.001
G:F, g/kg	427	431	4.7	0.046
Removals, %	3.40	3.63	0.579	0.776
Mortality, %	2.55	1.91	0.485	0.328
Total removals and mortality, %	5.96	5.55	0.708	0.689
Lightweight pigs, %[7]	3.17	7.23	2.595	< 0.001
Avg weight, kg	96.6	94.9	2.63	0.327
Wean to finish (d 23 to 183)[8]				
ADG, g	818	791	15.5	0.001
ADFI, g	1,768	1,691	47.5	0.001
G:F, g/kg	463	468	3.8	0.010
Removals, %	4.73	5.93	0.719	0.224
Mortality, %	2.97	2.59	0.517	0.598
Total removals and mortality, %	7.70	8.52	0.849	0.490

[1]Conventional farrowing strategy with sows and piglets housed in individual farrowing stalls.

[2]Pre-weaning socialization strategy defined as stall dividers removed between farrowing stall and walkway within 6 to 24 h post-farrowing such that 12 to 32 litters of piglets were co-mingled.

[3]After a 2 h acclimation period, 96 gilts per treatment (192 gilts total) were randomly selected for blood sample collection. Approximately 10 mL of blood was collected and analyzed for cortisol

concentrations using a commercial ELISA kit (Cortisol Parameter Assay Kit, R&D Systems, Minneapolis, MN).

[4]Represents the weighted average from all market events.

[5]A total of 4,313 pigs (initially 5.4 ± 0.15 kg) were used with 44 to 46 pigs per pen and 48 replications per treatment.

[6]A total of 2,073 pigs (initially 24.9 ± 0.97 kg) were used with 18 to 24 pigs per pen and 24 replications per treatment.

[7]Represents pigs that were too light and did not market with the rest of the pigs. These pigs were transported to a holding site to grow until appropriate market weight.

[8]Represents lifetime performance from weaning to market for 2,073 pigs that remained on site.

Table 6.3. Effects of farrowing strategy on carcass characteristics[1]

Item	Conventional[2]	Pre-weaning socialization[3]	SEM	*P* =
First marketing event				
Live BW, kg	127.7	123.7	3.26	0.001
HCW, kg	92.9	89.7	2.64	0.001
Carcass yield, %	72.7	72.5	0.22	0.285
Backfat depth, mm	14.5	14.5	0.51	0.997
Loin depth, mm	66.3	63.5	0.85	< 0.001
Lean, %	58.0	57.6	0.23	0.016
Final marketing event				
Live BW, kg	129.9	126.3	2.35	0.001
HCW, kg	96.7	93.8	2.09	0.001
Carcass yield, %	74.5	74.3	0.28	0.248
Backfat depth, mm	15.2	15.2	0.36	0.884
Loin depth, mm	67.8	65.8	0.93	0.001
Lean, %	57.7	57.4	0.14	0.102
Overall[4]				
Live BW, kg	129.2	125.5	2.71	0.001
HCW, kg	95.6	92.5	2.32	0.001
Carcass yield, %	74.0	73.7	0.26	0.094
Backfat depth, mm	15.0	15.0	0.41	0.995
Loin depth, mm	67.5	65.0	0.87	< 0.001
Lean, %	57.8	57.5	0.16	0.018

[1]A total of 2,073 pigs (initially 24.9 ± 0.97 kg) were used with 18 to 24 pigs per pen and 24 replications per treatment. The 6 heaviest pigs were sold at the first marketing event (d 154 for barrows and d 165 for gilts). The remaining pigs within pens were marketed on d 171 for barrows and d 183 for gilts.

[2]Conventional farrowing strategy with sows and piglets housed in individual farrowing stalls.

[3]Pre-weaning socialization strategy defined as stall dividers removed between farrowing stalls and walkway within 6 to 24 h post-farrowing such that 12 to 32 litters of piglets were co-mingled.

[4]Weighted average from all market events.