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EVALUATION OF BORON SUPPLEMENTATION IN SWINE AND POULTRY

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EVALUATION OF BORON SUPPLEMENTATION IN SWINE AND POULTRY

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

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ABSTRACT OF DISSERTATION

EVALUATION OF BORON SUPPLEMENTATION IN SWINE AND POULTRY

The objectives of this dissertation were to evaluate dietary boron (B) supplementation as sodium borate in various swine models and broiler chicks through a series of experiments. The initial experiment evaluated supplemental B (0, 25, 50, and 100 ppm B) on serum insulin, glucose, and clinical chemistry panel of growing pigs. Serum insulin/glucose ratio tended to decrease linearly with increasing supplemental B in a fasting state ($P = 0.08$). These findings were more noticeable (linear effect, $P = 0.02$) in a postprandial state (~ 50 minutes following a meal). A series of follow-up studies further evaluating B supplementation (0, 5, and 25 ppm B) in sows, piglets, and grower and market pigs were conducted. In multiparous sows, B supplementation tended to result in sows farrowing fewer piglets (quadratic, $P = 0.08$) yet piglet birth weight was increased quadratically ($P \leq 0.05$) with increasing B supplementation. Furthermore, B supplementation to multiparous sows resulted in a quadratic decrease in late gestation (d 80-90 of gestation) fasting serum insulin/glucose ratio ($P = 0.02$) whereas, in a postprandial state, serum insulin/glucose ratio was linearly increased ($P = 0.02$) with increasing B supplementation. In the second study, piglets from sows supplemented with dietary B exhibited an increase in the Mg content of the femur (quadratic effect, $P = 0.02$) at weaning. In the third study, both grower (70 kg BW) and market pigs supplemented with B had a greater kidney ash percentage (linear effect, $P = 0.03$; linear tendency, $P = 0.08$, respectively) compared to that of the control. Furthermore, grower pigs exhibited a linear increase in Mg concentration for the 3rd and 4th metacarpals ($P = 0.05$) with increasing B supplementation. In the final swine study, evaluating B supplementation on the apparent total tract digestibility (ATTD) and retention of nutrients in growing pigs resulted in a linear increase in both Mg absorption and digestibility regarding increasing B supplementation ($P = 0.01$ and $P = 0.04$, respectively). Lastly, an experiment evaluating B supplementation (0, 1, 2, 5, 10, 25, and 50 ppm B) to a semi-purified diet fed to broiler chicks resulted in a linear tendency for increasing tibia ash percentage ($P = 0.08$) with increasing supplemental B. Additionally, there was a greater tibia Mg concentration of birds supplemented B compared to control birds ($P < 0.01$). In summation, B supplementation appeared to affect insulin concentration in both grower pigs and sows. Furthermore, B improved Mg absorption and digestibility in growing barrows

while also affecting Mg concentrations in the bones of broilers, weaning pigs, and grower pigs, all suggesting B may play a key role in Mg metabolism.

KEYWORDS: boron, sows, reproductive performance, insulin, swine, broilers

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April 28th, 2023

Date

EVALUATION OF BORON SUPPLEMENTATION IN SWINE AND POULTRY

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Date

DEDICATION

-In memory of Henry “Pap” Lanham
Your influence will forever be imprinted in my heart and mind.

-To my beloved parents
Janet and Todd Chevalier
As their unwavering support, inspiration, and unconditional love has been the cornerstone of my academic career. This dissertation is dedicated to you all, as a humble tribute to your sacrifices and the immeasurable impact you all have had on my life.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ADFI,	Average daily feed intake
ADG,	Average daily gain
ALP,	Alkaline phosphatase
ANOVA,	Analysis of variance
AOAC,	Association of Official Agricultural Chemists
AST,	Aspartate aminotransferase
ATTD,	Apparent total tract digestibility
ATTR,	Apparent total tract retention
BMC,	Body mineral content
BSP,	Bone sialoprotein
BUN,	Blood urea nitrogen
BW,	Bodyweight
CCW,	Cold carcass weight
COL I,	Collagen type I protein
CP,	Crude protein
d,	Day
DM,	Dry matter
DNA,	Deoxyribonucleic acid
E2,	17β-estradiol
E.g.,	Exempli gratia
ESF,	Electronic sow feeding
FCR,	Feed conversion ratio
FDA,	Food and Drug Administration
g,	Gram
GE,	Gross energy

GLM,	Generalized linear model
GSH,	Glutathione
GSH-Px,	Glutathione peroxidase
GSR,	Glutathione reductase
h,	Hour
HCW,	Hot carcass weight
I.e.,	Id est or that is
i.v.,	Intravenous
ICP-OES,	Inductively coupled plasma-optical emission spectroscopy
IFN-γ,	Interferon-gamma
IL-8,	Interleukin-8
IM,	Intramuscular
kg,	Kilogram
L,	Liter
$LD_{50,}$	Lethal dose
LMA,	Longissimus dorsi muscle area
LPS,	Lipopolysaccharide solution
MDA,	Malondialdehyde
ME,	Metabolizable energy
mg,	Miligram
min,	Minute
mL,	Mililiter
mRNA,	Messenger ribonucleic acid
MTL,	Maximum tolerable level
NaBCl,	Sodium coupled borate transporter
NADPH,	Nicotinamide adenine dinucleotide phosphate
NEFA,	Non-esterified fatty acids
NRC,	National Research Council

OCN,	Osteocalcin
OPN,	Osteopontin
pH,	Potential of hydrogen
PHA,	Phytohemagglutinin
PPAR,	Peroxisome proliferator-activator receptor
ppb,	Parts per billion or μg/kg
ppm,	Parts per million or mg/kg or mg/L
PWM,	Preweaning mortality
RIA,	Radioimmunoassay
RNA,	Ribonucleic acid
ROI,	Return on investment
ROS,	Reactive oxygen species
RT-PCR,	Reverse transcription polymerase chain reaction
SBM,	Soybean meal
SEM,	Standard error of the mean
SID,	Standard ileal digestible
SOD,	Superoxide dismutase
STTD,	Standard total tract digestible
T_4,	Thyroxine
TNF-α	Tumor necrosis factor-alpha
UKVDL,	University of Kentucky Veterinary Diagnostic Laboratory
UL,	Tolerable upper intake level
UN DESA,	United Nations Department of Economic and Social Affairs
US,	United States
USDA,	United States Department of Agriculture

CHAPTER 1. Introduction

Over the past 50 years, global meat production has increased from 99.5 million tonnes of meat produced in 1970 to 337.2 million tonnes of meat produced in 2020 (Ritchie et al., 2017; FAO, 2022). Furthermore, in 1970 there was 35.8 million tonnes of pork produced worldwide. However, by 2020 global pork production had increased to 109.8 million tonnes. In 1970, pork made up ~ 36 % of the total meat produced globally whereas, in 2020, pork accounted for about 33 % of total meat production. Despite the minor decrease observed in pork production relative to total meat production over the last 50 years, pork has consistently represented about 30 to 40 % of the total meat produced globally. This is of importance because, on a global scale, pork has historically been considered the preferred source of animal protein compared to other types of meat. Starting in 1972 pork became the number one consumed meat globally on a per capita of total meat consumption basis; this number is especially surprising considering there are many regions of the world where pork consumption is negligible due to certain religious restrictions. This trend remained consistent up until 2014 when, in 2015, poultry meat consumption began to share the same global consumption footprint that pork had seen previously. Since then, poultry meat consumption has continued to rise but just marginally above the consumption levels of pork. Altogether in 2020, pork and poultry represent about 73% of total meat consumption worldwide. These historical data demonstrate that pork and poultry are the preferred sources of animal protein on a global scale.

It has been estimated that the world population will rise to around 9.5 billion people by 2050 (United Nations, 2022). Along with the increase in population over the

next several decades, there is an expected parallel increase in the demand for animal protein. Currently, poultry and pork represent the two most consumed types of animal protein worldwide (FAO, 2022). For the poultry and swine industries to continue to meet the growing world demand, there will have to be an increase in overall production accompanied by improvements in the efficiency of production.

Both swine and poultry industries have continuously improved genetics, management, and nutrition over the past century, however, continued efforts to improve production efficiencies are further necessary to supply the expected growth in demand for pork and poultry meat around the world. Historically, in the United States, swine and poultry production has often focused on a least-cost formulation method for nutritional programs as profit margins are tight and reducing the cost of feed reduces the cost of production. This is mostly due to feed and diet costs being estimated to account for 65-75% of total production costs. Nonetheless, developing or formulating diets on a least-cost basis may lead to potential deficiencies, either marked or marginal, in certain nutrients and thereby potentially reducing certain production efficiencies. Thus, because feed carries the bulk of production costs, altering the feed may have the greatest opportunity to yield improvements in production efficiencies with minimal changes in the costs associated with production. That is, if the improvements in production metrics result in a gross income that is greater than the cost of feed without any other changes in the cost of production. For this reason, it is important to evaluate the economics related to the change in production efficiencies that were a result of the dietary changes made to gain full insight on whether a certain ingredient should be used or not.

For example, Hagen et al. (2000) reported that the addition of chromium tripicolinate (200 ppb) in sow diets cost an additional $7.00 per ton of feed (an amount considered staggering by most nutritionist) but demonstrated that the improvements observed in sow productivity (litter size, wean to estrus interval, and sow mortality) with supplemental Cr yielded $40.08 more gross income over feed cost per sow per year. In another example, reducing morbidity and even more so, mortality as a result of a nutrient or management change will most likely provide huge economic returns as a sick or deceased animal will yield minimal to no value. However, a lot of times in which the effects of a nutrient on health are inadequately quantified or infrequently observed, the potential return on investment (ROI) for increased nutrient supplementation is underestimated. Thus, it is important to continue research efforts yet, when decisions about nutrient supplementation levels are made within a production entity, the decisions must consider the cost of the nutrient relative to the potential improvement in performance; this approach determines the potential ROI and ultimately provides a more precise way for producers to improve production while considering economic costs.

Overall, considering historical global meat production and consumption trends observed for the last 50 years, it is evident that pork and poultry represent the majority of total meat shares. Although production and consumption trends will continue to ebb and flow over time, it is hypothesized that both pork and poultry production and consumption will continue to grow in order to supply protein to the projected increase in world population growth. However, supplying both pork and poultry at a much larger rate of demand will require both industries to continue to grow and improve production practices. Furthermore, for producers in both industries, it is warranted that quality is not

sacrificed because of quantity. Moreover, through research and conceptualization, production efficiencies can continue to improve with minimal changes in the cost of production, optimizing producers' economic potential.

An area of research that has great opportunity to elicit biological improvements within swine and poultry production is that of dietary trace minerals. Research that evaluates different levels and sources of dietary trace minerals can further help understand the dietary requirement of the animal. Despite this, it is also equally important to understand the biological relevance as well as the bioavailability of certain minerals to identify the optimum level and ensure that animals are receiving that level with respect to their production phase. In the subsequent chapters, dietary supplemental boron (B) will be evaluated as a potential nutrient in various swine models and in broiler chickens. However, the biological significance of B as a potential nutrient for humans and animals has not clearly been understood. Without knowing the biological importance associated with B, it is difficult to understand the potential benefit of adding B to the diet. Thus, without knowing the biological response or change, one cannot calculate a potential return on investment (ROI) for B.

CHAPTER 2. Literature Review

2.1 Changes and challenges of swine production

The use of contemporary genetic analysis has led to hypotheses that the modern domesticated pig originated from the Eurasian wild boar (Sus scrofa) around 500,000 years ago (Giuffra et al., 2000). Many years after, wild pigs started to become domesticated as a reliable and efficient source of protein. It is thought that domestication occurred around 9,000 years ago (Bökönyi, 1974; Larson et al., 2011). Ever since domestication, pigs have continuously been raised to provide a source of protein and energy for human consumption. In 2020, it was estimated that 109.8 million tonnes of pork were produced globally. The amount of pork produced in 2020 was ~ 22 % higher than that of 20 years ago (Ritchie et al., 2017; FAO, 2022). The growth observed in the global pork production is associated with the increase in pork demand related to the growth in global human population. Interestingly, the United Nations Department of Economic and Social Affairs (UN DESA) Population Division estimated that the current world population is at 7.6 billion people, which is expected to grow to 8.6 billion people in 2030, 9.8 billion in 2050, and 11.2 billion by the year 2100 (UN DESA, 2022). The projected increases in global population over the next several decades suggest an expected parallel increase in demand for pork considering pork accounts for around 30 to 40 % of world meat consumption (FAO, 2022). In an attempt to meet expected consumer demands, global pork production will need to grow as well.

The United States, behind China and the European Union, is the third-highest pork-producing region in the world accounting for around 11% of the total global production (FAS, 2023). Combined, all three regions of the world account for almost

79% of the total global production of pork (FAS, 2023). Over the last several decades, continuous improvement in genetic selection, nutrition, and management has occurred on a global swine industry basis. An area that has been of great interest to improve within the swine industry is that of sow productivity. Sows play a vital role in fulfilling the demand for pork because their productivity determines the number of pigs that are available for pork production.

Sow productivity can be defined by many characteristics; however, the most common assessment is the number of pigs weaned per litter, per year, or per lifetime. In the US, annual sow productivity in terms of pigs weaned per litter has seen an ~ 33% increase from 2004 to 2022 (Table 2.1). Also, the average number of total pigs born per litter in the United States has increased from 11.5 in 2004 to 15.5 in 2022 (~35% increase in 18 years; Pig Champ, 2022). Consequently, increasing sow productivity in terms of increasing pigs weaned per litter per year without adjusting nutritional and management strategies accordingly can result in a reduction in sow wellbeing and overall production deficiencies as well as including the economic potential for the producer to plummet.

Table 2.1 Annual sow productivity improvement in the United States over the years[1].

	Year						
Items	2004	2010	2018	2019	2020	2021	2022
Total pigs per litter	11.5	12.8	14.4	14.7	15.0	15.2	15.5
Pigs born alive/litter	10.3	11.5	12.9	13.2	13.5	13.5	13.9
Pigs weaned per litter	9.1	10.2	11.2	11.5	11.8	11.9	12.1
Weaned pigs/sow/year	21.3	23.1	25.3	26.1	26.1	26.1	25.1
Average age at weaning	18.2	20.1	20.7	20.8	20.7	20.8	20.9

[1]Adapted from PigChamp (2022).

2.2 Modern sow production

As mentioned in the previous section sow productivity is one of the main constituents for success in pork production as the sow herd is responsible for the maximum productivity potential of the entire system (Ball et al., 2008). It comes as no surprise that reproducing females are often considered the most valuable animals in the herd due to their ability to raise and grow progeny (Schneck et al., 2008). However, it has been estimated that a replacement gilt must remain in the breeding herd for a minimum of three parities before she reaches a positive net present value (Stalder et al., 2003). Thus, sow longevity or the concept of how long a sow remains active and productive within the breeding herd should also be a key performance metric that evaluates a production system's culling and death rate. Culling is a term that is widely used in livestock industries referring to the removal of an animal(s) from the herd. Culling of non-productive animals is done routinely within a production system to maximize production efforts and gain insight into production costs and returns. In sow production, non-productive days is a production metric that represents the time when a sow is neither

gestating nor lactating. Minimizing non-productive days can result in more efficient production.

Over the past several decades, modern genetic selection has allowed the swine industry to focus on increasing sow productivity primarily in the form of producing more piglets per litter per sow. There has been a great effort to increase productivity and improve the number of pigs per litter for a sow. When an increase in the number of pigs produced is not associated with an increase of nutrient intake, or the efficiency with which the nutrients are used, this could lead to an increase in sow body nutrient mobilization that can ultimately affect the reproductive capacity and longevity of the sow because nutrient demand often exceeds nutrient intake and body reserves (Mahan, 1990). The modern sow requires a greater nutrient supply in gestation to support more, and/or heavier, fetuses prior to farrowing. Furthermore, in lactation, it is crucial to supply a greater amount of energy and nutrients to support her progeny given that milk production takes greater priority over individual health (Pettigrew and Moser, 1991), as larger litters require a greater demand for total energy and nutrients (Kim et al., 2013).

Work by Mahan and Newton (1995) demonstrated that body mineral contents, which included calcium (Ca), phosphorous (P), magnesium (Mg), potassium (K), sodium (Na), aluminum (Al), zinc (Zn), and copper (Cu), had drastically declined (~ 6 to 28 % lower depending on the mineral) in sows that completed three parities compared to those in similarly aged, nongravid gilts. Additionally, the same lab also reported that when sows had litter weights that were > 60 kg, the Ca, P, and Zn body mineral contents of the sow were lower (~ 15, 6, and 12 % lower respectively) than when litter weaning weights were < 55 kg. This work demonstrates that productive sows, over several reproductive

cycles, are susceptible to mineral deficiencies that may be exacerbated with larger litters as there is a larger nutrient demand on the sow. Under modern confinement production systems, essential nutrients are always supplemented; however, there is a large variation between nutrient sources and supplementation strategies that are used among different nutritionists and production systems. Furthermore, there are different nutrient requirements for different animals in a herd such as a gilt and a hyper-prolific sow that has raised multiple litters over her lifetime.

Parallel with the increases observed in sow productivity, there have been changes made to nutrient requirement estimates of both gestating and lactating sows which are reflected in NRC publications of the past 3 decades (Tables 2.2 and 2.3, respectively). The most recent NRC publication (2012) provided nutrient estimates for multiple stages of gestation (compared to a single gestation period in previous editions of the swine NRCs) demonstrating that sows in late gestation (> 90 d of gestation) require greater levels of energy and other nutrients (lysine, Ca, and P) than previous NRC editions (e.g., 1988 and 1998). Similarly, the most recent nutrient requirement estimates (NRC, 2012) for lactating sows have also been modified compared to previous editions to support greater demand for increased milk production.

Table 2.2 Comparison of NRC nutrient requirement estimates of gestating sows from 1988 to 2012[1].

Items	NRC 1988	NRC 1998	NRC 2012	
Breeding weight, kg	-	125 to 200	140 to 205	
Anticipated gestation weight gain, kg	-	35 to 55	45 to 65	
Days of gestation	-	-	< 90	> 90
Feed intake, kg/d	1.9	1.80 to 1.96	2.05 to 2.21	2.45 to 2.61
Requirement, amount/day				
ME, Mcal	6.1	6.0 to 6.4	6.4 to 6.9	7.7 to 8.2
Lysine, g[2]	8.2	9.4 to 11.4	7.7 to 12.4	13.1 to 19.3
Calcium, g	14.2	13.9	8.9 to 12.4	16.4 to 19.9
Phosphorus, g[3]	11.4	11.9	7.7 to 9.9	12.5 to 14.8
Copper, mg	9.5	9.3	21	
Iron, mg	152	148	168	
Manganese, mg	19	37	52	
Zinc, mg	95	93	210	

[1]Table adapted from Lu, 2018.
[2]Lysine requirement is presented on a total basis.
[3]Phosphorus requirement is presented on a total basis.

Table 2.3 Comparison of NRC nutrient requirement estimates of lactating sows from 1988 to 2012[1].

Items	NRC 1988	NRC 1998	NRC 2012	
Post-farrowing weight, kg	-	175	175	210
Anticipated daily weight gain of nursing pigs, g/d	-	150 to 250	190 to 270	
Feed intake, kg/d	5.3	4.31 to 6.40	5.93 to 5.95	6.61
Requirement, amount/day				
ME, Mcal	17.0	14.1 to 20.9	18.7	20.7
Lysine, g[2]	31.8	35.3 to 61.9	48.7 to 56.5	52.4 to 60.5
Calcium, g	39.8	39.4	35.3 to 45.0	37.7 to 48.1
Phosphorus, g[3]	31.8	31.5	17.7 to 22.6	18.9 to 24.0
Copper, mg	26.5	26.3	119.3	
Iron, mg	424	420	447	
Manganese, mg	53	105	149	
Zinc, mg	265	263	597	

[1]Table adapted from Lu, 2018.
[2]Lysine requirement is presented on a total basis.
[3]Phosphorus requirement is presented on a total basis.

Even though dietary nutrient estimates for gestating and lactating sows have changed to reflect the greater demands required for increased production, a conflict still exists between the selections for greater productivity and the target of a prolonged productive lifetime. A prolonged productive lifetime is considered beneficial to pig producers because of fewer unproductive days, greater acquired immunity to herd disease, and lower gilt replacement costs (Lucia et al., 2000a; Hoge and Bates, 2011). However, the intensive selection in the breeding herd has led to high national culling rates within commercial herds, which is often between 40 to 50%, as well as an increased sow mortality rate of 10.0 to 14.9 % during the past 6 years in the US (Figure 2.1).

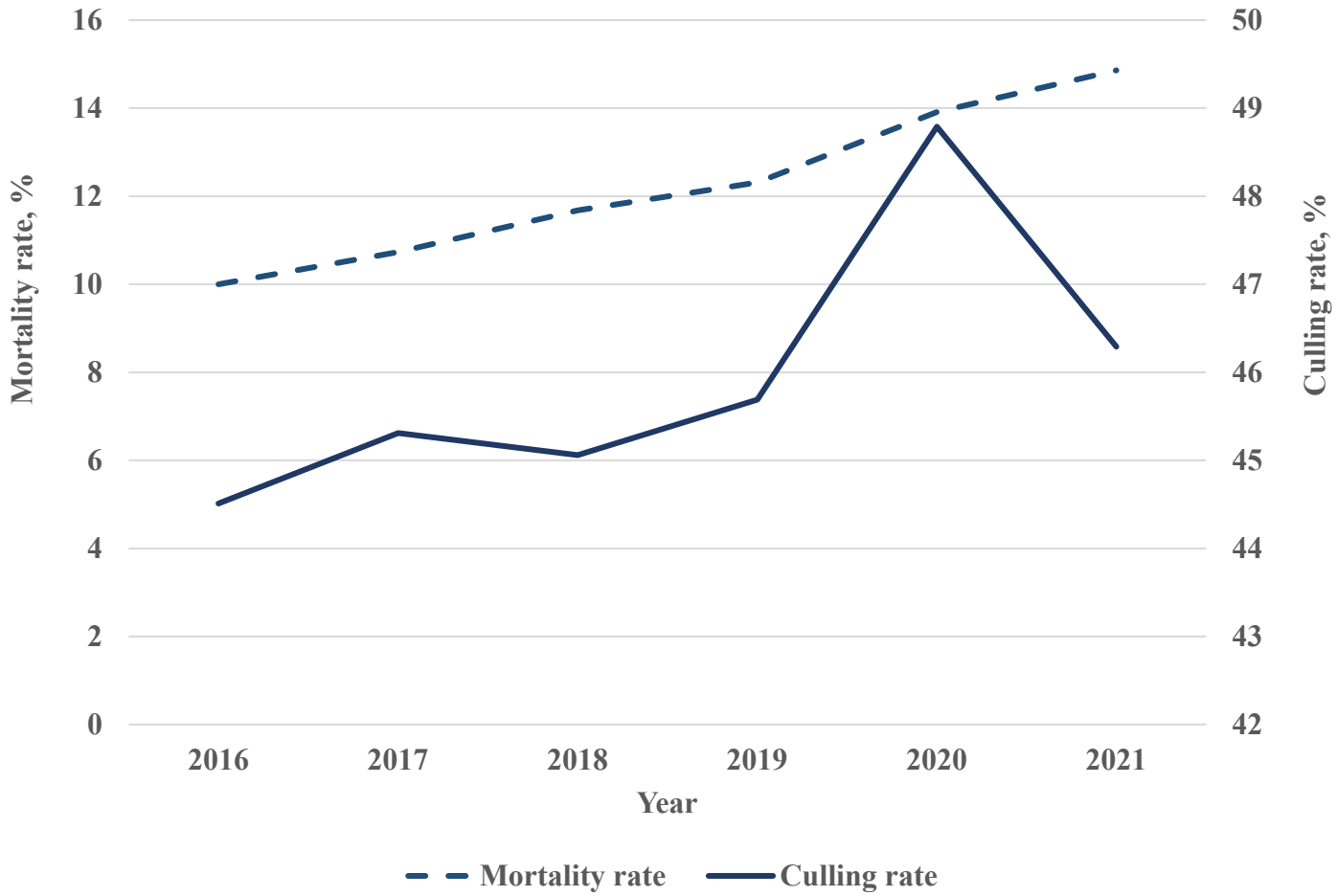

Figure 2.1 Sow mortality and culling rate in the US from 2016 to 2021 (Adapted from PigChamp, 2022).

There are many different reasons for sow removal (culling), the most common of them being either, or a combination of, reproductive failure, poor performance, lameness, and old age (Diaz et al, 2015). Lucia et al. (2000b) reported that on average, reproductive females finish 3.3 parities before being culled from breeding herds in the United States. A sow culled at early parities obviously produces fewer pigs in her lifetime, when compared to sows that stay in the breeding herd for a longer period (Stalder et al., 2004). Theoretically, it has been suggested that a sow must remain in the herd for at least 3 parities to produce enough piglets to pay for herself (Stalder et al., 2000; Stalder et al., 2003). However, it has been demonstrated by Carney-Hinkle et al. (2013) that older parity sows produce heavier pigs at birth and weaning. Therefore, the management practices and nutritional strategies of commercial producers must aim to improve sow longevity for better profitability and breeding herd efficiency (Thingnes et al., 2015).

The increase in sow productivity over the years has caused additional problems outside of high culling and mortality. The production of larger litter size has, in turn, resulted in a biological compensation of reduced individual piglet birth weight. Quesnel et al. (2008a) concluded that genetic selection for prolificacy (larger litter size and increased parity) in the sow has also resulted in greater variation of within-litter BW. Looking at retrospective data, when litter size increases from 9 to 16 pigs, the proportion of small piglets (< 1 kg BW) increased from 3 to 15 % (Quesnel et al., 2008a). It is well-known that smaller birthweight piglets have a decreased survival rate (Quiniou et al., 2002). Interestingly, in the same report, piglets weighing heavier than 1.4 kg at birth resulted in a survival rate of 90 % or greater whereas piglets with a birthweight of 1 kg or under had a lower survival rate of ≤ 71 % (Quiniou et al., 2002). More recent work using

sows from the maternal Norsvin Landrace line showed that with an increase in litter size there was a reduction in the number of successful nursings (defined as the proportion of initiated nursings resulting in milk ejection), with fewer piglets securing a teat during milk letdown, and higher maternal crushing and overall piglet mortality (Ocepek et al., 2017).

Sow productivity and longevity are major concerns for producers globally as they affect profitability within the swine industry. Thus, it is critical to find ways to mitigate any adverse events associated with these production changes within the swine industry. Areas like nutrition and management should be further investigated to facilitate positive changes in the productivity and longevity of the modern sow.

2.3 Nutrition and management of the sow

Proper nutrition and management can optimize sow productivity and longevity. Historically, feed costs account for roughly 65-70% of total swine operation costs. The modern, highly prolific sow requires a great amount of energy and nutrients to support her progeny through gestation and lactation (NRC, 2012; Pettigrew and Moser, 1991). The nutritional status of the sow can affect the survival and viability of her offspring as well as the longevity of the sow (Kim et al., 2007).

The energy requirements of the sow are dependent on several factors including bodyweight (BW), parity, housing and environmental conditions, and desired sow body conditioning. For these reasons, Noblet et al. (1990) suggested that it is not beneficial to feed sows on a herd basis but rather that feeding should be done on an individual sow basis. While this may be true, it can be very difficult to feed on an individual sow basis in

a large confinement operation making it challenging to meet the specific nutrient needs of each animal. However, with more modern equipment and ever-changing technologies, this may be more feasible than once thought. For instance, implementing electronic sow feeding (ESF) stations can permit individual nutrient supply more precisely than group feeding methods. Electronic sow feeding stations facilitate an easier method for producers to adjust feed allowance on an individual sow basis compared to traditional methods such as manually adjusting the feed drop or hand feeding for different stalls or pens.

2.4 Gestation feeding management

Gestational feeding regimes are designed to supply adequate nutrients for fetal and uterine tissue growth, mammary gland development, and maternal maintenance and growth. During gestation, 3 primary stages of pregnancy warrant different feeding strategies. The first stage of pregnancy is early pregnancy (d 0-21 following breeding) and should be solely focused on embryo survival and implantation (Boyd et al., 2000). The second stage (d 20-80) should focus on a nutritional program that is designed to replenish sow body reserves from the previous lactation to prepare her for the subsequent litter. In gilts, a diet should be fed that supports her growth and development as much as the development and growth of her litter. Also at this stage, sow and gilt nutrition should prioritize maternal gains that support fetal muscle fiber growth, ultimately determining the postnatal growth rate and efficiency potential of the litter (Stickland, 1994). Lastly, the third stage, (last 35 d of pregnancy), often considered late gestation, should account for the exponential growth in fetal and mammary tissues (Boyd et al., 2000). At all three

stages, it is critical to supply sows and gilts with adequate nutrient supply to optimize the targets respective to the phase of pregnancy.

On the other hand, it is crucial to not over-condition sows and gilts during gestation as overfeeding in gestation can lead to excessive fat deposition within the mammary tissue, negatively affecting mammary development (Farmer and Sorensen, 2001). Furthermore, overfeeding sows in gestation can reduce appetite during lactation (Young et al., 2004), ultimately resulting in greater mobilization of body nutrient stores and a greater risk for reproductive failures in the subsequent reproductive cycle. Hence, sows with a lower body condition at weaning because of reduced lactation feed intake cause challenges in the subsequent breeding, and if they conceive are more susceptible to future reproductive failures, lameness, and longer wean-to-estrus periods (Gill, 2007).

The first month of gestation is critical for sows since this is the period in which pregnancy is established and litter size is determined through embryo implantation (Langendijk, 2015). Historically, there has been concern that high feeding levels during these early stages of gestation lead to reduced embryo survival rate because a greater amount of feed reduces systemic concentrations of progesterone, inhibiting the uterus's capacity to produce an optimal uterine environment for successful implantation (Prime and Symonds, 1993; Jindal et al., 1996). In contrast, work by Quesnel et al. (2010) demonstrated that feeding 4 kg versus 2 kg of gestation diet per day throughout the first 3 weeks of gestation resulted in no effect on embryonic survival. More recent work by Ferreira et al. (2021), suggests that a gestational feeding regimen for sows that is close to the maintenance requirements can be harmful to maternal body reserves, blood metabolites, and sow longevity which all contribute to overall sow productivity. Other

work by Hoving et al. (2011) showed that feeding sows 3.25 kg/d compared to 2.50 kg/d during the first month of gestation resulted in an increase in sow BW recovery, and increased litter size, but did not affect the farrowing rate in the subsequent litter. In contrast, another recent study demonstrated that increasing feed allowance for the first month of gestation (comparing 1.8, 2.5, and 3.2 kg/d) did result in an increase in sow BW gain but reduced the number of total pigs born (Mallmann et al., 2020).

2.5 Lactation feeding management

It has been suggested that energy requirements are particularly higher during lactation compared to gestation and often cannot be met through voluntary feed intake (Noblet et al., 1990). The farrowing process is very demanding on the energy status of the sow as uterine contractions and abdominal straining during farrowing require a substantial amount of energy (Vallet et al., 2013; Feyera et al., 2018). In situations where farrowing duration is prolonged, sows are likely to exceed dietary energy supply which has been estimated to be absorbed from the gastrointestinal tract in the form of glucose 4 to 6 h postprandially (Serena et al., 2009; Theil et al., 2011). Hence, the importance of the time of farrowing relative to the sow's last meal as the energy from the diet may reduce the total amount of energy mobilized by the sow during the farrowing process. Following farrowing, if nutrient supply is inadequate during lactation, multiparous sows are known to mobilize body protein and fat stores to prioritize milk production for their litter (Pettigrew and Moser, 1991). This was further supported by earlier work demonstrating that multiparous sows produced more milk and faster-growing litters following a gastric infusion of additional nutrients, creating an anabolic state during lactation compared to sows having ad libitum access to feed (Matzat et al., 1990). However, first parity sows do

not necessarily respond to an excess of nutrients in the same manner as multiparous sows do. Unlike multiparous sows, Matzat et al. (1990) reported that first-parity sows partition extra energy into body growth rather than into milk production. Thus, feeding management must factor in the parity of the sow when considering nutrient levels and feeding allowance, ultimately minimizing the potential for excessive mobilization of body stores which will likely compromise the subsequent performance of the sow (Tritton et al., 1996).

During lactation, the sow is responsible for supplying nutrients in the form of milk to the offspring. In order to accomplish a healthy litter weaning weight with the increased number of pigs/litter, it is suggested that the sow needs to produce around 10 kg of milk/d assuming that 4 g of milk results in 1 g of piglet BW gain (Boyd et al., 2000). Moreover, for heavier pigs at weaning (litter growth of 3.48 kg/d), the sow needs to produce at least 13 kg of milk/d. Thus, the importance of nutrient intake during lactation is of great importance for the success of the sow and her litter.

Clowes et al. (2003) reported that sows can sustain a loss of 9 to 12% of their previously existing body protein mass during lactation without any detrimental effects on ovarian function and piglet growth. However, if body protein loss exceeds 12% then milk protein concentration and piglet growth begin to decline (Clowes et al., 2003). Other research estimated that for every 1% of body fat lost from sows during lactation, there will be a decrease of 0.1 piglets born in the subsequent litter (Whittemore, 2006). Additional work further illustrates that total feed intake as well as the pattern of intake during lactation affects subsequent reproductive performance as there is an inverse relationship between average daily feed intake (ADFI) and wean to 1st service interval

and wean to conception interval (King and Williams, 1984; Reese et al., 1982; Koketsu et al., 1996). Kirkwood et al. (1987) reported that sows fed two feeding levels (high or low feeding level), where the high level was based on 10, 13, and 14 % of each sow's immediate postfarrowing metabolic weight ($kg^{0.75}$) and given during weeks 1, 2, and 3-4 of lactation, respectively. Whereas the low feeding level was determined as 50% of the estimated high-level allowance in order to achieve a relatively severe weight and backfat loss. Sows receiving the low feeding level during a 28-d lactation resulted in an ~ 61% greater lactation BW loss coupled with an increase in the wean-to-estrus period (5.3 vs. 4.4 days) and about a 7% decrease in subsequent pregnancy rate. Overall, it is critical that nutrients are supplied to sows and gilts in the appropriate quantity during all phases of gestation to ensure optimal feed intake during the subsequent lactation period to facilitate successful future parities.

2.6 Mineral nutrition of the sow

It is imperative that sows receive proper nutrition to optimize her wellbeing and lifetime success. Mineral nutrition is an area of nutrition that does not get the same attention as other areas such as amino acids, energy, or fat. Minerals make up one of the smallest components of the diet, however, they are a key constituent for numerous biological and physiological processes all of which contribute to the overall productivity of the sow. It has been hypothesized that the length of time that a reproductive female stays in the breeding herd may be influenced by the extent of tissue mineral storage and their genetic productivity potential (Mahan, 1990). As modern sows are producing larger and heavier litters than their ancestors did several decades ago, there is a greater demand for mineral accumulation to prepare the body reserves for mobilization that occurs over

multiple reproductive cycles. Depleted mineral body reserves may depress the reproductive performance of sows, but more importantly, it increases the possibility of removal due to issues caused by a lack of body reserves (Mahan and Newton, 1995).

2.6.1 *Calcium (Ca), phosphorus (P), and magnesium (Mg)*

In sows, Ca and P are the most extensively studied minerals. It has been understood for some time that dietary requirements for Ca and P increase dramatically during late gestation (d 80 to farrowing) and lactation when demands for fetal development and milk secretion, respectively, are the highest (McDowell, 2003). Earlier studies reported that increasing dietary Ca and P (50 % more than NRC recommendations) levels during gilt development had no impact on subsequent litter performance (Nimmo et al., 1981b). Moreover, supplying Ca and P at levels 40% higher than NRC recommendations during gestation and lactation also resulted in no impact on litter performance (Mahan and Fetter, 1982). Although increasing Ca and P levels may not directly influence litter performance, they may serve more as an indirect influence that contributes to bone integrity and tissue mineral mobilization in the sow.

Locomotion problems or lameness is one of the most common reasons for sow removal (culling) and has been estimated to account for about 15.2% of sows that are removed from the breeding herd (Anil et al., 2009). Approximately 99% of Ca and 80% of P in the body is in bone (Crenshaw, 2001). Thus, influencing Ca and P metabolism in sows to prevent lameness or locomotion problems has been an area of great interest for many swine nutritionists and producers for several decades. Work by Nimmo et al. (1981a) revealed that 30 % of sows fed diets low in Ca (0.65%) and total P (0.50%) during growth and gestation were subsequently removed for locomotive problems

(lameness) whereas sows fed the control diets (0.98% Ca and 0.75% total P) had a 0% removal rate. In a similar study, gilts fed either a high or low Ca and P diet (0.94 and 0.72% vs. 0.81 and 0.65% of Ca and total P, respectively) during lactation for 3 consecutive reproductive cycles resulted in the low Ca and P diet group having worse ($P < 0.005$) Ca retention during parities 2 and 3 and tended ($P = 0.056$) to have a worse P retention during the 2nd parity (Everts et al., 1998).

Another vital constituent of bone, as well as a factor for many other biological processes, is Mg (Patience and Zijlstra, 2001). It is estimated that gestating and lactating sows require around 0.06% of Mg in diets (NRC, 2012). This would not seem to be an issue given that the Mg content in corn and soybean meal (SBM) found in typical breeding herd diets is estimated to be much higher (0.2 to 0.3% Mg). More recent work out of China demonstrated that supplementing increasing levels of MgSO4 (0, 200, 400, and 600 ppm $MgSO_4$) to sows linearly reduced ($P < 0.05$) weaning-to-estrus interval while also linearly increasing ($P = 0.02$) fecal moisture content during lactation (Hou et al., 2014). Similar work by Zang et al. (2014) reported that supplementing up to 0.045% of Mg (450 ppm Mg) from $MgSO_4$ from breeding to weaning resulted in around a 15% decrease (7.4 vs. 6.3 days) in wean to estrus interval in gilts and a linear decrease ($P \leq 0.03$) in constipation rate for both gilts and sows.

2.6.2 *Trace minerals*

Other trace minerals such as chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn) are also vital components in sow nutrition. Since trace minerals are required only at very low levels, it is likely that common ingredients (like corn and SBM) found in a typical gestation and lactation diet can provide adequate

amounts. However, it is common for commercial diets to contain vitamin and mineral premixes to ensure these nutrients are being supplied adequately. Nevertheless, trace minerals can be supplemented at very low levels (ppm and ppb of the diet) to provide optimal nutrient levels for the sow. For example, Lindemann et al. (1995) reported that dietary supplementation of Cr (0 vs. 0.2 ppm Cr) from chromium picolinate to sows increased the number of pigs born alive (11.25 vs. 8.93 pigs respectively) from sows completing 2 reproductive cycles compared to sows that received no Cr supplement. Other work that looked at feeding sows a diet supplemented with Cu (250 ppm Cu from $CuSO_4$) for up to 6 reproductive cycles resulted in a 78 and 14.5% reduction in failure to cycle or rebreed and weaning-to-estrus interval respectively, while also increasing piglet birth and weaning weights by 9 and 6% respectively, compared to sows fed diets supplemented with no Cu (Cromwell et al., 1993).

Iron is another trace mineral that is essential to swine. Iron has been extensively studied in swine primarily due to piglets being born with only about 50 mg Fe and low Fe concentrations in the sow milk (Venn, 1947). Over the years, Fe deficiency was exacerbated with the transition from pastured-based production to confinement system production. Thus, it is common practice within the swine industry to administer an injection of Fe (100-200 mg Fe) around birth to prevent iron-deficiency anemia. In the sow, it has previously been considered that the mature female is resistant to Fe deficiency due to the ability to accumulate Fe in body stores over a longer period of time compared to growing pigs (Mahan, 1990). However, more recent work evaluating sow Fe status on 11 commercial sow farms found that around 74.2% of the sows were considered anemic during lactation (Castevens, 2020). Moreover, higher parity sows (3+) were more likely

to be anemic during gestation. Work by Buffler et al. (2017) reported that higher dietary Fe levels (256 vs. 114 ppm Fe as $FeSO_4$) during gestation and lactation for multiparous sows resulted in increases in daily Fe retention, placental Fe content, and improved litter size and birth weight.

Manganese is another trace mineral that is found abundantly in grain diets and is not thought to be deficient in a typical corn-SBM diet for swine (Hill, 2013). However, in a recent study (Edmunds et al., 2022), increasing dietary supplemental Mn (0, 20, and 40 ppm Mn) to sows resulted in piglets born with a heavier birth weight and having a greater daily gain ($P < 0.05$).

Another essential mineral in swine nutrition is Se. Although Se is essential to swine in micro quantities, there is a large concern regarding potential environmental pollution by Se (Ullrey, 1992). Due to concerns regarding Se supplementation and environmental pollution, the United States Food and Drug Association (FDA) regulates that Se can only be supplied at levels up to 0.3 ppm in all swine diets (FDA, 1987).

The importance of dietary Se in biological systems is primarily due to Se being a major component of the antioxidant system within the body which may alleviate or limit the excessive reactive oxygen species observed during the reproductive cycle of a sow (Surai, 2006; Surai and Fisinin, 2016). Inorganic Se forms such as selenite and selenate have been supplemented to swine diets over the last 50 to 60 years (Surai and Fisinin, 2016). However, it is worth noting that inorganic Se can be highly toxic and interact with other minerals as well as having the inability to be stored and maintained as Se reserves within the body (Surai, 2006; Surai and Fisinin, 2014). Work from Mahan and Peters (2004) demonstrated that supplementing diets with increasing levels of Se (0, 0.15, and

0.30 ppm Se) in the form of either sodium selenite (inorganic) or Se yeast (organic) to gilts from the end of the nursery through the end of the 4th reproductive cycle resulted in higher Se concentration of sow tissues from the organic Se fed sows. Furthermore, in the same study, researchers found a higher Se concentration in the colostrum and milk of the sows fed diets supplemented with organic Se rather than inorganic Se. Additional studies evaluating dietary supplemental Se (0.3 ppm Se) to sows have confirmed the previous findings that organic Se may have a greater affinity to be transferred from the sow to the piglets through placental nutrient transfer and colostrum and milk (Quesnel et al., 2008b; Chen et al., 2016a,b).

Lastly, Zn is another trace mineral that is essential to swine due to its role in many metalloenzymes, including DNA and RNA synthetases and transferases. Furthermore, the presence of Zn in other digestive enzymes and the hormone insulin indicate that Zn is a key trace element needed in protein, carbohydrate, and lipid metabolism (NRC, 2012). It has been reported that inadequate intake of Zn during gestation and lactation may cause reproductive failures (Smith and Akinbamijo, 2000). The current NRC estimates that the dietary requirement of Zn for gestating and lactating sows is 100 ppm (NRC, 2012).

In an earlier study where gilts were fed diets supplemented with 0, 50, 500, and 5,000 ppm Zn (from ZnO) from 30 kg BW to the end of the 2nd reproductive cycle resulted in sows from the unsupplemented group (0 ppm Zn) having a greater number of abnormal pigs per litter, which were defined as piglets having either incomplete skin covering, abnormal skeletal features, spraddled legs, continuous shaking, or any other gross external abnormalities observed (Hill et al., 1983). Further results from the same study demonstrated that weaning weight was lower of piglets from sows receiving diets

supplemented with 5,000 ppm Zn compared to sows fed diets with lower Zn levels (50 and 500 ppm Zn).

Overall, macro- and microminerals only represent a small fraction of the whole diet fed to sows, yet they involved in nearly all biological and physiological mechanisms that contribute to the overall productivity of the animal. There has been an accumulation of information regarding mineral supplementation to reproducing sows over the past century allowing for dietary requirement estimates to be estimated. However, as the prolificacy and production of the modern sow are changing (increasing), nutrient demand is also changing. In addition, the nutrient profile of ingredients is constantly changing over time. Thus, nutrient supply must be intentional and accurate in order to optimize biological production as well as economic costs associated with production. In addition, it is important to continue to test and implement novel nutritional concepts that may provide potential benefits in biological production as well as the economic cost associated with production.

Many areas within swine nutrition warrant further investigation. However, one area of particular interest herein is the use of other trace minerals that have a known essentiality in other organisms like plants. Among these other trace minerals that have been identified as essential for plants but have limited information on higher-order animals is boron (B).

2.7 Boron (B)

Boron is the only nonmetal element in Group IIIA of the periodic table. Boron has an atomic number of 5 and contains two stable isotopes with a mass of 10 and 11. These

two isotopes are present in an approximate 20:80 (10:11, respectively) ratio, yielding an atomic weight of 10.81 g/mole (Power and Woods, 1997). Boron primarily exists in two forms depending on pH; at a pH less than 9.5, B exists primarily as a volatile undissociated boric acid, whereas at a pH greater than or equal to 10, B exists as the nonvolatile tetrahydroxy borate anion (Downing and Strong, 1999).

$$B(OH)_3 + H_2O \Leftrightarrow B(OH)_4^- + H^+ (pKa \approx 9.2)$$

Overall, there is a low natural abundance of B, but it is widely distributed in the lithosphere and the hydrosphere (Morgan, 1980). The ancient Egyptians have been credited with first using B compounds, as they used them for mummification and certain medicinal purposes (Travis and Cocks, 1984). Boron was later found to be an excellent addition to preserve and extend the palatability of foods like meat and dairy products (Gordon, 1987). In 1904, it was reported that human volunteers that had an intake of 500 mg boric acid/d (77 mg B/d) for 50 days resulted in disturbances in appetite, digestion, and overall health (Wiley, 1904). It was further established that boric acid at 4,000 mg B/d (699 mg B/d) was the limit beyond which humans experienced severe harm.

Today, the main industrial uses of B compounds can be found in the production of fiberglass, heat-resistant borosilicate glassware (Pyrex), detergents, enamels, herbicides, and fertilizers (Power and Woods, 1997). Boron is often obtained commercially through the mining process of mineral deposits that are formed from geological volcanic activity (Power and Woods, 1997). Boron can form complexes and interact with important biological substances like polysaccharides, pyroxidines, riboflavins, dehydroascorbic acid, and pyridine nucleotides. Boron is also found in sedimentary rock, seawater, coals,

and soils as borates (Samman et al., 1998). Boron has chemical properties that allow it to form complexes with hydroxyl group-containing molecules which are essential for cell growth, function, and proliferation (Park et al., 2005).

2.7.1 *Boron in plants*

Boron has been hypothesized to contribute to many different metabolic factors within biological systems. Although there has not been a nutrient requirement of B set for animals, it has long been understood that B is required in a continuous supply for proper cell wall development and integrity in plants (Warrington, 1923). More precisely, Warrington (1923) reported that in the presence of low B, broad bean growth was altered by short and thick roots, small and stunted shoots with the apex of the shoot being withered as well as the stem turning black. Later work by Berger (1949) further elucidated that in the absence of B, cell wall development in plants is disrupted. Over the years, B has widely been deemed and accepted as an essential element for the growth of vascular plants and diatoms (Loomis and Durst, 1992). In plants, B maintained the integrity of plasma membranes and stabilized the structure by forming complexes with phenolics to prevent oxidation from quinones and free radicals (Cakmak et al., 1995). Boron deficiency in vascular plants can cause a complete cessation of growth but, more importantly, it causes changes in the morphology, physiology, and reproduction of the plant (Dell and Huang, 1997). It has been proposed that the morphological and physiological alterations observed in the absence of B are most likely associated with the lack of complexes formed between B and compounds including hydroxyl groups in the cis position (Cakmak and Römheld, 1997). It is now well-accepted that there is an

absolute requirement of B for a multitude of plants and single-celled organisms (Hunt, 1996).

2.7.2 *Boron in human and animal nutrition*

Research regarding B supplementation in animals over the last century has been very limited and often unclear. Nonetheless, B is under growing scrutiny regarding its essentiality in animal nutrition. In the mid-1940's, a report supplementing B to potassium-deficient rats resulted in increased survival rates and liver glycogen concentrations (Skinner and Mchargue, 1945). However, a study that attempted to confirm these findings failed to demonstrate similar results (Follis, 1947). Subsequently, in the 1980s researchers found that supplemental B fed to cholecalciferol-deficient chicks resulted in an improvement in growth and a partial correction in leg abnormalities (Hunt and Nielsen, 1981). Eckhert (1998) reported that B supplementation to trout resulted in the stimulation of embryo growth. Also, it has been reported that an impairment of embryonic growth was observed in frogs, zebrafish, and rats fed a B-deficient diet (Fort et al., 1999a,b; Rowe and Eckhert, 1999; Lanoue et al., 1998, respectively). Since these early studies, there has been accumulating evidence using numerous species models indicating that B may have a greater impact on physiological and biochemical processes in animal models than previously known.

Boron can be a difficult mineral to quantify due to its chemical properties as well as its low presence in livestock feedstuffs. There is limited work reporting the concentrations of B found within common grains used as feedstuffs for livestock. However, work by Bhasker et al. (2015) reported B concentrations for corn and soybean meal. Corn had a mean B concentration (mean ± SE) of 2.3 ± 0.41 ppm with a minimum

and maximum analyzed content of 0.92 and 3.96 ppm respectively. Whereas soybean meal had a mean B concentration of 28.8 ± 1.18 with a minimum and maximum analyzed content of 20.43 and 35.79 ppm respectively.

Due to the limited studies regarding dietary B, there are limited established nutrient recommendations for B. However, there has been an upper intake limit of 20 mg B/d suggested by the Food and Nutrition Board (Food and Nutrition Board, 2002). Furthermore, NRC (2005) derived a maximum tolerable level (MTL) of 150 mg B/kg DM for poultry, swine, horses, and sheep by extrapolation of data from other species. The suggested lethal dose (Oral LD_{50}) of boric acid and borates for pigs is 200 – 350 mg B/kg BW (ATSDR, 2007; ESFA, 2004).

Inorganic B such as borates are readily absorbed across the gastrointestinal epithelium and the bulk of ingested B is secreted in the urine (Jansen et al. 1984 a, b). From studies using humans and rats as the model, it appears that the kidney regulates B homeostasis within the body and that glomerular filtration is the primary route of B excretion (Naghii and Samman, 1996; Naghii and Samman, 1997, Sharma et al., 2022). In healthy male human subjects, 84 to 85% of the dietary B consumed appeared in the urine (Sutherland et al., 1998). After an oral dose of a sodium borate solution was administered to rats, 99.6% of the dose was recovered in the urine within 24 h (Usuda et al., 1998). Excretion through urine, bile, sweat, and breath are all thought to be the major mechanisms for maintaining homeostasis (Naghii and Samman, 1993).

It is hypothesized that B found in animal tissue is often in the form of boric acid (Sutherland et al., 1998). Tissue B concentration varies greatly depending on the tissue, and the certain distinct function of a specific tissue (Newnham, 1991). Following a B

gavage (0.15 mg B/ kg BW as boric acid) in rats, B concentrations were increased in the blood, liver, testes, tibia, and muscle (Bai and Hunt, 1996). Furthermore, in Murrah Buffaloes, B concentration in the plasma, feces, and urine were all increased when the buffaloes were supplemented with increasing levels of B (200 and 400 ppm diet as boric acid) (Sharma et al., 2022). Overall, the B concentration in tissues increased with doses (2, 12.5, and 25 mg B/rat/d as boric acid), and among the organs examined, the kidney showed the highest concentration of B (Naghii and Samman, 1996).

Specifically in swine (barrows), supplementing 5 and 15 ppm B during the nursery and growing phases resulted in an increase ($P < 0.05$) in ADG and ADFI compared to control pigs (Armstrong and Spears, 2001). In the same study, the increase observed in ADG was a function of an increase in ADFI and not an improvement in feed efficiency This same group later found that there was a tendency for increased piglet birth weight and a reduction in the number of dead embryos after supplementing B (5 ppm B as Na borate) to a small number of gilts (8 gilts/treatment) starting at weaning and following them through maturity and their 1st reproductive cycle (Armstrong et al., 2002). Moreover, Armstrong et al. (2002) also found an increased B concentration in 35-d-old embryos from gilts supplemented with 5 ppm B diet indicating that B is transferred across the placenta.

2.7.3 *Boron relationship with other minerals*

Boron is a highly bioactive trace mineral that physiologically interacts with other minerals (Ca, P, Mg, etc.) in a biological setting. Nielsen et al. (1987) first reported that 3 mg of B per day decreased urinary Ca and Mg excretion in humans. In male sheep, dietary B supplementation (75 and 200 mg B/d as sodium borate) increased the apparent

absorption of Ca (Brown et al., 1989), while in rats, supplementation of B (3 ppm B as boric acid) increased apparent absorption of Ca and P (Hegsted et al., 1991). Furthermore, supplementation of B (150 or 300 ppm as sodium borate) in the drinking water of heifers resulted in a decrease in urinary phosphate excretion (Green and Weeth, 1997). Unlike humans, sheep, and rats, growing barrows supplemented with dietary B (5 and 15 ppm B as sodium borate) did not exhibit ($P > 0.05$) changes in urinary and fecal excretion nor retention of Ca and P (Armstrong and Spears, 2001). Interestingly in the same study, there were also no differences observed in plasma Ca and Mg but there was an increase in plasma P at the end of the nursery phase (Armstrong and Spears, 2001). Control of blood calcium is tightly regulated as calcium metabolism is ultimately dependent on controlling the rate of absorption of calcium and consequently bone metabolism (Mahan and Vallet, 1997). However, supplementing Murrah buffaloes with 200 and 400 ppm B (as boric acid) during the late stages of pregnancy did result in increased plasma Ca, Mg, and B concentrations during both the parturition and the postpartum periods (Sharma et al., 2020). Additionally, B supplementation resulted in an increase in the apparent absorption coefficient of Ca and Mg by 21 to 24% respectively; suggesting a possible relationship between B, Ca, and Mg (Sharma et al., 2022). In a 42-day study, broiler chickens that were supplemented with increasing dietary levels of B (0, 25, 75, 50, and 100 ppm B) from boric acid increased ($P < 0.05$) retention of calcium, and phosphorus but also reduced ($P < 0.001$) the retention of manganese and iron (Pradhan et al., 2020). Another study looking at dietary B supplementation (20 ppm B from boric acid) to broilers resulted in a 15 and 11% increase in Fe and Cu content of the excreta

respectively from birds supplemented with B compared to control birds (Kucukyilmaz et al., 2017). These findings suggest that B may have a role in trace-mineral metabolism.

A factorial study (2x2) where ram lambs were fed diets with either an adequate or low Ca level (0.54 and 0.36% Ca respectively) that was either supplemented with or without B (40 ppm B as sodium borate) demonstrated that B supplementation to a Ca-deficient diet led to similar growth rates, humoral immune response, total antioxidant activity, and degenerative changes in kidney and liver tissues compared to lambs fed a Ca-adequate diet (Bhasker et al., 2017). A very similar study that also utilized a factorial approach (2x2) where White Leghorn hens were fed with either an adequate or inadequate Ca diet (100 or 90% of the Ca requirement) that was either supplemented with or without B (40 ppm B) resulted in an amelioration of adverse effects associated with inadequate dietary levels of calcium (Adarsh et al., 2021). Furthermore, the authors reported that feed conversion ratio, eggshell thickness, and Ca retention were all positively influenced by B supplementation to the inadequate-Ca diet. Lastly, hens that were fed diets supplemented with B had lower cracked egg production compared to hens not supplemented with B (Adarsh et al., 2021). The findings above suggest that B supplementation may have more of an impact when dietary Ca, perhaps other minerals or vitamins, are deficient and thus provide amelioration to abiotic stress.

2.7.4 *Boron supplementation on bone responses*

Bone is the primary site of storage for many essential elements. Approximately 99% of Ca, 80 to 85% of P, and 70% of Mg in the body occur in the bone (McDowell, 2003). The ratio of Ca to P in bone is 2.1:1, and this ratio remains relatively constant even in instances of severe deficiencies of either or both minerals (Cromwell and Baer,

2005). It has been reported that B accumulates in bone dependent on the amount consumed (Chapin et al., 1998). Boron supplementation has been shown to affect the composition of bone. Supplementing B in the water of rats at 150 or 300 ppm B (as sodium borate) of drinking water resulted in a 53% decrease in the lipid percentage of the femur (Seal and Weeth, 1980). Dietary B supplementation (100 ppm B) to broiler breeder hens increased bone ash percentage (Qin and Klandorf, 1991). Similarly, Wilson and Ruszler (1997) reported B supplementation (50, 100, and 200 ppm B) fed to Leghorn pullets increased bone ash percentage. At lower supplemental levels (5 and 10 ppm B), Elliot and Edwards (1992) reported an increase in the bone ash percentage of broilers. Moreover, male broilers fed a diet supplemented with 5 ppm B (total of ~ 14.4 ppm B as boric acid) increased tibia ash percentage (Rossi et al., 1993). In a later study, Rossi et al. (1994) reported that there were no effects observed for tibia ash content of broilers supplemented 60 ppm B as boric acid to a Ca, P, and cholecalciferol deficient diet.

In rats, increasing dietary B intake from 0 to 9000 ppm boric acid (B levels of 0, 200, 1000, 3000, and 9000 ppm) for 12 weeks resulted in a ~10% increase in vertebral resistance to crushing force for all rats supplemented with 200 or more ppm B (Chapin et al., 1998). At much lower supplemental levels, supplementing 5 ppm B as boric acid increased tibial breaking load (Rossi et al., 1993) and increased shear fracture energy of the femur, tibia, and radius (Wilson and Ruszler, 1998) in chicks. Furthermore, supplying mature rabbits with a B gavage (sodium borate) every 96 hours that provided 50 mg B/kg BW resulted in a 27 to 46% increase in femur break force compared with the control treatments (Hakki et al., 2013). Additionally, it was observed that rabbits provided with 30 and 50 mg B/kg BW gavage had a much higher compression strength of the tibia and

greater tibia Ca and P concentrations compared to the control rabbits. Hakki et al. (2010) suggest that B is a bioactive beneficial element for bone strength and mineralization in rabbits that were fed a high-energy diet. In cultured osteoblasts that underwent quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis; an increase in mRNA expression of extracellular matrix proteins including Collagen Type I (COL I), Osteopontin (OPN), Bone Sialoprotein (BSP), and Osteocalcin (OCN) was observed when B was present (Hakki et al., 2010). Furthermore, B was observed to increase bone morphogenetic proteins -4, -6, and -7, which are crucial for bone morphogenesis (Hakki et al., 2010). Although these findings were observed in vitro which cannot always mimic in vivo conditions, they do support the hypothesis of Chen et al. (2011) who suggests that B is an indispensable mineral in bone development, cell proliferation, and mineralization in both bone and tissue.

A different study that fed diets supplemented with boric acid (400 ppm B) to calves for 90 days resulted in a 10, 5, and 18% increase in bone alkaline phosphatase (ALP), plasma osteocalcin, and Ca concentrations, respectively, compared to calves fed a diet without B supplementation (Singh et al., 2021). Moreover, a study conducted on athletic and sedentary female college students in the early 1990s indicated that dietary intake of B (3 mg B/d) for 10 months affected blood P and Mg which were both modified by exercise, however, there were no differences observed in bone density between the groups (Meacham et al., 1994). In swine, Armstong and Spears (2001) reported an approximate 15 % increase ($P < 0.05$) in the ultimate sheer force of the fibula from barrows fed a diet supplemented with 15 ppm B (as sodium borate) compared to barrows fed a diet supplemented with only 5 ppm B. In a later study, the same group also reported

that feeding diets supplemented with 5 ppm B (as sodium borate) to gilts from weaning through their first reproductive cycle resulted in beneficial effects observed on femur force at yield, the bending moment at yield point, and yield stress coupled with a 14% increase in serum osteocalcin compared to gilts fed diets not supplemented with B (Armstrong et al., 2002).

In poultry, feeding broiler chicks diets that were supplemented with increasing levels of dietary B (0, 25, 50, 75, and 100 ppm B) as boric acid for 42 days resulted in an increased ($P < 0.05$) femur bone ash, Ca, and P contents but also decreased ($P < 0.05$) Mn and Fe contents in the femur (Pradhan et al., 2021). The decreased Mn and Fe content previously observed in the femur of B-supplemented chicks was further supported by a decrease in Mn and Fe retention observed with increasing B supplementation (Pradhan et al., 2020). Moreover, serum Mn concentration was lower in rats that were fed a diet supplemented with graded B concentrations (5 to 40 ppm B as sodium borate) compared to rats fed a diet without supplemental B (Bhasker et al., 2016). In ostriches, supplementing B in the water at 400 ppm B via sodium borate for 45 days resulted in an 18% increase in bone ash content compared to ostriches receiving no supplemental B (Cheng et al., 2011). Hunt et al. (1994) suggested that B influences bone development by enhancing the major mineral content of the bone. Hence, the increases observed in bone ash in the previously reported literature are likely a function of increased mineral content within the bone.

2.7.5 *Boron supplementation effects on hormones, metabolism, and immune function*

The unique structure and nature of B permit it to form complexes with numerous biomolecules under normal physiological conditions (Hunt, 2008). More specifically, boric acid or borate ions form ester complexes with several biologically important sugars containing cis-hydroxyl groups like ribose (Nielsen and Meacham, 2011). Although the exact mechanism(s) for B action within biological systems is still unclear, Forrest and Meacham (2011) compiled substantial evidence indicating that B intake affects the presence or function of hormones, energy and mineral metabolism, immune function, and oxidative stress.

Nielsen et al. (1987) found that B supplementation (3 mg B/d) increased serum steroidal hormone (17β-estradiol and testosterone) concentrations in postmenopausal women. Naghii and Samman (1996) reported that rats supplemented with 2 mg B per day (as boric acid in the drinking water) resulted in an increase in plasma testosterone concentrations at both 3 and 6 weeks. Subsequently, the same group confirmed that supplementing B (2 mg B/rat/d as boric acid) through drinking water increased plasma testosterone concentration after 4 weeks of B supplementation (Naghii and Samman, 1997). In another instance, B was observed to enhance the action of 17β-estradiol (E2) activity on bone quality as it was found that trabecular bone surface was increased (~44 %) and trabecular plate separation was decreased (~31%) in ovariectomized rats fed a diet supplemented with 5 ppm B (as boric acid) for 5 weeks (Sheng et al., 2001). Also in the same report, the combination of B (5 ppm B boric acid) and E2 (30 μg/kg/d)

supplementation to ovariectomized rats resulted in approximately 30 and 44 % increase in trabecular bone volume and plate density, respectively.

In weaning pigs that were fed diets supplemented with B at inclusion levels of 0, 5, and 10 ppm B (from boric acid) for 14 days resulted in a linear increase in plasma Ca and cholesterol coupled with a linear decrease in plasma P and triglyceride levels (Cho et al., 2022). Lastly, B supplementation (0, 200, and 400 ppm B as boric acid) to diets fed to peripartum Murrah buffalos resulted in no effects observed on plasma triglycerides but did result in lower levels of plasma non-esterified fatty acids (NEFA) compared to animals fed a diet supplemented without B during the early lactating phase (Sharma et al., 2022).

Early work conducted in 1981, found that dietary B was necessary for the growth of vitamin D3-deficient chicks (Hunt and Nielsen, 1981). Vitamin D3 is a known influencer for tissue energy substrate utilization and mineral metabolism (Hunt, 1996). Moreover, a vitamin D3 deficiency often causes an increase observed in plasma glucose concentration primarily due to the impact that vitamin D3 has on beta cells (β-cells) and their production of insulin, which is needed to control blood glucose levels (Berridge 2017).

Interestingly, work using a vitamin D3-deficient chick as the model found that supplementing B (3 ppm B as boric acid) decreased a relatively high plasma glucose concentration (induced by vitamin D3 deficiency) by 29% compared to only a 6% decrease observed in the vitamin D3-adequate group (Hunt and Nielsen, 1987; Hunt, 1996). Since then, several other studies using chicks have continued to demonstrate that dietary B can mitigate the rise in plasma glucose concentrations that are associated with

vitamin D3 deficiency (Hunt and Herbel, 1993; Hunt, 1989; Hunt, 1988; Hunt et al., 1994). Hunt and Herbel (1991) reported that rats fed a diet supplemented with dietary B (2.4 mg ppm B as boric acid) had substantially lower plasma insulin, pyruvate concentrations, and creatinine kinase activity coupled with an increase in plasma thyroxine (T_4) concentration which are all indices of energy metabolism. The decrease in plasma insulin concentrations above is of particular interest because the overproduction of insulin can cause insulin resistance which is a function of deteriorated and weakened pancreatic beta cells (β-cells) which are responsible for insulin production (Sprietsma and Schuitemaker, 1994). Hunt (1996) hypothesized that B may reduce the stress that pancreatic β-cell endure with the overproduction of insulin.

Furthermore, Hunt and Herbel (1991) also reported that B-supplemented rats had a decrease in plasma aspartate aminotransferase (AST) compared to rats fed diets without supplemental B. The authors suggested that the reduction observed in plasma AST was likely a result of B supporting and maintaining normal liver function. Elevated plasma AST levels can be indicative of soft tissue damage as AST is a common metabolic enzyme measured to evaluate metabolic response with regard to the transamination of L-aspartate and α-ketoglutarate to oxaloacetate and glutamate (Kaneko, 1989).

Boron has also been considered to have a role in immune function and inflammatory response. Newly weaned pigs fed mycotoxin-contaminated diets supplemented with calcium fructoborate ($Ca\cdot[(C_6H_{10}O_6)_2B]_2\cdot 4H_2O$); supplied per manufacturers recommendations of 1.65 g/pig) for 24 days resulted in a drastic reduction in synthesis of liver tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8) which was accompanied by an ~ 48% increase in average weight gain compared to pigs fed

mycotoxin contaminated diets without calcium fructoborate supplementation (Taranu et al., 2011). In contrast, using in vitro studies, Nzietchueng et al. (2002) demonstrated that B modulates the turnover of the extracellular matrix and increases TNF-α release from the effects of B on specific enzymes in fibroblasts. Furthermore, Nzietchueng and colleagues (2002), hypothesized that B acts as a mediator for the synthesis of cytokines such as TNF-α.

A different study that fed broiler chicks diets supplemented with B as boric acid (0, 25, 50, 75 ppm B), found an increase in total antioxidant capacity that was measured by ferric reducing antioxidant power (Pradhan et al., 2021) for B supplemented birds compared to birds not supplemented with B. Furthermore, the previous authors also reported a decrease in plasma glutathione (GSH) but no effect on plasma malondialdehyde (MDA) in broilers fed diets with increasing B levels which suggests that B may protect cellular damage caused by lipid peroxidation.

In rats, supplying 40 ppm B of drinking water (as boric acid) resulted in an increase in absolute and relative weight, antioxidant capacity, and tissue structure of the spleen, however at concentrations of 80 ppm B of drinking water or greater resulted in adverse effects that were harmful to the rats (Hu et al., 2014). Thus, it was proposed that lower B concentrations (i.e. 40 ppm B of drinking water) may play a protective role in the development of the spleen while higher B concentrations (in this study ≥ 80 ppm B of drinking water) can lead to damaging and toxic effects to the spleen likely caused by increased lipid peroxidation as previous authors reported a decrease in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities while MDA activity was increased. The work above suggests that B may have a protective role on cellular

integrity when B is supplemented at optimum levels, however, when B is supplemented at higher levels there is a potential for B to produce toxic effects potentially damaging tissues and cells. The previous work is illustrative of the complexity of B supplementation; supplementing at an optimum level can produce advantageous responses but too much supplementation can lead to adverse results. Altogether determining the optimum supplemental level of B can be of great benefit, nonetheless, the optimum B supplementation level likely is dependent on a wide array of considerations including species, age, production phase, nutritional status, etc.

The potential effects of B supplementation on cellular integrity have also been observed in vitro using dermal cells. Demirci et al. (2016) demonstrated that adding B derivatives (boric acid and sodium borate) to dermal cells resulted in both B compounds increasing proliferation, migration, vital growth factor, and gene expression levels while also displaying antimicrobial effects against bacteria yeast, and fungi. Another in vitro study revealed that 19.5 μg B/mL (or 19.5 ppm B) of boric acid or sodium borate incubated with B-cells of the pancreas in a hypoxic state increased cell viability and antioxidant enzyme activity (Aydin et al., 2019). Furthermore, authors reported that either boric acid or sodium borate impacted (increased) gene expression levels of antioxidant enzymes, insulin biosynthesis and secretion, beta cell maturation, and glucose metabolism. The results reported by Aydin et al. (2019) may help explain the results reported by Kucukkurt et al. (2015) where serum insulin levels were decreased (0.20 vs. 1.18 ng/mL, respectively) in rats fed diets supplemented with or without sodium borate (100 ppm B vs. 0 ppm B). Moreover, rats fed the B-supplemented diet (sodium borate)

had an approximate 48 and 49% decrease in serum leptin and glucose concentrations respectively, compared to rats fed the diet without supplemental B.

Differently, in gilts that were fed diets supplemented with B (5 ppm B as sodium borate) and later injected with an intradermal injection of phytohemagglutinin as a skin test (PHA; used to elicit a cell-mediated immune response) to measure changes in skin thickness resulted in B supplemented gilts having a decreased skinfold difference observed at 6, 12, 24 and 48 h following the injection (Armstrong et al. 2001). The results above were later repeated and accompanied by increased production of TNF-α and interferon-gamma (IFN-γ) following an intramuscular injection of lipopolysaccharide solution (LPS) (Armstrong and Spears, 2003). Although the direct mechanisms related to B and immune function have only been speculated, Hunt and Isdo (1999) suggests that B increases the activity of enzymes related to the respiratory burst cascade. This, in short, can be explained by specific enzymes destroying reactive oxygen species (ROS) that are generated by superoxide radical that was converted from molecular oxygen through the activation of NADPH oxidase on the surface of phagocytic cells.

2.7.6 *Boron on male reproduction*

More recently, B has been considered to influence male reproduction in several different animal species. In 10-month-old male rabbits that were fed diets supplemented with 17.5, 35, or 70 ppm B from boric acid increased sperm concentration and sperm output (Elkomy et al., 2015). Moreover, the semen from B-supplemented male rabbits was found to have an increase in live sperm (3.1, 3.7, and 4.1%, respective of increasing B) accompanied by a reduction in dead or abnormal sperm compared to the semen from bucks not supplemented with B. More recently, Ibrahim et al. (2019) reported that intact

male sheep (rams) supplemented with 70 ppm B as boric acid had greater serum testosterone levels in the 12th and 16th weeks compared to the rams fed a diet without B supplementation. Like Elkomy et al. (2015), Ibrahim et al. (2019) also found that semen from rams fed a B-supplemented diet had an increased sperm concentration and total volume of ejaculate, with a reduction in secondary sperm abnormalities. Congruent with previous reports, male goats supplemented orally with 40 ppm B (sodium borate) had a higher total sperm motility, total progressive motility, and average sperm concentration of the total ejaculate and per mL of semen compared to goats supplemented with no B (Krishnan et al., 2019). The same group also reported that B-supplemented goats had greater levels of glutathione reductase (GSR; an antioxidant enzyme) in the seminal plasma compared to the goats not receiving B which is of particular interest considering the role GSR has on glutathione (GSH) and its ability to fight cellular oxidative stress and thus potentially protecting sperm cells.

Different than dietary B supplementation, B has recently been considered a beneficial additive used to supplement extended semen due to its metabolic and antioxidant activities. Work by Tirpan and Tekin (2015) demonstrated that the addition of B from sodium borate to extended male goat semen had positive effects on spermatozoa viability and reduction in abnormal spermatozoa ratios. Following work evaluating B (sodium borate) addition to extended bull semen in replacement of glucose resulted in no statistical differences between treatments suggesting that B addition may alter energy metabolism resulting in sufficient energy levels needed for the intracellular metabolism of spermatozoa during the freeze and thawing processes (Tirpan et al., 2018). Another group evaluated the effects of adding B directly to semen (ram semen) as an extender and

its effects associated with the freeze-thawing practice of semen samples (Yeni et al., 2018). Authors reported that adding 1mM of B (sodium borate) to diluted ram semen samples that were frozen and thawed resulted in a 10% increase in sperm motility with a 27% decrease in sperm DNA damage compared to semen that had no B added. Furthermore, a later study utilizing cryopreserved brown trout spermatozoa found that adding 0.1 to 0.4 mM B to the ionic semen extender increased fertilization and eyeing rates and decreased DNA damage at two different freezing rates (Bozkurt et al., 2019). The potential beneficial effects observed by adding B to semen appear to be generated through improving energy metabolism and antioxidant status of the semen ultimately favoring optimal conditions for spermatozoa.

2.7.7 *Boron toxicity*

Although B has shown numerous beneficial properties in various animal models and metabolic pathways, there is still a valid concern for B toxicity with high levels and chronic exposure. The first report of B toxicity occurred in 1904 when human volunteers having an intake of 500 mg boric acid/d (77 mg B/d) for 50 days exhibited negatively impacted appetite, digestion, and overall health (Wiley, 1904). The authors concluded that 500 mg boric acid/d was too much for a normal man to consume regularly and that consumption of boric acid at 4,000 mg/d (699 mg B/d) was the limit to which humans experienced severe harm.

Other studies have shown that higher levels of B can negatively affect reproduction. For instance, male rats that consumed 1,170 ppm B for 90 days became sterile (Weir and Fisher, 1972). The same lab also reported that male dogs consuming 1,750 ppm B for 90 days resulted in testicular atrophy. However, the same authors also

reported that a different feeding trial lasting two years illustrated that both rats and dogs could tolerate up to 350 mg B/kg diet with no adverse effects observed on reproduction. Similarly, male rats supplemented with 1,000 or 2,000 ppm B for 60 days (Lee et al., 1978) resulted in decreased diameter of the seminiferous tubule and a loss of spermatocytes, spermatids, and mature spermatozoa; the infertility observed in that study continued for eight months after the termination of the B diet. More recent work by Singh et al. (2021) reported that supplementing B at 10.8 g B/d (equivalent to 600 ppm B) in the form of boric acid to crossbred calves for 120 days did not result in any detrimental effects. Detrimental effects of B consumption and exposure appear to only be present at levels greater than 1000 ppm B. Moreso, adverse effects of B supplementation may be more associated with the species and the ability of the species to handle certain quantities of B. Currently, the WHO has indicated that a safe upper intake would be 0.4 mg B/kg BW (or ~ 28 mg B/d) for a 70 kg person (WHO, 1996). Whereas the Institute of Medicine has established a B intake of 20 mg/d as Tolerable Upper Intake Level (UL) for adults (Food and Nutrition Board, 2002). The lethal dose (LD_{50}) of ingested B has not been determined precisely, however, Locatelli et al. (1987) estimated that a lethal dose of boric acid (17.5% B) for infants is 2-3 g and for children is 5-6 g. Overall, B has a relatively low order of toxicity when administered orally. Nonetheless, it should be noted that acute toxicity can occur at very high intakes (> 1,000 ppm B) and chronic toxicity can occur with prolonged exposure to high dietary intake of B. Alternatively, toxicity should be expressed and evaluated on a species and life stage basis as there are great differences between species and their respective life phase that influences B tolerance.

2.8 Conclusion

The productivity of the modern sow continues to improve over time. One major improvement in sow production over the last several decades is the increase in litter size. Through advancements made in genetic selection, the average number of total piglets born per litter in the US has seen an approximate 35% increase from 2004 to 2022 (PigChamp, 2022). Consequently, the US swine industry has also experienced a dramatic increase in sow mortality and removal from the breeding herd. Ultimately, this increase in sow removal and mortality directly correlates with decreased sow longevity and overall lifetime productivity of the sow. It is well understood that a decrease in sow lifetime productivity, results in an overall reduction in the economic potential for producers. Thus, advances in sow nutrition and management must improve in parallel with the sow's genetic potential to optimize production and economic potential.

An area that is often understudied in sow nutrition is trace mineral supplementation. Further understanding the potential effects of supplementing different sources and levels of trace minerals on modern sow production may yield economic advantages to producers given that production or productivity is improved with minimal change in the cost of production. It is well understood that sow's have a dietary requirement for certain trace minerals. However, B is a trace mineral that has been overlooked and perhaps understudied in swine.

Evaluating B as a supplemented trace mineral to sows is an area that warrants great interest mainly due to the numerous reports where dietary B resulted in enhanced reproduction, metabolism, bone structure, and immune function in a wide range of animal models. However, there is limited information available on supplementing dietary B to

swine. Therefore, evaluating dietary B supplementation to sows may result in findings that help improve the overall nutrition status of the sow and consequently improve sow productivity and economic potential for swine producers.

CHAPTER 3. Effects of dietary boron supplementation on fasting and postprandial serum insulin, glucose, and clinical chemistry profile of growing pigs

3.1 Abstract

A 49-d experiment was conducted to evaluate the effects of supplemental boron (B) on serum insulin, glucose, and clinical chemistry profile of growing pigs. Crossbred pigs (n = 48; initial body weight [BW] 19.18 ± 0.29 kg) were blocked on BW and sex and randomly allotted to 1 of 4 dietary treatments. Diets were corn-SBM-based, formulated to meet or exceed NRC (2012) nutrient requirement estimates, and were supplemented with 0, 25, 50, or 100 ppm B as sodium tetraborate decahydrate. Blood samples were collected on d 20 and 41 after a fasting period. Fasting samples were collected following an overnight fast; then a postprandial sample was taken approximately 50 minutes after the pigs had 10 minutes of ad-libitum access to feed. Samples were processed and serum analyzed for insulin and glucose concentration. On d 20, supplemental B resulted in a quadratic response on fasting serum glucose concentration (5.25, 4.80, 4.65, and 5.01 mmol/L, $P = 0.03$) and a linear decrease in postprandial serum insulin concentration (29.1, 25.5, 18.2, and 18.1 μU/mL, $P = 0.02$). Furthermore, there was a tendency for a linear decrease in fasting insulin/glucose ratio (0.85, 0.84, 0.42, and 0.59, $P = 0.08$), which became more noticeable during the postprandial state (3.96, 3.63, 2.63, and 2.73, $P = 0.02$). Again on d 41, there was a quadratic response on fasting serum glucose concentration (4.32, 4.07, 3.91, and 4.68, $P = 0.01$) with supplemental B. Results suggest that supplemental B may impact serum insulin and glucose concentrations by reducing the amount of insulin needed to maintain glucose concentrations. However, higher levels of supplemental B did result in suppressed growth performance. Thus, additional research is warranted to determine the optimum level of supplemental B.

Keywords: boron, glucose, insulin, pig

3.2 Introduction

Currently, there is not an established nutrient requirement estimate regarding dietary boron (B) supplementation in swine, however, NRC (2012) acknowledges that B may be essential in very low dietary levels as it has been for other species but not yet proven in swine. Over the last 40 years, there has been an accumulation of data reported for several different species supporting the biological importance of B and its essentiality in animal nutrition (Nielsen, 2008). Other reports have found that dietary B supplementation may affect energy metabolism (Hunt, 1997), insulin sensitivity, and immune function (Hunt and Idso, 1999; Armstrong et al., 2001). Furthermore, dietary B decreased peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats (Bakken and Hunt, 2003). In these studies, B supplementation (2 ppm B) to rats fed a low-B diet (0.2 ppm B) reduced plasma insulin but did not change plasma glucose concentrations after an overnight fasting period. The same authors reported that B-deprived chicks had about a 75% higher peak insulin release from isolated, perfused pancreata compared to the pancreata of B-supplemented chicks. Also, feeding diets that were supplemented with B (100 ppm B) to rats for four weeks resulted in a much lower plasma glucose and insulin concentration compared to the rats fed a control diet (6.4 ppm B) (Kucukkurt et al., 2015). These findings suggest that B may affect energy metabolism by possibly reducing the amount of insulin needed to maintain glucose within normal ranges for a variety of species. Thus, the objective of this experiment was to evaluate increasing levels of dietary B supplementation to a common corn-soybean meal basal diet fed to growing swine on growth performance, and serum glucose, insulin, and clinical chemistry during a fasted and postprandial state after 3 and 6 weeks of receiving dietary B.

3.3 Experimental procedures

This experiment was conducted at the University of Kentucky Swine Research Center under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

3.3.1 *Animals, housing, and experimental design*

A total of 48 pigs (24 barrows and 24 gilts) with a mean initial bodyweight (BW) of 19.18 ± 0.29 kg were allotted to pens based on initial BW, sex, and litter of origin. Pens were randomly assigned to receive one of four dietary treatments as follows: 0, 25, 50, and 100 mg supplemental B per kg of diet. All pigs were housed by sex and 6 per pen, with 2 pens per treatment (6 barrows and 6 gilts per treatment). Boron was supplemented as sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc. Boron, CA).

All experimental diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates for growing swine with respect to BW (Table 3.1). Pigs were maintained on their respective experimental diets throughout a grower period (49 d) and averaged a final BW of 60.66 ± 0.86 kg. Experimental diets were fed in a two-phase feeding regimen (Phase I, 0-20d; Phase II, 20-49 d). All pigs had ad libitum access to feed and water throughout the 49-d grower period. Animal weights and feed disappearances were monitored and recorded weekly.

On d 20 and d 41, all pigs underwent an overnight fasting period where feeders were removed at approximately 1700 h. At 0600 h on the following day, all pigs were bled for a fasting sample. At the completion of the last blood sample, feeders were placed

back in the pen and pigs were allowed ad libitum access to feed for a total of 10 minutes, then the feeder was removed once again. Approximately 50 minutes after the pigs had access to feed, pigs were bled for a postprandial sample. Venous blood samples were obtained from the jugular vein and collected using a needle holder and Vacutainer blood collection tube with polymer gel (Becton, Dickson and Company, Franklin Lakes, NJ) for serum separation. Serum was obtained by centrifugation of the blood at 2500×g and 4°C for 20 minutes. Serum samples were stored at -80 °C until the chemical assays were performed.

Table 3.1 Formulation and calculated composition of basal diet (as-fed basis)[1].

	Basal diet	
Items	Phase I	Phase II
Ingredient, %		
Corn	60.81	69.39
Soybean meal, 48% CP	33.40	25.00
Grease, choice white	2.60	2.60
L-Lysine•HCl	0.30	0.25
DL-Methionine	0.16	0.09
L-Threonine	0.12	0.08
Dicalcium phosphate	0.88	0.88
Limestone	1.07	1.05
Salt	0.50	0.50
Vitamin premix[2]	0.04	0.04
Trace mineral premix[3]	0.10	0.10
Santoquin[4]	0.02	0.02
Total	100.00	100.00
Calculated composition		
Metabolizable energy, kcal/kg	3,397.79	3,404.93
Crude protein, %	21.42	18.00
SID Lysine, %[5]	1.23	0.98
Ca, %	0.70	0.67
Total P, %	0.56	0.52
STTD P, %[6]	0.33	0.31

[1]Phase I = 0 -20 d on test; Phase II = 20 – 49 d on test.
[2]Vitamin inclusion per kg of total diet: 9,361 IU of vitamin A; 2,342 IU of vitamin D3; 62 IU of vitamin E; 6.9 IU of vitamin K; 0.03 mg of vitamin B12; 0.23 mg of biotin; 0.17 mg of folic acid; 41.5 mg of niacin; 20.85 mg of pantothenic acid; 4.16 mg of riboflavin; 0.23 mg of biotin; 0.17 mg of folic acid; 41.5 mg of niacin; 4.16 mg of vitamin B6; and 1.15 mg thiamin.
[3]Mineral inclusion per kg of total diet: 50 mg of Mn as manganous sulfate, 100 mg of Fe as ferrous sulfate, 125 mg of Zn as zinc sulfate, 18 mg of Cu as copper sulfate, 0.35 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.
[4]Santoquin (Novus International, Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diets.
[5]Calculated composition of lysine is presented on a standardized ileal digestible (SID) basis.
[6]Calculated composition of phosphorus is presented on a standardized total tract digestible (STTD) basis.

3.3.2 *Laboratory and statistical analysis*

Aliquots of serum were sent to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) where a serum chemistry panel was performed using an Alfa Wassermann Vet Axel chemistry analyzer (Alfa Wassermann Diagnostic Technologies LLC., West Caldwell, NJ). Serum glucose concentration was determined using an automated enzymatic assay using a YSI 2700 biochemical analyzer (YSI Incorporated, Yellow Springs, OH). Insulin concentration was determined using a commercial porcine insulin radioimmunoassay (RIA) kit (EDM Millipore Corporation, Billerica, MA). Standards and samples were analyzed in duplicates.

The experimental data were subjected to ANOVA using Generalized Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). The model included terms for treatment, sex, replicate, and sex by treatment interaction with the individual pig serving as the experimental unit. All data are presented as least squares means. Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of dietary B supplementation. Coefficients used in the polynomial contrast were obtained using the PROC IML procedure in SAS. Differences were considered significant when $P < 0.05$ and tendencies were detected at $P \leq 0.10$.

3.4 Results and discussion

In the present study, there were no sex by treatment interactions observed for any of the response measures.

3.4.1 *Growth performance*

Currently, nutrient requirement estimates have yet been established for dietary B in swine (NRC, 2012). Nonetheless, the National Research Council (NRC) proposed a maximum tolerable level (MTL) of 150 mg B/ kg of DM (or ppm) for poultry, swine, horses, and sheep by extrapolation of data from other species (NRC, 2005). In the current study, the highest level of supplemental B was 100 ppm which is under the MTL estimate (NRC, 2005). The effect of dietary B supplementation on BW and ADG is presented in Table 3.2. There were sex effects ($P < 0.05$) present regarding pig BW at all weighing dates. Sex effects were also present for ADG at selective periods as well as the overall experimental period (d 0-49). The sex effects present in the current study are likely due to the difference in BW of barrows compared to gilts at the initiation of the experiment. On d 0 or the initiation of the experiment, barrows were ~ 8% heavier than gilts (initial BW of 43.85 vs. 40.72 kg, respectively). By the end of the experiment (d 49), the difference in BW between barrows and gilts remained separated as barrows were ~ 9% heavier than gilts (final BW of 139.67 vs. 127.88 kg, respectively). A linear decrease in ADG ($P < 0.01$) in relation to B supplementation was observed as early as d 14-20 which contributed to a linear decrease in BW ($P < 0.05$) from d 20 through to d 49. Overall (d 0-49), there was a linear decrease in ADG ($P = 0.02$) resulting in the 100 ppm B treatment group having the lowest final BW. The lower ADG that was observed for the high B diet may be a result of the supplemental B level approaching the MTL set by NRC (2005), however, lethargy or any other health concern was not observed for any of the pigs in the present study. Armstrong and Spears (2001) reported that dietary B supplementation (5 and 15 ppm B) to barrows resulted in increased ADG and ADFI during the nursery (0-35

d) and growing (36-66 d) phases. In the current experiment, dietary B was supplemented at higher levels with 25 ppm B being the lowest supplemental level, and thus in the current experiment the dietary supplemental levels may be too high which would explain the reduced ADG observed with dietary B supplementation.

Table 3.2 Effects of dietary boron (B) supplementation on bodyweight (BW) and average daily gain (ADG)[1].

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
BW, kg							
0 d[S]	19.2	19.1	19.2	19.2	0.29	-	-
7 d[S]	22.9	22.7	22.5	22.6	0.38	-	-
14 d[S]	29.5	29.2	28.8	29.0	0.36	-	-
20 d[S]	33.9	33.5	33.2	32.3	0.40	0.01	-
28 d[S]	40.1	39.9	39.2	38.8	0.46	0.04	-
35 d[S]	48.0	47.2	46.4	45.0	0.58	< 0.01	-
41 d[S]	54.6	54.3	52.8	52.5	0.74	0.03	-
49 d[S]	61.9	61.0	60.6	59.2	0.86	0.03	-
ADG, kg							
0-7 d	0.52	0.52	0.47	0.49	0.03	-	-
7-14 d[S]	0.94	0.92	0.90	0.91	0.03	-	-
14-20 d	0.73	0.72	0.73	0.56	0.04	< 0.01	0.10
21-28 d	0.78	0.80	0.76	0.81	0.03	-	-
28-35 d[S]	1.13	1.04	1.02	0.88	0.05	< 0.01	-
35-41 d[S]	1.10	1.18	1.08	1.25	0.05	0.07	-
41-49 d[S]	0.91	0.84	0.97	0.84	0.04	-	-
0 -20 d[S]	0.73	0.72	0.70	0.66	0.01	< 0.001	-
20 –49 d[S]	0.97	0.95	0.95	0.93	0.02	-	-
0-49 d[S]	0.87	0.86	0.85	0.82	0.02	0.02	-

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). There were no sex by treatment interactions present for any growth response measures.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$; where barrows were heavier than gilts.

3.4.2 *Serum insulin and glucose concentration*

Serum insulin and glucose concentrations at d 20 and d 41 are reported in Table 3.3 and 3.4, respectively. On d 20, postprandial serum insulin concentration decreased linearly ($P = 0.02$) with increasing dietary B concentration. Additionally, on d 20 there was a quadratic decrease ($P = 0.03$) in fasting serum glucose concentration with the 50 ppm B treatment having the lowest concentration. There was a sex effect ($P < 0.05$) present for postprandial serum glucose concentration where the gilts had ~ 13% lower serum glucose concentration compared to barrows (6.51 vs. 7.36 mmol/L, respectively). Altogether the fasting insulin to glucose ratio on d 20 had a linear tendency to decrease ($P = 0.08$) with dietary B levels which became more pronounced in the postprandial state ($P = 0.02$). However, on d 41, serum insulin concentration was not affected by B supplementation. Like d 20, fasting serum glucose quadratically decreased ($P = 0.01$) with 50 ppm B treatment having the lowest glucose concentrations compared to the other treatments. The results observed in Table 3.3 agree with Kucukkurt et al. (2015), where authors reported that rats receiving a diet supplemented with 100 ppm B (sodium borate) for 4 weeks resulted in a large reduction in both fasting plasma glucose and insulin. Additionally, work supplementing much lower levels of dietary B (1.65 ppm B as boric acid) to broiler chicks undergoing in-situ pancreatic perfusion (B and glucose solution) resulted in lower peak insulin release compared to chicks that were deprived of dietary B (Bakken and Hunt, 2003).

However, Bakken and Hunt (2003) reported that B supplementation (2 ppm B as boric acid) to diets fed to rats resulted in lower fasting plasma insulin concentrations with no change to fasting plasma glucose levels. In the current experiment, fasting glucose

concentration was quadratically decreased on both d 20 and 41 with pigs receiving diets supplemented with 50 ppm B having the lowest glucose levels. The differences observed between Bakken and Hunt (2003) compared to the study herein may be due to differences in supplementing B as either boric acid or sodium borate. Ultimately, Kucukkurt et al. (2015) supplemented rats with 100 ppm B as either boric acid or sodium borate and found that sodium borate stimulated a larger decrease in fasting plasma insulin and glucose concentrations. In the present study, B supplementation as sodium borate resulted in several instances of serum insulin and glucose being affected. Nonetheless, the reductions in fasting serum insulin/glucose concentrations in the present study agree with Bakken and Hunt (2003), who suggests that dietary B may lower the amount of insulin needed to maintain glucose concentrations.

Table 3.3 Effects of dietary boron (B) supplementation on fasting and postprandial serum insulin and glucose concentration at d 20[1]

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
Insulin, μU/mL							
Fasting	4.51	4.16	1.96	3.09	0.73	-	-
Postprandial	29.13	25.49	18.15	18.10	3.27	0.02	-
Glucose, mmol/L							
Fasting	5.25	4.80	4.65	5.01	0.19	-	0.03
Postprandial[S]	7.05	6.89	7.08	6.72	0.28	-	-
Insulin/glucose							
Fasting	0.85	0.84	0.42	0.59	0.13	0.08	-
Postprandial	3.96	3.63	2.63	2.73	0.38	0.02	-

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to feed. There were no sex by treatment interactions present for any serum measures.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[S]Sex effect present, $P \leq 0.05$; where gilts had a lower serum glucose concentration compared to barrows.

Table 3.4 Effects of dietary boron (B) supplementation on fasting and postprandial serum insulin and glucose concentrations at d 41[1]

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
Insulin, μU/mL							
Fasting	3.83	2.50	2.43	3.44	0.72	-	-
Postprandial	27.03	30.30	27.87	27.53	5.67	-	-
Glucose, mmol/L							
Fasting	4.32	4.07	3.91	4.68	0.18	-	0.01
Postprandial	6.27	5.98	6.32	6.02	0.26	-	-
Insulin/glucose							
Fasting	0.84	0.59	0.62	0.68	0.14	-	-
Postprandial	4.16	5.16	4.47	4.48	0.86	-	-

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to feed. There were no sex by treatment interactions present for any serum measures.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B *P*-values greater than 0.10 are replaced with "-".

3.4.3 *Serum mineral and clinical chemistry profile*

Serum mineral profiles at d 20 and d 41 are reported in Tables 3.5 and 3.7, respectively. At d 20 there were no differences observed for Na, Cl, Ca, and P in both the fasting and postprandial state. However, there was a tendency for a quadratic effect (P = 0.07) observed for fasting serum Ca:P ratio. Also at d 20, there were tendencies for quadratic effects (P = 0.06 and P = 0.09, respectively) for postprandial serum K and Mg concentration.

Later at d 41 (Table 3.7), there was a linear increase in fasting serum Na and Mg concentrations (P = 0.05 and P = < 0.01, respectively). In the postprandial state, serum phosphorus tended to decrease quadratically (P =0.06) with increasing dietary B supplementation. Additionally, there was a linear increase observed for postprandial serum chloride concentration (P = 0.02). Armstrong et al. (2001), reported that gilts supplemented with 5 ppm B had greater plasma Ca concentrations during the nursery period but no other differences in plasma Ca, P, and Mg concentrations were observed during multiple growth periods (nursery, grower, and finisher phases). Conversely, in the current experiment, there were no effects observed in both fasting or postprandial serum Ca concentrations. Also, dissimilar to Armstrong et al. (2001), the current study demonstrated an increase in fasting serum Mg concentration at d 41. In ruminant species (heifers and sheep), it has been hypothesized that B may be linked to the metabolism of macro-minerals such as Ca, P, and Mg (Green and Weeth, 1977; Brown et al., 1989). Although not significant, it is worth noting that there were tendencies for a linear and quadratic effect for fasting and postprandial serum P content at d 41, respectively. Interestingly, at d 41, fasting and postprandial serum P concentrations were affected by

sex ($P < 0.05$), as gilts had greater serum P concentrations at both fasting and postprandial compared to barrows (8 and 5% greater, respectively).

Fasting and postprandial serum biochemical profiles at d 20 and 41 are reported in Tables 3.6 and 3.8, respectively. Serum bilirubin production is related to the turnover rate of heme found in the spleen and liver (Kaneko, 1989). Various hepatobiliary disorders can cause serum bilirubin concentrations to rise. At d 20, there was a linear tendency for fasting serum bilirubin to increase with increasing dietary B supplementation ($P = 0.06$). However, pigs fed the 25 ppm B treatment had numerically the lowest total bilirubin concentration, in the postprandial state, and all treatments were within the reference range of Merck (2010). Similar at d 41, pigs supplemented 25 ppm B had the lowest fasting serum total bilirubin resulting in a tendency for a quadratic effect ($P = 0.06$).

At d 20 postprandial serum aspartate aminotransferase (AST) increased linearly ($P = 0.04$) with increasing B concentration. Aspartate aminotransferase is a common enzyme measured to evaluate metabolic response with regards to the transamination of L-aspartate and α-ketoglutarate to oxaloacetate and glutamate (Kaneko, 1989). Severe elevations of AST can be linked with hepatic disorders. Alkaline phosphatase (ALP) has been recognized as a key clinical measure for bone and liver function as the enzyme is associated with osteoblastic activity in bone (Kaneko, 1989). In young, growing animals, it is very common to see an elevated ALP concentration due to the high osteoblastic activity in these animals as bones undergo development and growth (Kaneko, 1989), Interestingly gilts supplemented with 5 ppm during the growing phase (mean BW = 59.6 kg) had approximately a 19% increase in serum ALP concentration (Armstrong et al.,

2001). However, in the current study, there were no effects of B on serum ALP concentrations at any point of the experiment, but it is noteworthy that all treatments did express higher serum ALP concentrations than the reference ranges provided by Merk (2010). These greater ALP concentrations observed in the present study may be linked to the age of the pigs as they are considered to be in a rapid growth phase and are undergoing bone development.

Unlike ALP, AST concentrations remained within the reference range. At d 41, there were no differences observed for fasting and postprandial serum AST and ALP concentrations. But once again, the ALP concentrations for all treatments remained above the reference ranges provided by Merck (2010) at d 41.

Serum albumin, globulin, and total protein concentrations are all indicators of hepatic protein synthesis (Kaneko, 1989). In the current experiment, there were no differences observed in total protein during any of the time points. Interestingly at d 21, the fasting serum globulin concentration decreased linearly ($P = 0.05$) with a tendency for a quadratic response ($P = 0.10$) with the pigs receiving 50 ppm B having the lowest concentration. Furthermore, fasting serum albumin/globulin ratio increased linearly ($P = 0.03$) with increasing dietary B. Conversely at d 41, there were no differences between treatments observed for both fasting and postprandial albumin, globulin, and albumin/globulin ratio.

It has previously been reported that gilts fed 5 ppm B expressed serum cholesterol concentrations approximately 16% greater than those of the control pigs (Armstrong et al., 2001). In the current study, serum cholesterol was only affected at d 41, where there

was a quadratic response ($P = 0.05$) in relation to dietary B as the pigs supplemented with the 50 ppm B had the lowest serum cholesterol concentration of the treatments.

Blood urea nitrogen (BUN) and creatinine can be used as screening tools to measure gross renal dysfunction (Kaneko, 1989). Kidney dysfunction occurs when about 2/3rds of the nephrons are disturbed causing poor glomerular filtration. In the present study, it is noted that all treatments had BUN and creatinine values that were in the normal reference ranges (Merck, 2005). Blood urea nitrogen, creatinine, and the ratio were not affected at d 21. However, there was a tendency for a quadratic effect on postprandial serum creatinine concentration and a linear effect ($P = 0.04$) for fasting serum BUN/creatinine ratio.

Table 3.5 Effects of dietary boron (B) supplementation on fasting and postprandial serum mineral analysis at d 20[1].

Items	Reference values[2]	Supplemental B, ppm				SEM	*P*-value[3]	
		0	25	50	100		L	Q
Sodium, mmol/L								
Fasting	139-153	141.00	142.33	140.67	142.50	0.60	-	-
Postprandial		141.17	143.83	141.25	142.17	0.57	-	-
Potassium, mmol/L								
Fasting	4.4-6.5	6.72	7.13	6.67	6.75	0.17	-	-
Postprandial		6.33	6.66	6.54	6.12	0.17	-	0.06
Chloride, mmol/L								
Fasting[S]	97-106	102.50	102.75	102.08	102.42	0.55	-	-
Postprandial		101.25	101.5	100.92	101.42	0.44	-	-
Calcium, mg/dL								
Fasting	9.3-11.5	11.21	11.42	10.96	11.13	0.13	-	-
Postprandial		11.66	11.99	11.64	11.51	0.15	-	-
Phosphorus, mg/dL								
Fasting	5.5-9.3	9.59	10.29	10.05	10.04	0.25	-	-
Postprandial		9.87	10.51	10.23	10.01	0.30	-	-
Ca:P								
Fasting	-	1.17	1.11	1.10	1.11	0.02	-	0.07
Postprandial[S]		1.19	1.15	1.15	1.16	0.03	-	-
Magnesium, mg/dL								
Fasting	2.3-3.5	2.28	2.47	2.37	2.42	0.07	-	-
Postprandial[S]		2.27	2.53	2.34	2.32	0.07	-	0.09

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to feed. There were no sex by treatment interactions present for any serum measures.
[2]Values represent the lower and upper limits of reference ranges of serum biochemical constituents in Table 7 of the Reference Guides in Merck (2005).
[3]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.

Table 3.6 Effects of dietary boron (B) supplementation on fasting and postprandial serum clinical chemistry profile at d 20 [1].

Items	Reference values[2]	Supplemental B, ppm				SEM	*P*-value[2]	
		0	25	50	100		L	Q
BUN, mg/dL								
Fasting	8.25-25	13.00	12.67	13.92	12.83	0.77	-	-
Postprandial		13.75	13.92	14.67	13.67	0.80	-	-
Creatinine, mg/dL								
Fasting[S]	0.8-2.3	1.28	1.37	1.25	1.29	0.03	-	-
Postprandial		1.31	1.37	1.33	1.38	0.04	-	-
BUN/creatinine								
Fasting	-	10.33	9.33	11.33	10.17	0.61	-	-
Postprandial		10.42	10.17	11.08	10	0.61	-	-
Total bilirubin, mg/dL								
Fasting	0.0-0.5	0.48	0.40	0.81	0.63	0.08	0.06	-
Postprandial[S]		0.31	0.26	0.48	0.33	0.06	-	-
ALP, U/L								
Fasting[S]	41-176	283.00	268.08	323.92	277.92	15.28	-	-
Postprandial[S]		270.50	266.17	313.83	262.42	15.20	-	0.06
AST, U/L								
Fasting	15-55	24.67	23.42	27.08	26.58	2.24	-	-
Postprandial[S]		28.58	33.67	39.75	40.58	4.07	0.04	-
Total protein, g/dL								
Fasting	5.8-8.3	5.98	6.07	5.79	6.01	0.09	-	-
Postprandial		5.73	5.98	5.69	5.70	0.09	-	-

Albumin, g/dL								
Fasting	2.3-4.0	4.51	4.61	4.54	4.67	0.07	-	-
Postprandial		4.33	4.53	4.41	4.38	0.06	-	-
Globulin, g/dL								
Fasting[S]	3.9-6.0	1.47	1.46	1.25	1.34	0.05	0.05	0.10
Postprandial		1.40	1.45	1.28	1.33	0.06	-	
Albumin/globulin								
Fasting[S]	-	3.16	3.23	3.71	3.54	0.14	0.03	-
Postprandial		3.18	3.17	3.49	3.39	0.15	-	-
Cholesterol, mg/dL								
Fasting	81-134	87.17	90.17	84.42	91.75	1.81	-	-
Postprandial		85.67	89.67	84.33	89.33	2.18	-	-

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to feed. There were no sex by treatment interactions present for any serum measures. BUN = blood urea nitrogen; ALP = alkaline phosphatase; AST = aspartate aminotransferase.

[2]Values represent the lower and upper limits of reference ranges of serum biochemical constituents in Table 7 of the Reference Guides in Merk (2005).

[3]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[S]Sex effect present, $P \leq 0.05$.

Table 3.7 Effects of dietary boron (B) supplementation on fasting and postprandial serum mineral profile at d 41[1].

Items	Reference values[2]	Supplemental B, ppm				SEM	*P*-value[3]	
		0	25	50	100		L	Q
Sodium, mmol/L								
Fasting	139-153	141.42	142.33	141.50	142.92	0.44	0.05	-
Postprandial		141.25	141.33	140.17	143.50	1.11	-	-
Potassium, mmol/L								
Fasting	4.4-6.5	5.93	5.84	6.03	6.09	0.13	-	-
Postprandial		6.04	6.16	6.19	6.29	0.22	-	-
Chloride, mmol/L								
Fasting	97-106	100.25	100.67	101.08	101	0.39	-	-
Postprandial		99.75	99.58	100.00	101.83	0.69	0.02	-
Calcium, mg/dL								
Fasting	9.3-11.5	10.73	10.77	10.74	10.81	0.09	-	-
Postprandial		11.56	11.29	11.22	11.47	0.18	-	-
Phosphorus, mg/dL								
Fasting[S]	5.5-9.3	11.03	10.54	11.00	11.43	0.24	0.09	-
Postprandial[S]		11.78	10.93	11.35	11.70	0.26	-	0.06
Ca:P								
Fasting	-	0.98	1.03	0.98	0.95	0.02	-	-
Postprandial		0.99	1.04	0.99	0.99	0.02	-	-
Magnesium, mg/dL								
Fasting	2.3-3.5	2.27	2.33	2.38	2.53	0.06	< 0.01	-
Postprandial		2.26	2.35	2.33	2.41	0.08	-	-

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to feed. There were no sex by treatment interactions present for any serum measures.

[2]Values represent the lower and upper limits of reference ranges of serum biochemical constituents in Table 7 of the Reference Guides in Merk (2005).

[3]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[S]Sex effect present, $P \leq 0.05$.

Table 3.8 Effects of dietary boron (B) supplementation on fasting and postprandial serum clinical chemistry profile at d 41[1].

Items	Reference values[2]	Supplemental B, ppm				SEM	*P*-value[3]	
		0	25	50	100		L	Q
BUN, mg/dL								
Fasting	8.25-25	14.08	11.75	14.50	14.67	0.76	-	-
Postprandial		15.50	13.75	15.92	15.42	0.73	-	-
Creatinine, mg/dL								
Fasting	0.8-2.3	1.44	1.42	1.38	1.38	0.04	-	-
Postprandial		1.52	1.39	1.35	1.44	0.04	-	< 0.01
BUN/creatinine								
Fasting	-	10.00	8.50	10.67	10.92	0.53	0.04	-
Postprandial		10.33	9.92	11.83	10.67	0.48	-	-
Total bilirubin, mg/dL								
Fasting[S]	0.0-0.5	0.56	0.36	0.48	0.53	0.05	-	0.06
Postprandial		0.33	0.33	0.28	0.31	0.03	-	-
ALP, U/L								
Fasting	41-176	212.42	180.83	235.50	203.00	15.06	-	-
Postprandial		208.42	176.58	223.58	196.00	13.65	-	-
AST, U/L								
Fasting	15-55	33.33	38.75	30.83	34.25	4.52	-	-
Postprandial		56.83	50.92	37.42	42.58	11.31	-	-
Total protein, g/dL								
Fasting	5.8-8.3	6.13	6.17	5.99	6.19	0.08	-	-
Postprandial		6.06	6.03	5.81	6.09	0.13	-	-
Albumin, g/dL								

Fasting	2.3-4.0	4.37	4.38	4.32	4.43	0.07	-	-
Postprandial		4.32	4.23	4.17	4.37	0.09	-	-
Globulin, g/dL								
Fasting	3.9-6.0	1.77	1.78	1.68	1.76	0.06	-	-
Postprandial		1.74	1.79	1.64	1.73	0.07	-	-
Albumin/globulin								
Fasting	-	2.55	2.49	2.61	2.57	0.12	-	-
Postprandial		2.53	2.41	2.55	2.6	0.10	-	-
Cholesterol, mg/dL								
Fasting	81-134	96.75	99	96.67	100.42	2.08	-	-
Postprandial		95.17	95.08	91.42	100.25	2.18	-	0.05

[1]Means represent a total of 48 pigs, 12 pigs per treatment (6 barrows and 6 gilts per treatment). Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to feed. There were no sex by treatment interactions present for any serum measures. BUN = blood urea nitrogen; ALP = alkaline phosphatase; AST = aspartate aminotransferase.
[2]Values represent the lower and upper limits of reference ranges of serum biochemical constituents in Table 7 of the Reference Guides in Merk (2005).
[3]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.

3.5 Conclusion

In summary, the majority of the current literature surrounding dietary B supplemented to pigs has not supplemented levels greater than 15 ppm B (Armstrong et al., 2000; Armstrong and Spears, 2001; Armstrong et al., 2002;). However, in the current experiment, dietary B was supplemented at greater levels (25, 50, and 100 ppm). Overall, these higher levels of supplemental B in the current study did not demonstrate any overt detrimental effects on health. It is also worth noting that pigs supplemented 100 ppm B had the lowest ADG during the 49-d study. Although the levels of dietary B supplementation in the current study were lower than the MTL estimate (150 ppm DM) provided by NRC (2005), the reduction in growth observed in the current experiment may well be due to the supplemental levels approaching the MTL. Furthermore, while diets were not analyzed for B concentration, the diets supplemented with B likely had a greater total B concentration than the expected supplemented level due to the B content of other ingredients within the basal diet. Despite that, the response observed for serum insulin and glucose in the present study further builds on previous literature that suggests that B may play a vital role in insulin secretion and glucose metabolism. However, further studies evaluating the impact of supplemental B on insulin secretion and glucose clearance are warranted to help further understand the potential benefits of B on insulin homeostasis and its effects on glucose metabolism.

CHAPTER 4. Evaluation of dietary boron supplementation to multiparous sows for multiple reproductive cycles on reproductive performance and late gestation insulin/glucose ratio.

4.1 Abstract

The objective of this experiment was to evaluate the effects of dietary boron (B) supplementation on sow and gilt reproductive performance and late gestation serum insulin and glucose concentration through 3 reproductive parities. A total of 72 multiparous sows and gilts [Yorkshire x Landrace x Large White] were selected at estrus detection, bred twice via artificial insemination, and allotted to 1 of 3 dietary treatments. Dietary treatments consisted of a gestation and lactation corn-soybean meal-based basal diet that was supplemented with either 0, 5, or 25 ppm B. Sows remained on their respective gestation and lactation treatment until they were either culled or completed their 3rd reproductive cycle. Venous blood samples were obtained in late gestation (d 80-90 of gestation) during a fasting and postprandial state to determine serum insulin and glucose concentrations. A quadratic tendency for fewer piglets born ($P = 0.08$) was observed with increasing dietary B supplementation A quadratic decrease in preweaning mortality ($P < 0.01$) was observed with increasing supplemental B. Furthermore, B supplementation resulted in a quadratic increase in piglet birth weight (total born and born alive; $P = 0.04$ and $P = 0.05$, respectively) which subsequently resulted in a 5 % heavier weaning weight of piglets from sows fed diets supplemented with 5 ppm B compared to control sows (6.01 vs. 5.69 kg, respectively). Furthermore, sows supplemented with 5 ppm B had a lower fasting serum insulin concentration in late gestation (quadratic effect, $P = 0.05$) that was coupled with no change in serum glucose concentration, ultimately resulting in a quadratic decrease in the fasting insulin/glucose ratio ($P = 0.02$). Conversely, in a postprandial state,

there was a linear tendency for serum glucose concentration to increase with increasing dietary B supplementation ($P = 0.07$). More pronounced, the postprandial insulin/glucose ratio increased linearly with increasing supplemental B (9.74, 9.57, and 12.15; $P = 0.02$). In summary, dietary B supplementation resulted in heavier piglet weights at birth however, this was also accompanied by fewer total pigs born. Late gestation fasting serum insulin was lower when B was supplemented in the diet at 5 ppm B, suggesting that B may impact energy metabolism by providing greater efficiency in metabolizing and maintain homeostatic glucose levels. Nonetheless, further work is warranted to help better understand the relationship that dietary B supplementation may have on reproductive traits including the number and size of piglets born as well as the mechanisms at which B supplementation impacts insulin and glucose concentrations in a variety of biological and physiological phases of a gilt or sow.

Keywords: Boron, supplementation, sows, gilts, insulin, glucose

4.2 Introduction

Boron (B) has commonly been accepted as an essential nutrient for vascular plant growth (Warrington, 1923; Lovatt and Dugger, 1994). However, in animals, the essentiality of B is unclear as the physiological role of B has not clearly been understood. Nonetheless, there has been an accumulation of information from a variety of animal models (chicks, fish, rodents, and frogs) suggesting that B may play a key role in animal nutrition (Hunt and Nielsen, 1981; Eckhert, 1998; Rowe and Eckhert, 1999; Lanoue et al., 1998; Fort et al., 1999a, b).

In pigs, Armstrong et al. (2002) reported long-term B supplementation (5 mg B/kg of diet) to gilts resulted in increased piglet weaning weight and tended to increase average piglet birth weight. More recent work reported that rats fed a diet supplemented with B (2 ppm B) had reduced plasma insulin without changing plasma glucose concentrations (Bakken and Hunt, 2003). The same lab also reported that peak insulin release from isolated, perfused pancreata of B-deprived chicks was almost 75 % higher than that of pancreata from B-supplemented chicks. These data suggest that B may reduce the amount of insulin needed to maintain plasma glucose, a benefit that could be useful given the demonstration that sows can be gestationally diabetic in late gestation, and that the glucose tolerance of pregnant sows is related to postnatal pig mortality (Kemp et al., 1996).

Thus, the objective of this experiment was to evaluate the effects of boron supplementation to multiparous sows for multiple reproductive cycles on reproductive performance, and late gestation serum insulin and glucose concentration.

4.3 Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Research Center. The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

4.3.1 *Animals, housing, management, and experimental design*

A total of 72 multiparous sows and gilts [Yorkshire x Landrace x Large White] were selected at estrus detection, they were all bred twice (24 hrs between services) via artificial insemination, weighed, and randomly allotted to the experimental diets while balancing parity and genetics across treatments. Experimental diets consisted of three dietary supplemental levels of boron (0, 5, 25 ppm B) from sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc. Boron, CA) to either a gestation or lactation basal diet that was formulated to meet or exceed the NRC (2012) nutrient requirement estimates for parity 1 gestating and lactating sows, respectively (Table 4.1). The gestation diets were formulated to contain 3,305 kcal/kg of ME, 15.43% of CP, and 0.73% of lysine; meanwhile, the lactation diets contained 3,298 kcal/kg of ME, 18.57% of CP, and 0.91% of lysine. There was no antibacterial agent included in the gestation and lactation diets.

All experimental animals received their respective treatment diet until they were removed from the experiment. Once allotted to treatments, gilts and sows were floor-fed 1.9 kg/d of their gestation diet until farrowing; during the first 3 d after farrowing, they were provided with 3.2 kg/d of the lactation diets, and then increased gradually until daily feed intake reached at least 6.4 kg, thereafter sows were allowed to consume diets on an

ad-libitum basis during lactation. All the experimental animals had free access to water throughout the entire experiment.

Sows and gilts were kept in individual gestation stalls (0.57 × 2.13 m^2) in an environmentally controlled building with partially slatted concrete floors. On d 109 to 112 of gestation, they were moved to environmentally controlled farrowing rooms and placed in farrowing crates (1.52 × 2.13 m^2) that were equipped with a plastic-coated woven wire floor area, heating lamps, and nipple waterer for piglets, as well as drinking nipple and feed trough for sows. Sows remained in the farrowing room until the end of the lactation period (20.3 ± 1.7 d).

All sows were weighed at breeding, pre-farrowing (d 109 to 112 of gestation), post-farrowing (within 24 h following parturition), and weaning, and piglet BW was also recorded at birth and weaning. The total number of piglets at birth (total born and born alive) and weaning were recorded to determine litter size and preweaning mortality. Feed offering and disappearance was recorded daily to determine average daily feed intake (ADFI) of the sow during lactation.

After farrowing (within 24 h of parturition), piglets were processed by normal farm-specific procedures including clipping needle-teeth, tail docking, iron administration (1.5 mL of iron dextran 100 mg/mL, Henry Schein Animal Health, Dublin, OH), and ear notching. No creep feed was offered to piglets during lactation, but access to the sow's feed was not restricted. In addition, each sow was intramuscularly injected with 1 mL of oxytocin (OXOJECT, Henry Schein Animal Health, Dublin, Ohio) and 4 mL of penicillin (PENJECT, Henry Schein Animal Health, Dublin, Ohio) in the trapezius muscle on the farrowing day. On the day of weaning, sows and piglets were

moved to gestation stalls and nursery rooms, respectively. Sows were exposed to boars starting on d 3 post-weaning until they were bred or had ample time to cycle. Sows were removed from the experiment if they failed to rebreed or farrow, developed unsoundness, showed evidence of low milk production, or became excessively thin (Appendix 2).

Table 4.1 Formulation and calculated composition of experimental basal diets (as-fed basis).

	Basal	
Items	Gestation	Lactation
Ingredient, %		
Corn	76.48	68.55
Soybean meal, 48% CP	19.00	27.00
Grease, choice white	1.00	1.00
Dicalcium phosphate	1.55	1.60
Limestone	1.00	0.90
L-Lysine·HCl	0.06	0.04
Chromax[1]	0.05	0.05
Salt	0.50	0.50
Vitamin mix[2]	0.10	0.10
Trace mineral premix[3]	0.10	0.10
Choline chloride - 60%[4]	0.10	0.10
Santoquin[5]	0.02	0.02
Copper sulfate pentahydrate	0.04	0.04
Total	100.00	100.00
Calculated composition		
Metabolizable energy, kcal/kg	3,305	3,298
Crude protein, %	15.43	18.57
SID Lysine, %[6]	0.73	0.91
Calcium, %	0.80	0.80
Total P, %	0.62	0.66
STTD Phosphorus, %[7]	0.42	0.45
Analyzed composition, %		
Ca	1.05	1.13
P	0.65	0.68
Mg	0.13	0.16

[1]Supplied 200 µg chromium/kg diet (Prince AgriProducts).
[2]Supplied the following per kg of total diet: 11,025 IU of vitamin A; 1,103 IU of vitamin D3; 77 IU of vitamin E; 2.2 IU of vitamin K; 0.03 mg of vitamin B12; 0.40 mg of biotin; 4.96 mg of folic acid; 30.32 mg of niacin; 27.56 mg of pantothenic acid; 4.96 mg vitamin B6; 1.65 mg thiamin; and 8.27 mg of riboflavin.
[3]Supplied the following per kg of total diet: 110 mg of zinc; 110 mg of iron; 18 mg of copper; 50 mg of manganese; 0.70 mg of iodine; and 0.30 mg of selenium.
[4]Supplied 600 mg choline chloride (520.8 mg choline)/ kg diet.
[5]Santoquin (Novus International Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diet.
[6]Calculated composition of lysine is presented on a standard ileal digestible (SID) basis.
[7]Calculated composition of phosphorus is presented on a standard total tract digestible (STTD) basis.

4.3.2 *Data and sample collection*

4.3.2.1 Feed sample collection

The experiment started in April 2020 and finished in January 2022, so multiple batches of experimental diets were mixed. Representative samples of corn, soybean meal, and mixed diets were collected at the feed mill for every batch of experimental diet mixing. Feed samples were stored at -20°C until analyzed.

4.3.2.2 Sow and litter performance

All sows and gilts were weighed at the time of breeding, pre-farrow (d 109-112), within 24 h after farrowing, and at weaning. The total number of piglets born, born alive, and stillborn were recorded within 24 h after farrowing; and the number of live piglets at weaning was also recorded. Cross-fostering was kept to a minimum and only performed within the same treatments. All cross-fostering was performed within the first 48 h following parturition and termed as adjusted born alive. Individual piglet weight was measured at the time of processing (< 24 h following birth) and weaning. Adjusted born alive weight (litter and piglet) is representative of the weight after cross-fostering was performed. Because of different weaning ages across litters, the weight of the piglet and litter at weaning were standardized to the end of 21-d lactation by the following equation modified from Jang et al. (2017):

$$Adjusted\ weight = weight\ at\ birth + \frac{(weight\ at\ weaning - weight\ at\ birth)}{weaning\ age} \times 21$$

4.3.2.3 Blood sample collection

Blood samples were obtained from all pregnant sows and gilts during a fasting and post-prandial state. Blood sampling occurred during late gestation (d 80-90 of gestation, mean day = 85.4 ± 0.12), where the fasting sample was obtained prior to the sow's morning meal (~ 20 h fast); and the post-prandial sample was obtained 40 to 55 minutes following the feeding of the sow. Blood was collected through vena cava puncture into 16 × 100 mm Vacutainer blood collection tubes with polymer gel (Becton, Dickinson and Company, Franklin Labs, NJ) and allowed to clot for an hour. Once the samples were clotted, blood tubes were centrifuged at 2500 × g for 20 minutes at -4° C. Following centrifugation, samples were aliquoted into 1.5 mL Eppendorf Safe-Lock tubes (Eppendorf North America, Hauppauge, NY) and stored at -80°C until analyzed.

4.3.3 *Sample processing and laboratory analysis*

Feed samples were analyzed for Ca, P, and Mg composition at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO) and presented in Table 4.1. Diet mineral analysis (Ca, P, and Mg) was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) under methods described by AOAC (method 985.01; AOAC, 2006). Diets were also analyzed for B composition. The B analysis of the diet samples confirmed the rank of the treatments but variability between duplicate samples precluded acceptance of the absolute values.

Serum samples obtained from all sows were analyzed for serum insulin and glucose concentrations. Serum glucose concentrations were determined enzymatically using an automated analyzer (YSI 2700 Select Analyzer, YSI Inc. Life Sciences). Serum

insulin levels were assayed using a commercially available radioimmunoassay kit (Porcine insulin RIA, Millipore Sigma). All samples for each assay were run in duplicate and on the same day. The insulin inter-assay coefficient of variation for the low- and high-quality controls was 6.25 %.

4.3.4 *Statistical analysis*

All data were subject to ANOVA using the GLM procedure in SAS (Statistical Analysis System, Cary, NC) for a completely randomized design. The individual sow and litter served as the experimental unit and results are reported as least squares means

The data of reproductive performance, and serum insulin and glucose were analyzed by a model that included 1 main effect as follows:

$$Y_{ij} = \mu + B_i + testparity_j + (B \times testparity)_{ij} + e_{ij}$$

Y_{ij} = response variables (reproductive performance; serum insulin and glucose)

μ = constant common to all observations.

B_i = dietary boron level

$Testparity_j$ = test parity of the sow

e_{ij} = error term of the model

Orthogonal polynomial contrasts were performed to further determine the linear and quadratic effects of increasing dietary B level. Linear and quadratic coefficients regarding dietary B supplementation were obtained using the PROC IML procedure in SAS. Results are reported as least squares means by using the LSmeans function in SAS.

The α level for determination of statistical significance was set at 0.05, with ≤ 0.10 used to declare a tendency for significance.

4.4 Results

4.4.1 *Sow and litter performance*

A total of 72 sows were enrolled in this study (n = 24/treatment). However, data from only 61 sows were used in the final analysis. There was a total of 11 litters (4 sows), 5 litters (4 sows), and 6 litters (3 sows) removed from the dataset, respectively, for all three dietary B supplementation levels. Thus, a total of 11 sows and 23 litter observations were removed from the dataset due to irregularities observed during the study. Irregularities and removed sows are listed in Appendix 2 as Table A.2.1.

Throughout the experiment, a total of 12, 11, and 12 sows respective to dietary treatment had 3 successful reproductive parities. Distribution of first- to fourth-parity litters were similar across treatment (Table 4.2).

The effects of sow test parity on sow and litter performance are presented in Appendix 3 as Table A.3.1 and A.3.2, respectively.

Table 4.3 shows the effects of dietary B on sow performance. Sow BW was linearly affected by increasing B supplementation at pre-and post-farrowing times (P = 0.05). At weaning, sows tended to have a greater BW with B supplementation (P = 0.08). Furthermore, there was a treatment by test parity interaction present for sow gestation and lactation BW change (P = 0.03, P = 0.04, respectively; Table 4.3). Independent of dietary

B treatment sows gained more BW during gestation for their 1st and 2nd test parity compared to their 3rd test parity.

However, it is worth noting that 1st test parity sows fed diets supplemented with dietary B exhibited the largest BW gain in contrast to sows fed the control diet having the largest gestation BW gain during the second test parity. In conjunction with the treatment by test parity interactions present for sow BW change during gestation and lactation, sow BW during pre-farrow and weaning was impacted by test parity ($P \leq 0.01$). Overall, sows receiving diets supplemented with 5 ppm B had a net positive BW change (0.3 kg) during lactation compared to sows receiving either the control diet or 25 ppm B which both lost weight (-3.4 and -2.7 kg, respectively) during the lactation period.

Sow weaning BW increased as test parity increased for all treatments. Irrespective of dietary B supplementation, first-parity sows lost weight during their first lactation. Interestingly, sows fed diets supplemented with B gained weight during lactation in their 2nd and 3rd test parities whereas sows fed the control diet lost weight during lactation in their 2nd test parity. There were no effects observed with B supplementation on lactation ADFI. However, as test parity increased for all treatments, daily feed intake during lactation also increased. Days to estrus was linearly increased ($P = 0.01$) in sows with increasing levels of dietary B supplementation. There was also an effect of test parity on days to estrus.

The effects of dietary B supplementation on sow reproductive and litter performance are provided in Tables 4.4 and 4.5. There were no treatment by test parity interactions observed for litter performance. The total number of piglets born tended to decrease quadratically ($P = 0.08$) with increasing dietary B supplementation as sows

receiving the control diet had ~ 1 more pig born compared to sows receiving the 5 and 25 ppm B supplemented diets. Furthermore, there was a quadratic decrease observed for adjusted born alive litter size with increasing dietary B supplementation ($P = 0.04$). Altogether, sows fed diets supplemented with 5 ppm B had the lowest number of pigs born alive (12.5) compared to sows fed diets without B supplementation (13.7) and 25 ppm B (13.2). There was a linear decrease in the number of stillborn piglets with dietary B supplementation ($P = 0.04$). The number of mummified fetuses farrowed was affected by sow test parity ($P = 0.03$), as test parity increased, the number of mummified fetuses born decreased. No effects regarding dietary B supplementation or test parity were observed for litter size at weaning.

Despite the lack of response observed on litter size at weaning, preweaning mortality was quadratically decreased for sows receiving diets supplemented with increasing dietary B ($P < 0.01$). Sows fed diets supplemented with 5 ppm B had an ~ 45 and 16 % lower preweaning mortality compared to sows fed either the control or 25 ppm B diets, respectively. In addition, a tendency for test parity to affect preweaning mortality was observed ($P = 0.09$).

There were no effects observed for litter weight with dietary B supplementation ($P > 0.10$). However, there was a tendency for test parity to affect weaning litter weight ($P = 0.10$). Irrespective of dietary treatment, first-test parity sows had heavier litter weaning weights compared to second and third-test parity sows. Furthermore, regarding adjusted litter weight gain (equalized to a 21-d lactation period), an effect of test parity was observed where adjusted litter weight gain was lower as test parity increased ($P < 0.05$). Although not significant, it is worth noting that litters from sows fed diets

supplemented with 5 and 25 ppm B had ~ 4 % greater litter weight gain during lactation compared to control sows that were fed diets without supplemented B.

There were no effects observed between piglet weight and test parity. However, sows fed diets supplemented with 5 ppm B had increased piglet total and born alive birth weights (quadratic effect, $P \leq 0.05$). Subsequently, sows fed diets supplemented with 5 ppm B weaned numerically heavier piglets (~ 5% heavier) at weaning compared to the sows fed diets without B supplementation. Similar to the findings observed for litter weight gain, there was ~ 5 and 6 % numerical increase in adjusted piglet weight gain during lactation for sows fed diets supplemented with 5 and 25 ppm B diet compared to sows fed the control diet.

Table 4.2 Distribution of number of litters for gilts and sows[1].

	Supplemental B, ppm		
Items	0	5	25
Farrowed litters on test, n[2]			
1 Litter	20	20	21
2 Litters	19	20	21
3 Litters	12	11	12
No. of total litters, n[3]			
1st-parity litter	17	18	17
2nd-parity litter	16	18	18
3rd-parity litter	15	13	15
4th-parity litter	3	2	4

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.
[2]Representative of the test parity.
[3]Representative of the true parity (i.e., test parity 1 sow enrolled on test during her second parity).

Table 4.3 Effects of dietary boron (B) supplementation on sow performance[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
No. Obs.	51	51	54			
Mean Parity	2.08	1.98	2.11	0.11	-	-
Gestation length, d	115.9	116.0	115.7	0.30	-	-
Lactation length, d	20.3	20.4	20.1	0.31	-	-
Sow BW, kg						
Breeding	174.0	172.2	176.3	3.12	-	-
Pre-farrow[T]	209.4	204.6	216.0	3.39	0.05	-
Post-farrow	196.1	195.5	203.7	3.21	0.05	-
Weaning[T]	192.7	195.8	201.0	3.36	0.08	-
BW Change, kg						
Gestation[T]	35.3	32.4	39.7	2.64	0.10	-
Lactation[T]	-3.4	0.3	-2.7	2.35	-	-
Lactation ADFI, kg/d[T]	5.3	5.2	5.4	0.12	-	-
Days to estrus[T]	4.76	4.98	5.26	0.12	0.01	-

[1]Pre-farrow BW was recorded on d 109-112 of gestation whereas post-farrow BW was recorded within 24 h following parturition.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[T]Test parity effect present, $P \leq 0.05$. P-values for test parity are reported in Appendix 3 as Table A.3.1.

Table 4.4 Effects of dietary boron (B) supplementation on litter size[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
No. Obs.	51	51	54			
Litter Size, No.						
Total born	15.1	14.0	14.2	0.4	-	0.08
Born alive	13.7	12.8	13.4	0.4	-	-
Adjusted born alive[3]	13.7	12.5	13.2	0.4	-	0.04
Stillborn	1.4	1.1	0.8	0.2	0.04	-
Mummified[T]	0.03	0.26	0.10	0.2	-	-
Weaning	11.7	11.5	11.6	0.3	-	-
Preweaning mortality	1.92	1.06	1.61	0.22	-	< 0.01

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[3]Adjusted born alive represents number of piglets alive after cross-fostering.

[T]Test parity effect present, $P \leq 0.05$. P-values for test parity are reported in Appendix 3 as Table A.3.2.

Table 4.5 Effects of dietary boron (B) supplementation on litter performance[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
No. Obs.	51	51	54			
Litter weight, kg						
Total born	19.82	19.56	18.92	0.60	-	-
Born alive	18.37	18.19	18.10	0.59	-	-
Adjusted born alive[3]	18.33	18.15	18.10	0.52	-	-
Weaning	64.82	66.69	66.11	1.96	-	-
Gain[T]	46.45	48.50	48.01	1.73	-	-
Adjusted litter weight, kg[4]						
Weaning	66.34	68.05	68.16	1.88	-	-
Gain[T]	48.02	49.90	50.06	1.60	-	-
Piglet weight, kg						
Total born	1.35	1.44	1.38	0.03	-	0.04
Born alive	1.37	1.46	1.39	0.03	-	0.05
Adjusted born alive[3]	1.37	1.46	1.39	0.03	-	0.04
Weaning	5.54	5.89	5.79	0.15	-	-
Gain	4.17	4.43	4.40	0.13	-	-
Adjusted piglet weight, kg[4]						
Weaning	5.69	6.01	5.96	0.14	-	-
Gain	4.32	4.55	4.58	0.12	-	-

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[3]Adjusted born alive represents number of piglets alive after cross-fostering.

[4]Adjusted for a 21 d lactation period.

[T]Test parity effect present, $P \leq 0.05$. P-values for test parity are reported in Appendix 3 as Table A.3.3.

Table 4.6 Effects of dietary boron (B) supplementation on sow performance through 3 test parities[1].

Supplemental B, ppm:	0			5			25			
Test parity:	1	2	3	1	2	3	1	2	3	SEM
No. Obs.	20	19	12	20	20	11	21	21	12	
Gestation length, d	115.61	115.79	116.38	115.75	115.93	116.20	115.84	115.62	115.66	0.71
Lactation length, d	20.65	19.89	20.42	20.60	20.00	20.55	20.19	20.10	20.08	0.51
Sow BW, kg										
Breeding	170.43	172.86	178.84	168.64	171.22	176.83	171.62	174.30	182.84	6.46
Pre-farrow	212.63	217.28	198.26	216.12	208.98	188.70	219.29	211.06	217.65	7.02
Post-farrow	197.76	201.93	188.51	197.26	195.92	193.36	204.21	197.08	209.75	6.64
Weaning	189.75	193.29	195.01	181.97	199.18	206.27	187.82	199.70	215.50	6.96
BW Change, kg										
Gestation[X]	42.20	44.42	19.43	47.48	37.76	11.87	47.66	36.76	34.81	5.47
Lactation[X]	-8.00	-8.64	6.50	-15.28	3.27	12.90	-16.39	2.61	5.74	4.86
Lactation ADFI, kg/d	4.90	5.44	5.63	4.36	5.63	5.71	4.81	5.61	5.65	0.24
Days to estrus	4.71	4.94	4.64	4.94	5.22	4.80	4.94	5.53	5.30	0.26

[1]Pre-farrow BW was recorded on d 109-112 of gestation whereas post-farrow BW was recorded within 24 h following parturition.
[X]Treatment by test parity interaction present, $P \leq 0.05$.

Table 4.7 Effects of dietary boron (B) supplementation on litter size through 3 test parities[1].

Supplemental B, ppm:	0			5			25			
Test parity:	1	2	3	1	2	3	1	2	3	SEM
Litter Size, No.										
Total born	14.80	14.68	15.75	13.85	13.84	14.18	13.85	14.14	14.58	0.85
Born alive	13.85	13.26	14.00	13.10	12.63	12.64	12.95	13.38	13.92	0.86
Adjusted born alive[2]	13.75	13.26	14.00	13.00	12.00	12.64	12.43	13.38	13.92	0.82
Stillborn	0.95	1.42	1.75	0.75	1.15	1.55	0.86	0.76	0.67	0.44
Mummies	0.10	0.00	0.00	0.45	0.15	0.18	0.52	0.05	0.00	0.20
Weaning	12.10	11.47	11.67	12.05	10.95	11.45	11.43	11.71	11.75	0.66
Preweaning mortality	1.65	1.79	2.33	0.95	1.05	1.18	1.00	1.67	2.17	0.45

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.

[2]Adjusted born alive represents number of piglets alive after cross-fostering.

Table 4.8 Effects of dietary boron (B) supplementation on litter performance through 3 test parities[1].

Supplemental B, ppm:	0			5			25			
Test parity:	1	2	3	1	2	3	1	2	3	SEM
Litter weight, kg										
Total born	19.26	20.57	19.63	19.28	19.65	19.74	18.15	19.30	19.30	1.24
Born alive	18.31	18.86	17.93	18.33	18.03	18.22	17.17	18.47	18.67	1.22
Adjusted born alive[2]	18.17	18.88	17.93	18.16	18.09	18.22	17.17	18.47	18.67	1.07
Weaning	69.72	64.54	60.19	70.56	64.29	65.23	67.15	65.27	65.92	4.07
Gain	51.41	45.68	42.25	52.23	46.26	47.01	49.99	46.80	47.25	3.58
Adjusted litter weight, kg[3]										
Weaning	70.54	66.86	61.64	71.58	66.51	66.08	69.02	67.47	67.99	3.90
Gain	52.37	47.98	43.71	53.42	48.42	47.86	51.86	48.99	49.32	3.32
Piglet weight, kg										
Total born	1.34	1.43	1.27	1.40	1.50	1.41	1.39	1.38	1.36	0.07
Born alive	1.37	1.44	1.30	1.41	1.51	1.46	1.41	1.40	1.38	0.07
Adjusted born alive[2]	1.36	1.44	1.30	1.41	1.51	1.46	1.39	1.40	1.38	0.07
Weaning	5.86	5.64	5.12	5.92	5.92	5.81	5.90	5.65	5.81	0.31
Gain	4.49	4.20	3.82	4.52	4.41	4.36	4.51	4.25	4.43	0.28
Adjusted piglet weight, kg[3]										
Weaning	5.94	5.88	5.23	6.01	6.15	5.86	6.06	5.86	5.97	0.29
Gain	4.58	4.44	3.94	4.60	4.63	4.41	4.68	4.46	4.59	0.25

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.

[2]Adjusted born alive represents number of piglets alive after cross-fostering.

[3]Adjusted for a 21 d lactation period.

4.4.2 *Serum insulin and glucose concentrations*

Late gestation fasting and postprandial serum insulin and glucose concentrations from sows fed increasing supplemental levels of dietary B are presented in Table 4.9. Sows were blood sampled during a late gestation period defined as day 80 to 90 of gestation. During the late gestation phase, sows underwent a fasting period of ~ 20 hours where they were then bled to obtain a fasting serum sample. Following the fasting blood sample, sows received their morning meal and ~40 to 55 minutes following the meal underwent a second bleeding which allowed for a postprandial serum sample. There were no treatment by test parity interactions observed for late gestation serum insulin and glucose concentration at fasting or postprandial.

Regarding increasing dietary B supplementation, there was a quadratic decrease in fasting serum insulin levels ($P = 0.05$) where sows fed diets supplemented with 5 ppm B had the lowest serum fasting insulin concentration. Conversely, there were no effects observed regarding B supplementation on fasting glucose concentration. However, the decreased fasting serum insulin concentration coupled with no change in glucose resulted in a quadratic decrease in fasting serum insulin/glucose ratio ($P = 0.02$) with sows supplemented with 5 ppm B exhibiting the lowest insulin/glucose ratio. Both fasting and postprandial serum glucose concentrations were affected by the test parity of the sow ($P < 0.0001$).

In addition, increasing dietary B supplementation resulted in a linear tendency for decreased postprandial serum glucose concentration ($P = 0.07$). A more pronounced linear increase ($P = 0.02$) for postprandial serum insulin/glucose ratio was observed with increasing dietary B supplementation. Moreover, sows that were fed diets supplemented

with 25 ppm B had an ~ 25 % increase in postprandial serum insulin/glucose ratio compared to sows fed diets without B supplementation.

Table 4.9 Effects of dietary boron (B) supplementation on gilt and sow late gestation fasting and postprandial serum insulin and glucose concentrations[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Insulin, μU/mL						
Fasting	6.23	5.01	6.30	0.49	-	0.05
Postprandial	36.84	37.37	42.98	3.38	-	-
Glucose, mmol/L						
Fasting[T]	3.62	3.68	3.66	0.06	-	-
Postprandial[T]	3.79	3.90	3.63	0.09	0.07	-
Insulin/glucose						
Fasting[T]	1.72	1.36	1.72	0.12	-	0.02
Postprandial	9.74	9.57	12.15	0.90	0.02	-

[1]All animals underwent a fasting period of about 20 h, following the fasting sample, animals were fed their morning meal. Around 40 to 55 minutes after access to the morning meal, sows were sampled again for a postprandial sample.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[T]Test parity effect present, $P \leq 0.05$. P-values for test parity are reported in Appendix 3 as Table A.3.4.

Table 4.10 Effects of dietary boron (B) supplementation on late gestation serum insulin and glucose concentration of sows through 3 test parities[1].

Supplemental B, ppm:	0			5			25			
Test parity:	1	2	3	1	2	3	1	2	3	SEM
Insulin, μU/mL										
Fasting	6.77	6.02	5.91	4.26	7.16	3.60	6.28	6.90	5.73	1.06
Postprandial	41.40	36.46	32.65	37.79	34.55	39.76	39.81	40.96	48.16	7.30
Glucose, mmol/L										
Fasting	3.78	3.46	3.60	3.83	3.63	3.56	3.92	3.49	3.56	0.13
Postprandial	3.89	3.57	3.90	4.19	3.59	3.92	4.08	3.50	3.32	0.19
Insulin/glucose										
Fasting	1.79	1.74	1.63	1.12	1.95	1.01	1.56	2.00	1.60	0.26
Postprandial	10.69	10.14	8.38	9.11	9.51	10.08	9.61	12.01	14.84	1.95

[1]All animals underwent a fasting period of about 20 h, following the last sample, animals were fed their morning meal. Around 40 to 55 minutes after access to the morning meal, sows were sampled again for a postprandial sample. There were no treatment by test parity interactions present for serum measures.

4.5 Discussion

4.5.1 *Sow and litter performance*

The study of dietary B supplementation in pigs has been limited. However, there has been several studies that report specific responses regarding dietary B supplementation. For example, work by Liao et al. (2010) demonstrated that mRNA from a borate transporter (NaBCl) is expressed in the intestinal epithelial (jejunal and ileal) and kidney tissues of growing pigs. In the same report, the authors further reported that dietary B supplementation increases the jejunal NaBCl mRNA expression while decreasing the mRNA expression in the kidney (Liao et al., 2010). This work illustrates that B has a unique transporter (NaBCl) within the body, and that B may have biological importance that permits B to be transported more readily in one tissue versus another. This is of particular interest because B has been considered an essential mineral for proper growth in plants but in higher-order animals, the essentiality of B has yet been defined.

Work in the late 1990s reported that B supplementation to a low-B culture medium stimulated embryonic trout growth (Eckhert, 1998), and increased survivability of zebrafish embryos (Rowe and Eckhert, 1999). Furthermore, in B-deficient mice, embryonic development was impaired and delayed (Lanoue et al., 1998). Low B exposure in Xenopus laevis embryos increased necrosis, mortality, and malformation frequencies (Fort et al., 1999). However, in swine, Armstrong et al., (2002) reported that there were no differences in the total number of piglets born and born alive from sows fed a diet supplemented with B (5ppm) compared to sows fed a diet without B

supplementation. In the current study, sows fed a diet supplemented with B (5 and 25 ppm) tended to have fewer piglets born and born alive compared to the control sows fed a diet without B supplementation. Furthermore, in the present study, there was a quadratic decrease observed in the number of stillborn piglets at birth with increasing dietary B supplementation represented by sows receiving 5 ppm B having the lowest number of stillbirths. This further disagrees with the work by Armstrong et al. (2002), who reported sows supplemented with 5 ppm B having an increase in the number of stillborn piglets at parturition. The conflicting results observed for number of stillborn piglets between the study herein and Armstrong et al. (2002) may be a simple function of boron supplemented sows having fewer total piglets born in the current study and thus, a reduced occurrence of a stillbirth is more likely with fewer total births. Moreover, the differences observed in litter size between the work reported by Armstrong et al. (2002) and the study herein are likely related to the improvements in genetic potential of the modern sows. More specifically, Armstrong et al (2002) reported an average of 10.0 and 9.6 total pigs born with respect to dietary treatment (0 vs. 5 ppm B) compared to 15.1, 14.0, and 14.2 total pigs born with respect to dietary treatment (0, 5, and 25 ppm B) for the current study. Thus, the 4 to 5 pig increase in total born observed in the current study is likely to inflate the number of stillborn occurrences. Furthermore, it is likely that the overall increase in total number of pigs born resulted in a wider range, and as a result increased the variation. Yet, the larger sample size in the study herein compared to Armstrong et al. (2002) provides enough statistical power to generate a quadratic tendency.

A key production metric that is often measured in sow production to determine sow productivity is the number of days it takes a sow to return to estrus following weaning (days to estrus). An increase in the time it takes a sow to return to estrus following weaning increases the number of non-productive days. Non-productive days are days that a sow is not supporting a litter, whether that is during gestation or lactation, and often can be expressed as the period following weaning and breeding. In the current study, increasing dietary B resulted in a linear increase in days to estrus. Despite the statistical increase reported, it is worth noting that the increase observed was merely half of a day (0.5 d).

Armstrong and colleagues (2002) reported that piglet birth weight was around 9% greater for sows fed diets supplemented with 5 ppm B compared to sows fed diets without B supplemented. These findings are consistent with the current study, where a quadratic increase in piglet birth weight (total born and born alive) was observed. More specifically in the current study, sows fed diets supplemented with 5 ppm B had about a 7 % increase in both total born and born alive piglet birth weight compared to sows fed diets without B supplementation. However, the increase observed in piglet birth weight could also be a product of the sow having fewer pigs (total born and born alive), and thus there is more space for piglets to grow in-utero, subsequently producing a heavier pig at birth. Despite the increases observed in the number of piglets born over the last several decades, the uterus of the dam is limited in uterine capacity. The uterine capacity represents a combination of the ability of the uterus to provide nutrients, the ability of the placenta to transfer nutrients to the fetuses, and the ability of the fetuses to efficiently use those nutrients for growth and development (Vallet and Freking, 2005).

Again, Armstrong et al. (2002) reported that piglet weaning weight was numerically greater in piglets from the sows fed diets supplemented with 5 ppm B. Although not significant, in the current study, piglet weight at weaning was 6 and 5% greater in B supplemented sows respectively, compared to control sows. Moreover, in the present study, piglet weight gain from farrowing to weaning was numerically increased by 5 and 6%, respective of B supplementation compared to the control. The findings herein are consistent to those of Armstrong et al. (2002) suggesting that B supplementation may result in marginal advantages observed for piglet weaning weight and weight gain through lactation. Conversely, the advantage observed for piglet birth weight of B-supplemented sows observed in both studies may contribute to some of the differences in weaning weight and weight gain observed in both studies. There have been several previous reports suggesting that low-birthweight piglets ultimately end up as the lighter pigs at subsequent production phases (Quiniou et al., 2002; Gondret et al., 2005; Smith et al., 2007).

In the current swine industry, preweaning mortality (PWM) is considered a hot topic as PWM has increased alongside increases in litter size. It is common for producers to suffer a PWM of 10 to 20% (PigChamp, 2022). In the current study, piglet mortality was expressed on an absolute number basis rather than a percentage basis. Nonetheless, sows fed diets supplemented B had an ~ 45 and 19% reduction in piglet deaths compared to sows fed the control diet. There have been several attempts to determine causes of PWM, with the most common cause being low birthweight (Fix et al., 2010). A more recent study evaluating birth weight and piglet survival data from 3 European and 1 U.S. commercial herds, identified a birthweight threshold associated with increased risk for

PWM of less than or equal to 1.11 kg (Feldpausch et al., 2019). Although the number of low birthweight piglets were not categorized by dietary treatment in the current study, sows supplemented with 5 ppm B had the lowest PWM and greatest piglet birth weight.

4.5.2 *Serum insulin and glucose concentrations*

Although direct mechanisms are unclear, it is hypothesized that B may reduce the stress that pancreatic β-cells experience during the overproduction of insulin (Hunt, 1996). In one report, dietary B supplementation decreased peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or Mg status (Bakken and Hunt, 2003). In these studies, rats that were fed a low-boron diet (0.2 ppm B) supplemented with B (2 ppm B) resulted in a reduction in plasma insulin but did not change plasma glucose concentrations. Furthermore, Bakken and Hunt (2003) reported that peak insulin release from isolated, perfused pancreata of boron-deprived chicks was almost 75% higher than that from pancreata of boron-supplemented chicks. More recently, B supplementation to rabbits resulted in downregulation of the peroxisome proliferator-activator receptor (PPAR) in the liver, which is actively involved in energy metabolism (Baspinar et al., 2015). Interestingly the previous authors also reported that the downregulation of PPAR was more pronounced with boric acid compared to sodium borate. Nonetheless, Aydin et al. (2019) reported that incubating hypoxic β-cells of the pancreas with either boric acid or sodium borate (19.5 ppm B) led to an increased cell viability.

These findings suggest that B may support various mechanisms within energy metabolism and ultimately reduce the amount of insulin needed to maintain plasma glucose. All of which could potentially benefit reproductive sows as past research has

indicated that sows become less tolerant to glucose and have diabetic tendencies during pregnancy (George et al., 1978; Bouillon-Hausman et al., 1986; Scheafer et al, 1991) which can be exacerbated during the later stages of pregnancy (Kemp et al., 1996). Furthermore, George et al (1978), and Anderson et al (1971), reported that sows with a decreased glucose tolerance during pregnancy, also happened to produce heavier piglets at birth suggesting that there is a heightened risk of gestational induced diabetes for more productive sows. These findings were later confirmed by Kemp et al. (1996) in which the authors reported that the extent of gestational induced glucose intolerance in sows appears to affect both piglet birth weight and survival rate during lactation.

The study herein evaluated serum insulin and glucose concentrations of late gestating gilts and sows during a fasting and post-prandial state. In the present study, there was a quadratic decrease in fasting serum insulin concentration regarding increasing dietary B supplementation. Fasting serum insulin concentration was ~ 20% lower for sows and gilts fed diets supplemented with 5 ppm B compared to sows fed either the control or 25 ppm B diet.

Similarly, fasting serum insulin levels were also decreased in rats that were fed diets supplemented with 100 ppm B (1.18 vs. 0.20 ng/mL respectively), however, the lowered fasting insulin concentration was accompanied by an ~ 49% decrease in fasting serum glucose levels compared to rats fed diets without B supplementation (Kucukkurt et al., 2015). Different from the previous study, dietary B supplementation in the current study did not affect fasting serum glucose concentration. Although, the unaffected glucose concentrations coupled with the decrease in serum insulin concentration resulted in a quadratic decrease for fasting insulin/glucose ratio with respect to increasing dietary

B supplementation. These data suggests that in a fasting state, sows fed diets supplemented with 5 ppm B may be more sensitive to insulin, as a lower fasting insulin concentration with no effect on glucose concentration further suggests that these sows may have an improved efficiency in maintaining a maintenance glucose concentration.

In the postprandial state, there were only numerical differences observed for serum insulin concentration coupled with a linear tendency for postprandial serum glucose to decrease with dietary B supplementation. This resulted in serum insulin/glucose ratio to increase linearly with dietary B supplementation as sows supplemented with the 25 ppm B having ~ 25 and 27% higher ratio compared to sows fed the control diet and 5 ppm diet, respectively.

During pregnancy, fetal development relies solely on nutrient transfer via the placenta. In particular, the growth rate of the fetuses increases exponentially during the last third of pregnancy (~ d 70 – 115 of gestation), resulting in a taxing nutrient demand from the fetuses onto the sow (Boyd et al., 2000; McPherson et al., 2004; Kim et al., 2009; Kim et al., 2013). Sows address this large nutrient demand in the latter parts of pregnancy by increasing uterine blood flow which can be partly adapted by the number of fetuses the sow is supporting (Pére et al., 2000). In the same work, the authors reported that sows are less sensitive to insulin during the last quarter of gestation (~ d 85 to 115 of gestation), which further increases until partition by demonstrating an increase in plasma glucose half-life as well as a slower decrease in plasma free fatty acids (FFA) after a meal. Interestingly, these findings regarding insulin sensitivity are independent of the feeding level of the sow supporting (Pére et al., 2000). The importance of glucose metabolism in the sow is not only crucial for the health and longevity of the sow, but it

has also been suggested that glucose metabolism can have direct effects on the progeny. Reports from the 1970s showed that there is a positive correlation ($R^2 = 0.58$ and 0.70, respectively) between mean birth weight of the piglets and time for glucose to return to fasting levels (Anderson et al., 1971; George et al., 1978).

Furthermore, Pére et al. (2007) suggests that both postprandial insulin and glucose concentrations differ in different physiological states in sows. This work revealed that gilts become more resistant to insulin at the end of pregnancy compared to sows which was characterized by the greater plasma glucose and insulin concentrations after a meal, as well as an increased glucose half-life, a delayed return of insulin to basal concentrations after an i.v. glucose load. Interestingly, in the current study, the test parity 1 females (primarily gilts) that were fed B diets had ~ 37 and 7% lower fasting serum insulin concentration compared to control test parity 1 females. This was further observed in the postprandial state where B test parity 1 sows had ~ 9 and 4% lower serum insulin concentration relative to the control test parity 1 sows. This may suggest that the positive effects of B on insulin and glucose metabolism may be more specific to the biological age and production phase of the animal.

4.6 Conclusion

In conclusion, sows that were fed gestation and lactation diets supplemented with 5 and 25 ppm B had fewer stillborn piglets and fewer piglet deaths during lactation. However, these findings may be conflicted with the numerical differences observed in litter size as there was a quadratic tendency for B supplemented sows to have fewer piglets born. When B was supplemented to sows at 5 ppm B, sows farrowed heavier

piglets. Although not significant, sows from both B treatments numerically had an increased litter and piglet weaning weight and weight gain during lactation. These numerical increases observed in litter and piglet weight gain coupled with lower preweaning mortality could be related to glucose metabolism and may prove beneficial for swine producers.

CHAPTER 5. Effects of maternal boron supplementation to multiparous sows on sow and litter tissue and bone composition at birth and weaning

5.1 Abstract

This experiment used a total of 15 multiparous sows [Yorkshire x Landrace x Large White; Mean parity: 3.8 ± 0.3] that were selected from a larger group of sows on experiment based on farrowing dates. The initial experiment assigned sows to 1 of 3 dietary treatments following estrus detection and artificial insemination. Experimental diets consisted of three dietary supplemental levels of boron (0, 5, 25 ppm B) from sodium borate decahydrate. Sows were fed dietary treatments for all of gestation and lactation. At birth and weaning, 2 piglets/litter (5 litters/treatment) were randomly selected and euthanized for tissue and bone mineral determination. In addition, sows were humanely slaughtered following weaning and select tissues and metacarpals were harvested for mineral determination. Sows fed diets supplemented with 5 ppm B had a greater kidney phosphorus concentration (quadratic effect, $P = 0.04$) and a tendency for greater kidney calcium concentration (quadratic effect, $P = 0.07$). Metacarpals from sows fed diets supplemented with boron had a linear decrease ($P = 0.04$) in bone breaking strength. Litter femur lipid content at birth was quadratically effected ($P = 0.004$) by boron supplementation with the 5 ppm B litters having the highest lipid content. At weaning, magnesium content of the femur was increased quadratically ($P = 0.02$) for litters in the 5 ppm B treatment. In conclusion, maternal B supplementation resulted in no adverse effects on the sow or litter. The inconsistent results regarding maternal B supplementation on litter tissue and bone content at birth and weaning may be a function of B transfer to piglets in-utero versus milk supply.

Keywords: boron, tissues, bone, sow, piglet

5.2 Introduction

Currently, the NRC (2012) does not have a recommendation for dietary boron (B) supplementation in swine. However, current literature has suggested that B influences a multitude of different biochemical functions that may have physiological importance to biological systems (Hunt, 1998). More so, B is considered a trace element that plays a bioactive role in mineral metabolism and utilization (Nielsen, 2017). Supplementing B to magnesium-deficient chicks led to an amelioration in leg abnormalities that were observed with inadequate magnesium intake (Hunt and Nielsen, 1986). The same lab also studied the interaction between B and vitamin D in chicks. Boron supplementation led to an improvement in growth, increased plasma ionized Ca, and decreased plasma alkaline phosphatase levels accompanied by a decrease in the incidence of rickets associated with vitamin D deficiency (Hunt and Nielsen, 1981). Hunt and Neilsen (1986) suggested that B has an indirect effect on vitamin D metabolism through an alteration of Ca, P, and Mg metabolism. More recent work using growing barrows demonstrated that dietary B supplemented at 5 and 15 ppm as sodium borate resulted in an increase in the ultimate sheer force of the fibula from barrows fed diets supplemented with 15 ppm B (Armstrong and Spears, 2001). Subsequently, the same group also reported that long-term supplementation of B improved bone characteristics in gilts (Armstrong and Spears, 2002). Thus, the objective of this study was to evaluate the effects of maternal dietary B supplementation on the dam and litter tissue and bone composition at birth and weaning.

5.3 Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Unit and Animal Laboratory. The animal slaughter was

performed at the University of Kentucky Meat Laboratory in the Department of Animal and Food Sciences. The experiments were conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

5.3.1 *Animals, housing, management, and experimental design*

This experiment used a total of 15 multiparous sows [Yorkshire x Landrace x Large White; Mean parity: 3.8 ± 0.3] that were selected from a larger group of sows that were on the experiment in Chapter 4. Sows were selected at estrus detection and bred twice (24 h between services) via artificial insemination, weighed, and randomly allotted to experimental diets while balancing parity and genetics across treatments. Experimental diets consisted of three dietary supplemental levels of boron (0, 5, 25 ppm B) from sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc. Boron, CA). The gestation and lactation basal diets (Table 5.1) were formulated to meet or exceed the NRC (2012) nutrient requirement estimates for parity 1 gestating and lactating sows. During gestation, sows were floor-fed 1.9 kg/d of their respective gestation diets until farrowing. Following farrowing they were provided 3.2 kg/d of their respective lactation diets for the first 3 days of lactation, and then increased gradually until daily feed intake reached at least 6.4 kg/d, thereafter sows were allowed to consume diets on an ad-libitum basis throughout lactation. Sows received their respective dietary treatment until they were slaughtered.

Following breeding and treatment allotment sows were kept in individual gestation stalls (0.57 × 2.13 m^2) in an environmentally controlled building with partially slatted concrete floors. On d 109 to 112 of gestation, sows were moved to environmentally controlled farrowing rooms and placed in farrowing crates (1.52 × 2.13

m^2) that were equipped with a plastic-coated woven wire floor area, heating lamps, nipple drinker, and a feed trough.

All sows were weighed at breeding, pre-farrowing (d 109 to 112of gestation), post-farrowing (within 24 h following parturition), and weaning, and piglet BW was also recorded at birth and weaning. The total number of piglets at birth (total born and born alive) and weaning were recorded to determine litter size and preweaning mortality. Cross-fostering was kept to a minimum and only performed within the same treatments. All cross-fostering was performed within the first 48 h following parturition and termed as adjusted born alive. Feed offering and disappearance was recorded daily to determine average daily feed intake (ADFI) of the sow during lactation. After farrowing (within 24 h following processing), piglets were processed by normal farm-specific procedures including clipping needle-teeth, tail docking, iron administration (1.5mL of iron dextran 100 mg/mL, Henry Schein Animal Health, Dublin, OH), and ear notching. Individual piglet weight was measured at the time of processing (< 24 h following birth) and weaning. Adjusted bon alive weight (litter and piglet) is representative of the weight after cross-fostering was performed. Because of different weaning ages across litters, the weight of the piglet and litter at weaning were standardized to the end of 21-d lactation by the following equation modified from Jang et al. (2017):

$$Adjusted\ weight = weight\ at\ birth + \frac{(weight\ at\ weaning - weight\ at\ birth)}{weaning\ age} \times 21$$

No creep feed was offered to piglets during lactation, but access to the sow's feed was not restricted. In addition, each sow was intramuscularly injected with 1 mL of

oxytocin (OXOJECT, Henry Schein Animal Health, Dublin, Ohio) and 4 mL of penicillin (PENJECT, Henry Schein Animal Health, Dublin, Ohio) in the trapezius muscle on the farrowing day.

At birth (within 24 h of parturition) and at weaning 2 pigs/litter (5 litters/treatment) were randomly selected and euthanized for tissue and bone mineral determination at each time point. Piglets were euthanized via intravenous injection of sodium pentobarbital (Fatal-Plus solution, Vortech Pharmaceuticals LTD., Dearborn, Michigan). Tissue (liver, kidney, and heart) and femur samples were collected, weighed, and stored at -20° C until further laboratory analysis was performed. At weaning, sows were moved to the abattoir and killed for tissue (liver, kidney, heart, and ovaries) and metacarpal collection. Sows were electrically stunned, suspended by a rear leg, and exsanguinated. Following exsanguination tissues and metacarpals (3^{rd} and 4^{th}) of sows were harvested, weighed, and stored at -20° C until further laboratory analysis was performed.

Table 5.1 Formulation and calculated composition of basal diet (as-fed basis).

	Basal diet	
Items	Gestation	Lactation
Ingredient, %		
Corn	76.48	68.55
Soybean meal, 48% CP	19.00	27.00
Grease, choice white	1.00	1.00
Dicalcium phosphate	1.55	1.60
Limestone	1.00	0.90
L-Lysine·HCl	0.06	0.04
Chromax[1]	0.05	0.05
Salt	0.50	0.50
Vitamin mix[2]	0.10	0.10
Trace mineral premix[3]	0.10	0.10
Choline chloride - 60%[4]	0.10	0.10
Santoquin[5]	0.02	0.02
Copper sulfate pentahydrate	0.04	0.04
Total	100.00	100.00
Calculated Composition		
Metabolizable energy, kcal/kg	3,305	3,298
Crude protein, %	15.43	18.57
SID Lysine, %[6]	0.73	0.91
Calcium, %	0.80	0.80
STTD Phosphorus, %[7]	0.42	0.45
Analyzed Composition, %		
Ca	1.05	1.13
P	0.65	0.68
Mg	0.13	0.16

[1]Supplied 200 µg chromium/kg diet (Prince AgriProducts).
[2]Supplied the following per kg of total diet: 11,025 IU of vitamin A; 1,103 IU of vitamin D3; 77 IU of vitamin E; 2.2 IU of vitamin K; 0.03 mg of vitamin B12; 0.40 mg of biotin; 4.96 mg of folic acid; 30.32 mg of niacin; 27.56 mg of pantothenic acid; 4.96 mg vitamin B6 and 1.65 mg thiamin; and 8.27 mg of riboflavin.
[3]Supplied the following per kg of total diet: 110 mg of zinc; 110 mg of iron; 18 mg of copper; 50 mg of manganese; 0.70 mg of iodine; and 0.30 mg of selenium.
[4]Supplied 600 mg choline chloride (520.8 mg choline)/ kg diet.
[5]Santoquin (Novus International Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diet.
[6]Calculated composition of lysine is presented on a standard ileal digestible (SID) basis.
[7]Calculated composition of phosphorus is presented on a standard total tract digestible (STTD) basis.

5.3.2 *Sample collection and processing*

Representative samples of corn, soybean meal, and mixed diets were collected at the feed mill for every batch of experimental diet mixing. Feed samples were stored at -20°C until analysis.

Due to the limited size and quantity of tissue from piglets at birth, tissue samples were pooled together based on the litter of origin. Tissue samples were placed through a kitchen-grade meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ) to provide a homogenous tissue sample. After samples were ground and mixed, 1-2 g of tissue was digested with nitric acid in a pressurized microwave digester (MARS 6 CEM, Matthews, NC) according to the recommendations by the manufacturer, and appropriately diluted for mineral analysis. Femurs were cleaned of excess muscle and fat and placed at -20 °C until further analysis.

5.3.3 *Laboratory analysis*

Feed samples were analyzed for Ca, P, and Mg composition at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO) and presented in Table 5.1. Diet mineral analysis (Ca, P, and Mg) was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) under methods described by AOAC (method 985.01; AOAC, 2006). Diets were also analyzed for B composition. The B analysis of the diet samples confirmed the rank of the treatments but variability between duplicate samples precluded acceptance of the absolute values.

To assess bone-breaking strength, femurs (piglets) and metacarpals (sows) were thawed to room temperature and subjected to breaking strength determinations using an

Instron Materials tester (Model TM 1123; Instron Corp., Canton, MA, USA). Breaking strength is defined as the peak amount of force, before fracture, applied by a wedge mounted on a pressure-sensitive compression cell at a loading rate of 40 mm/min at the center of the fresh bone when placed horizontally on two supports 3.2 cm apart. The femurs and metacarpals were cut in half to remove the marrow. After drying in an oven at 107 °C overnight, they were wrapped in cheesecloth and extracted with fresh petroleum ether three times at 24-h intervals or until the solvent was clear. The percentage ash content of fat-free bone was determined by weighing the bone into a dried and preweighed porcelain crucible. Samples were then placed into a muffle furnace and heated to 600°C overnight (method 942.05; AOAC, 2003). Ash weight was recorded and the ash percent in dry, fat-free bone was determined. The ash from the femur and metacarpal samples were then acid-digested and diluted to 250 mL for mineral analysis.

Tissue dry matter (DM) was determined for all tissue samples by placing around 2-3 g of ground sample into a gravity convection drying oven (Precision Scientific Co., Chicago, IL) at 107°C for approximately 24 hours and weighing the sample again to observe the moisture content lost (AOAC International, 2006). The percentage ash content of tissues was determined by weighing 2 to 3 g of sample into a dried and preweighed porcelain crucible. Samples were placed into a muffle furnace and heated to 600°C overnight (method 942.05; AOAC, 2003). All tissue, femur, and metacarpal samples were analyzed for Ca and Mg by flame atomic absorption spectrophotometry (AAnalyst 200, PerkinElmer, Waltham, MA, USA). Before Ca analysis, samples were prepared in a 0.1 % NaCl solution (to help capture free Ca ions) and ran with a Ca reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for the development of

the standard curves. Calcium determination was performed using a nitrous oxide-acetylene gas mixture and a wavelength of 422.7 nm (modification of method 927.02; AOAC, 2003). Magnesium determination was performed using acetylene gas, at a wavelength of 285.21 nm (modification of method 927.02; AOAC, 2003) and ran with a Mg reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for the development of the standard curves. Liver samples from sows were also analyzed for Fe, Zn, and Cu by flame atomic absorption spectrophotometry (same as previous) using acetylene gas and measured at wavelengths of 248.3, 213.9, and 324.8 nm respectively (modification of method 927.02; AOAC, 2003). Phosphorus was analyzed using a gravimetric method (modification of method 968.08; AOAC, 1990), where 20 mL of the digested sample mixed with Quimociac solution, was filtered, and the precipitate obtained was weighed to calculate the P concentration (Appendix 6). Calcium, P, and Mg analysis of experimental basal diets are presented in Table 5.1

5.3.4 *Statistical analysis*

All data were subjected to an outlier analysis prior to the final statistical analyses. The process involved identifying potential outliers that were 3 standard deviations from the mean of the response measure, as well as reviewing study notes before a final decision was made whether to exclude that value. Data were subjected to ANOVA using the GLM procedure of SAS (SAS 9.4, Cary, NC). All sow data (performance, tissue, and bones) were analyzed using the litter or the sow as the experimental unit with the treatment as the only term in the model. The final model for sow performance, tissue, and bone composition data were analyzed by the model:

$Y_i = \mu + \text{Boron}_i + + e_i$, where

Y_i = response variable (absolute and relative organ size, tissue, and metacarpal composition)

μ = a constant common to all observations

$boron_i$ = supplemental boron level

e_i = error term of the model

Piglet tissues and bones at birth and weaning were pooled by litter to facilitate proper laboratory analysis. Thus, piglet tissue and bone data at both birth and weaning were also analyzed using the litter as the experimental unit and litter BW as a covariate. The final model for piglet tissue and bone data at birth and weaning were analyzed by the model:

$Y_{ij} = \mu + \text{Boron}_i + \text{LitterBW}_j + + e_{ij}$, where

Y_{ij} = response variable (absolute and relative organ size, tissue, and femur composition of piglets)

μ = a constant common to all observations

$boron_i$ = supplemental boron level

$LitterBW_j$ = average BW of the piglets euthanized within a litter

e_{ij} = error term of the model

Orthogonal polynomial contrasts were performed to evaluate the linear and quadratic effects of increasing supplemental B levels. Linear and quadratic coefficients for unequal spacing of treatments were obtained using the PROC IML function in SAS.

Results are reported as least squares means by using the LSmeans function in SAS. The α level for determination of statistical significance was set at 0.05, with ≤ 0.10 used to declare a tendency for significance.

5.4 Results

5.4.1 *Sow and litter performance*

All sows and piglets were in good health and condition during the experimental period. Tables 5.2 and 5.3 present the performance data of the 15 sows that were used for tissue and bone harvest. Two piglets from each litter were euthanized at both birth and weaning, affecting the litter performance data. Thus Tables 5.2 and 5.3 only represent the least squares means for dietary treatment with no *P*-value reported. Respective P-values for sow and litter performance can be found in Appendix 4; Tables A.4.1 to A.4.3.

Table 5.2 Effects of dietary boron (B) supplementation on sow bodyweight (BW) and lactation feed intake[1].

	Supplemental B, ppm			
Items	0	5	25	SEM
No. of sows	5	5	5	
Mean parity	3.8	3.6	4.0	
Gestation length, d	115.8	114.6	116.2	0.42
Lactation length, d	22.8	23.8	22.2	0.49
Sow BW, kg				
Breeding	197.5	206.3	216.3	8.38
Pre-farrow	237.2	251.9	249.3	7.34
Post-farrow	229.8	236.8	239.8	8.33
Weaning	231.4	226.4	240.0	10.97
Sow BW change, kg				
Gestation	39.6	45.6	33.0	9.26
Lactation	1.6	-10.4	0.2	8.85
Lactation daily feed intake, kg	4.7	4.4	5.0	0.38

[1]Statistical analysis is reported in Appendix 4. Pre-farrow BW was recorded on d 109-112 of gestation whereas post-farrow BW was recorded within 24 h following parturition.

Table 5.3 Effects of dietary boron (B) supplementation on litter performance of sows[1].

	Supplemental B, ppm			
Items	0	5	25	SEM
No. of litters	5	5	5	
Litter size, n				
Total born	13.0	13.6	14.6	1.23
Born alive	10.6	11.6	14.2	1.07
Adjusted live born[2]	9.6	10.4	12.4	1.14
Stillborn	2.4	2	0.4	0.69
Mummified	0.2	0.4	0.4	0.35
Weaning	8.4	9.6	10.4	1.09
Preweaning mortality	1.2	0.8	2.0	0.73
Litter weight, kg				
Total born	14.66	18.85	19.75	1.80
Born alive	12.58	16.73	19.41	1.75
Adjusted live born[2]	11.44	15.31	17.65	1.71
Weaning	49.10	66.22	61.91	8.02
Gain	36.52	49.48	42.50	6.65
Individual piglet weight, kg				
Total born	1.12	1.38	1.37	0.09
Born alive	1.17	1.42	1.39	0.09
Adjusted live born[2]	1.17	1.45	1.45	0.08
Weaning	5.99	6.87	5.88	0.42
Gain	5.99	6.87	5.88	0.42
Adjusted weight, kg[3]				
Litter weight at weaning	45.99	60.10	59.14	7.08
Litter weight gain	34.55	44.79	41.48	5.81
Piglet weight at weaning	5.61	6.23	5.64	0.35
Piglet weight gain	4.44	4.78	4.19	0.33

[1]Statistical analysis is reported in the Appendix 4.
[2]Adjusted born alive represents number of piglets alive after cross-fostering.
[3]Adjusted for a 21 d lactation period.

5.4.2 *Sow tissue and metacarpal composition*

The effect of dietary B supplementation on sow organ weights are presented in Table 5.4. There were no dietary treatment effects observed on absolute and relative organ weights of sows supplemented B through gestation and lactation. Table 5.5 presents the liver and kidney content of sows fed diets supplemented with B during gestation and lactation. There were no statistical effects observed for the liver ($P > 0.05$), however, the iron content of the liver was numerically greater (62% and 19% higher, respectively) in sows fed diets with increasing B supplementation compared to the sows fed the control diet without B supplementation. Additionally, the liver copper concentration was numerically lower (39% and 14% lower, respectively) in sows supplemented with increasing B compared to the control sows.

There was a quadratic decrease ($P = 0.05$) observed for the dry matter content of the kidney with increasing B supplementation. Furthermore, there was a linear tendency ($P = 0.06$) coupled with a quadratic increase ($P = 0.04$) observed in the kidney P content of sows where sows fed diets supplemented with 5 ppm B had the greatest P content of the kidney. Additionally, there was a quadratic tendency ($P = 0.07$) for kidney Ca content to increase in sows fed diets supplemented with increasing B levels. Similar to the kidney P content, the kidney Ca content for sows fed diets supplemented with 5 ppm B having the greatest Ca content. Also, although not significant, sows supplemented with 5 ppm B had approximately a 5 % increase in kidney Mg content compared to sows fed diets without B supplementation.

The effects of increasing dietary B supplementation on sow metacarpal composition are presented in Table 5.6. Interestingly, there was a linear decrease in the

3rd and 4th metacarpal bone-breaking strength ($P = 0.04$) with increasing B supplementation. Additionally, a quadratic tendency for the Mg content of the metacarpal to decrease ($P = 0.06$) with increasing B supplementation.

Table 5.4 Effects of dietary boron (B) supplementation on absolute and relative sow organ weights.

	Supplemental B, ppm				*P*-value[1]	
Items	0	5	25	SEM	L	Q
No. of sows	5	5	5			
Sow BW, kg	227.6	222.6	237.6	12.61	-	-
Absolute weight, g						
Liver	3,229.6	2,962.0	3,203.5	344.63	-	-
Kidney	549.7	603.2	615.1	44.53	-	-
Heart	716.9	674.2	781.1	41.04	-	-
Ovaries	31.3	21.5	20.6	6.46	-	-
Relative weight, %[2]						
Liver	1.42	1.32	1.33	0.12	-	-
Kidney	0.24	0.27	0.26	0.02	-	-
Heart	0.32	0.31	0.33	0.02	-	-
Ovaries	0.015	0.010	0.009	0.004	-	-

[1]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

[2]Relative weight is calculated as a percentage of bodyweight (BW).

Table 5.5 Effects of dietary boron (B) supplementation on sow liver and kidney composition[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
No. of sows	5	5	5			
Liver						
DM, %	27.72	26.70	26.57	0.40	-	-
Ash, %	1.32	1.31	1.34	0.02	-	-
Ca	223.1	243.3	236.2	16.4	-	-
P	11,895.3	11,629.9	11,969.6	413.7	-	-
Mg	333.2	350.0	344.8	6.8	-	-
Fe	280.1	455.9	335.3	77.4	-	-
Cu	555.8	338.5	473.1	124.7	-	-
Zn	230.9	265.8	230.6	26.3	-	-
Kidney						
DM, %	19.86	17.27	19.37	0.88	-	0.05
Ash, %	1.03	1.01	1.04	0.03	-	-
Ca	310.6	379.8	325.1	25.9	-	0.07
P	11,433.1	12,492.7	10,736.0	407.3	0.06	0.04
Mg	425.7	445.5	425.0	17.4	-	-

[1]Calcium, P, Mg, Fe, Cu, and Zn concentrations are reported as mg/kg DM.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 5.6 Effects of dietary boron (B) supplementation on sow 3rd and 4th metacarpal composition.

	Supplemental B, ppm				*P*-value[1]	
Items	0	5	25	SEM	L	Q
No. of sows	5	5	5			
Bone weight, g						
Fresh	45.24	43.49	46.65	2.45	-	-
Dry fat-free	25.09	23.99	24.76	1.27	-	-
Length, cm	8.70	8.45	8.51	0.21	-	-
Breaking strength, kg	372.6	360.5	316.3	18.41	0.04	-
Bone composition						
Marrow Wt., g	6.52	6.27	7.04	0.49	-	-
Marrow, %	14.34	14.57	15.00	0.70	-	-
Lipid Wt., g	1.94	1.74	1.80	0.10	-	-
Lipid, %	5.02	4.69	4.55	0.18	-	-
Ash, %	35.36	35.97	35.82	0.56	-	-
Ca, %	25.55	25.42	25.68	0.41	-	-
P, %	11.43	11.39	11.48	0.11	-	-
Mg, %	0.306	0.292	0.297	0.004	-	0.06

[1]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

5.4.3 *Piglet tissue and bone composition at birth and weaning*

A total of two piglets from each litter were euthanized at both birth and weaning to assess litter tissue and bone content. At birth, a quadratic decrease for absolute heart weight ($P = 0.04$), accompanied by a quadratic tendency ($P = 0.06$) for relative heart size to decrease with increasing maternal B supplementation (Table 5.7). This is supported by litters from sows that were supplemented with 5 ppm B having piglets with smaller absolute and relative heart sizes (14% and 12% lower, respectively) compared to piglets from the control sows. In addition, there was a linear tendency observed for liver Ca to decrease ($P = 0.06$) at birth with increasing maternal B supplementation, which was illustrated by litters from B-supplemented sows having a 40% lower liver Ca level compared to litters from control sows (Table 5.8).

The femur composition of piglets at birth from sows supplemented with B is presented in Table 5.10. There was a quadratic increase ($P < 0.01$) observed in femur lipid percent, where the femur of piglets from sows supplemented with 5 mg ppm B having the greatest femur lipid concentration. Not significant but worth noting, the femur P content of piglets from sows fed diets supplemented with 5 and 25 ppm B were 6 and 12% greater, respectively compared to piglets from sows fed diets without B supplementation.

Effects of supplemental B to sows on litter tissue size at weaning are presented in Table 5.8. There was a quadratic increase ($P = 0.05$) along with a quadratic tendency ($P = 0.06$) for absolute and relative liver weight, respectively, with increasing maternal dietary B supplementation. Furthermore, a quadratic decrease ($P = 0.02$) in liver Mg concentration of weaning pigs from sows fed diets with increasing B supplementation

(Table 5.9). There was a quadratic effect ($P = 0.04$) observed on kidney DM content where piglets from sows supplemented with 5 ppm B had the lowest DM content at weaning (Table 5.9). Additionally, litters had a linear increase ($P = 0.02$ and $P = 0.04$, respectively) in both kidney Ca and Mg concentration at weaning with regards to increasing maternal B supplementation.

Lastly, the effects of maternal B supplementation on femur content at weaning are presented in Table 5.11. There was a quadratic decrease ($P = 0.02$) along with a linear tendency ($P = 0.08$) in femur Mg concentration to decrease with increasing maternal B supplementation.

Table 5.7 Effects of maternal dietary boron (B) supplementation on piglet tissue composition at birth and weaning[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Birth, n	5	5	5			
Piglet BW, kg	1.11	1.21	1.01	0.13	-	-
Organ absolute weight, g						
Liver	33.98	31.63	33.29	2.15	-	-
Kidney	9.69	9.67	8.73	0.80	-	-
Heart	9.32	7.98	9.18	0.43	-	0.04
Organ relative weight, g						
Liver	3.14	2.85	2.79	0.22	-	-
Kidney	0.87	0.89	0.84	0.07	-	-
Heart	0.84	0.74	0.83	0.04	-	0.06
Weaning, n	5	5	5			
Piglet BW, kg	5.48	6.84	5.21	0.77	-	-
Organ absolute weight, g						
Liver	168.72	192.72	164.50	8.77	-	0.05
Kidney	34.32	35.36	32.70	2.83	-	-
Heart	34.95	34.70	32.96	2.10	-	-
Organ relative weight, g						
Liver	2.97	3.48	2.87	0.19	-	0.06
Kidney	0.58	0.62	0.58	0.05	-	-
Heart	0.60	0.64	0.57	0.04	-	-

[1]Treatment means represent 5 litters per treatment. Each litter sacrificed 2 random piglets/litter at birth and weaning.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 5.8 Effects of maternal dietary boron (B) supplementation on litter tissue composition at birth [1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Birth, n	5	5	5			
Liver						
DM, %	23.05	24.31	23.35	0.90	-	-
Ash, %	1.23	1.36	1.34	0.08	-	-
Ca	454.89	347.41	316.34	39.44	0.06	-
P	10,365.89	10,037.33	11,521.21	858.59	-	-
Mg	678.30	641.66	754.07	64.26	-	-
Kidney						
DM, %	18.46	18.37	18.75	0.60	-	-
Ash, %	1.16	1.15	1.23	0.03	-	-
Ca	639.65	665.95	546.28	36.047	0.04	-
P	13,770.73	14,126.21	14,841.63	450.67	-	-
Mg	793.50	803.54	838.53	39.590	-	-

[1]Treatment means represent 5 litters per treatment. Each litter sacrificed 2 random piglets/litter at birth and weaning. Calcium, P, and Mg concentrations are reported as mg/kg DM.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 5.9 Effects of maternal dietary boron (B) supplementation on litter tissue composition at weaning[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Weaning, n	5	5	5			
Liver						
DM, %	24.45	23.78	24.42	0.55	-	-
Ash, %	1.37	1.34	1.42	0.03	-	-
Ca	304.17	327.19	307.21	19.53	-	-
P	13,109.36	12,046.00	13,359.19	495.28	-	-
Mg	424.13	380.11	410.70	11.44	-	0.02
Kidney						
DM, %	19.30	17.96	19.46	0.45	-	0.04
Ash, %	1.20	1.18	1.22	0.02	-	-
Ca	501.76	583.04	613.99	24.76	0.02	0.10
P	13,719.01	13,246.63	13,726.92	273.09	-	-
Mg	479.74	471.42	500.08	8.23	0.04	-

[1]Treatment means represent 5 litters per treatment. Each litter sacrificed 2 random piglets/litter at birth and weaning. Calcium, P, and Mg concentrations are reported as mg/kg DM.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 5.10 Effects of maternal dietary boron (B) supplementation on piglet femur composition at birth[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Birth						
Femur weight, g						
Fresh	6.10	5.38	5.58	0.22	-	0.05
Dry fat-free	1.91	1.70	1.72	0.09	-	-
Length, cm	4.90	4.68	4.80	0.09	-	-
Breaking strength, kg	18.69	21.22	20.78	1.16	-	-
Femur composition						
Marrow weight, g	1.04	1.26	0.94	0.27	-	-
Marrow, %	17.90	22.05	17.57	4.19	-	-
Lipid weight, g	0.11	0.11	0.11	0.01	-	-
Lipid, %	5.69	6.38	5.98	0.14	-	< 0.01
Ash, %	42.45	44.80	43.45	2.29	-	-
Ca, %	14.92	14.93	14.79	0.70	-	-
P, %	9.28	9.85	10.41	0.47	-	-
Mg, %	0.246	0.255	0.269	0.012	-	-

[1]Treatment means represent 5 litters per treatment. Each litter sacrificed 2 random piglets/litter at birth and weaning. Calcium, P, and Mg concentrations are reported as mg/kg DM.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 5.11 Effects of maternal dietary boron (B) supplementation on piglet femur composition at weaning[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Weaning						
Femur weight, g						
Fresh	28.66	28.75	29.40	0.74	-	-
Dry fat-free	9.50	9.43	9.40	0.18	-	-
Length, cm	8.04	8.05	8.23	0.16	-	-
Breaking strength, kg	85.04	80.35	77.00	3.92	-	-
Femur composition						
Marrow weight, g	0.52	0.89	0.71	0.23	-	-
Marrow, %	2.06	2.94	2.25	0.70	-	-
Lipid weight, g	0.52	0.48	0.50	0.02	-	-
Lipid, %	5.19	4.92	5.08	0.16	-	-
Ash, %	57.10	58.42	57.17	0.71	-	-
Ca, %	17.39	16.91	17.13	0.46	-	-
P, %	8.75	8.44	9.03	0.28	-	-
Mg, %	0.295	0.274	0.302	0.006	0.08	0.02

[1]Treatment means represent 5 litters per treatment. Each litter sacrificed 2 random piglets/litter at birth and weaning. Calcium, P, and Mg concentrations are reported as mg/kg DM.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

5.5 Discussion

In a variety of different species, B supplementation has been reported to have effects on mineral metabolism. For example, pregnant Murrah buffaloes that were supplemented with 200 and 400 ppm B (as boric acid) resulted in increased plasma Ca, Mg, and B concentrations during the partum and postpartum phases (Sharma et al., 2020). In addition, the same lab found that Ca and Mg both responded similarly to dietary B supplementation as their apparent absorption coefficient increased by 21 to 24%, respectively (Sharma et al., 2022). In poultry species there has also been several reports that demonstrate a positive relationship between B supplementation and bone characteristics, including bone-breaking strength and bone ash content (Qin and Klandorf, 1991; Wilson and Rutzler, 1997, 1998; Elliot and Edwards, 1992; Rossi et al., 1993; Cheng et al., 2011; Pradhan et al., 2021). In rats, boron supplementation resulted in a 5 to 10% increase in vertebral resistance crush force (Chapin et al., 1998). In rabbits, boron supplementation led to a 27 to 46% increase in femur break force (Hakki et al., 2013).

In young pigs (2-month-old), the femur, humerus, and ribs were the only bones to exhibit a response to increasing dietary levels of Ca and P, suggesting that these should be the bones that are selected to assess bone mineralization in pigs with a similar age (Crenshaw et al., 1981). More recently using x-ray absorptiometry, it has been determined and suggested that femur ash content is a better indicator of total body mineral content (BMC) compared to fibula ash as there was a greater correlation to BMC using the femur ash compared to fibula ash (R^2= 0.94 vs. R^2= 0.74, respectively) in 25 kg

pigs (Crenshaw et al., 2009). Thus, in the present study, piglet bone composition utilized the femur whereas sow bone composition was determined using the metacarpals.

Results from the sow metacarpal composition in the current study disagree with previous studies demonstrating dietary B has positive effects on bone strength characteristics. In the current study, there was a linear decrease observed in sow metacarpal bone-breaking strength with increasing B supplementation. A possible explanation regarding this response may simply be due to the age of the sows selected for tissue collection as these sows were mature animals that had only received dietary B supplementation for 1 parity (~115 days), suggesting a potential loading period required for B to impact mature animals. Additionally, the bone and mineral status in these older sows at the time of being allotted to dietary treatments may have varied which would make assessments after a single period potentially misleading.

Interestingly, unlike the sows, the piglets from B-supplemented sows at birth had numerically greater femur bone-breaking strength. However, the results at birth were not consistent with the results from weaning pigs in the current study. The inconsistency between bone breaking strength of piglets at birth and weaning is also like those of the Mg concentration in these femurs. At birth, piglets from B-supplemented sows had a numerical increase in Mg content with increasing maternal B supplementation, however, this was not consistent in weaning pigs. The differences in observations at birth and weaning may be a function of in-utero nutrient supply versus the nutrient supply during lactation. Moreover, both time points (birth and weaning) in which piglets were sacrificed, are critical biological periods of growth and development. Prior to farrowing, piglets undergo rapid change illustrated by an exponential increase in growth and

development during the last trimester of pregnancy, all of which prepare the pig for success in the environment change it undergoes during the farrowing process. Also, weaning pigs are a product of rapid growth during the lactation period which is illustrated by an accumulation of body mass that can be two- or three-fold in a period of 3 weeks. Thus, the magnitude of growth and development changes that newborn and weaning piglets undergo in a short period are likely to affect various responses that were measured, resulting in differing results.

Armstrong et al. (2002) reported that supplementation of 5 ppm B resulted in a decreased Ca content of the embryo at d 35 of gestation. Interestingly, in the current study, the Ca content of the liver and kidney of newborn piglets from sows supplemented with 5 and 25 ppm B were also decreased. More so, the P content of the liver and kidney of piglets at birth from sows receiving 25 ppm B was increased 11% and 7%, respectively. However, by weaning, the effects observed on the Ca and P content of the liver and kidney were nonexistent. Other work has suggested that B interacts with Ca and P metabolism (Nielsen et al., 1987; Hegsted et al., 1991). Although these observations have not clearly been understood, Armstrong et al. (2002) suggest that B has a role in cell membrane function, composition, or stability. Moreover, the contradicting results between Ca and P content of the liver and kidney of piglets from B-supplemented sows at birth and weaning may be a result of cell membrane function or biological distribution of Ca and P through major tissues at different physiological stages.

5.6 Conclusion

In conclusion, dietary supplementation of B to the diet of sows at levels of 0, 5, and 25 ppm B resulted in no adverse effects on reproduction, litter performance, and litter tissue and femur composition. The results for tissue mineral concentrations were inconsistent between sows and their litters at birth and weaning. This may be attributed to the fact that sows were only supplemented with dietary B for 1 reproductive cycle. The inconsistent results regarding maternal B supplementation between the litter tissue and bone content at birth compared to at weaning may be a function of B transfer to piglets in-utero versus milk supply. Thus, further experimentation to further understand the maternal transfer of B via placental and mammary nutrient supply is warranted.

CHAPTER 6. Effects of dietary boron supplementation on grower and market pig tissue, bone, and carcass composition

6.1 Abstract

A total of 40 crossbred pigs (27 barrows and 15 gilts; Yorkshire x Landrace x Large White) were used to evaluate the effects of boron (B) supplementation on the determination of tissue, bone, and carcass composition at two different time points. Pigs originated from sows fed gestation and lactation diets supplemented with varying levels of B (0, 5, and 25 ppm B). Pigs were fed diets from weaning to slaughter that contained the same level of B as their dam had received. Experimental diets were formulated to meet or exceed NRC (2012) nutrient recommendations and were provided ad libitum access during the experiment. A total of 18 grower pigs (6 pigs/treatment) and 22 finisher pigs (8, 7, and 7 pigs/treatment respectively) were humanely slaughtered at approximately 70 kg BW (grower period) and 120 kg BW (finisher period) respectively, for mineral determination of tissues and metacarpals. Finisher pigs were also evaluated for specific carcass measures. There was a linear tendency for liver Mg concentration to be greater ($P = 0.07$) in grower pigs supplemented increasing levels of B. Furthermore, grower pigs fed diets supplemented with increasing B demonstrated a linear increase ($P = 0.03$) in kidney ash percentage whereas in finishing pigs, kidney ash only tended to increase ($P = 0.08$) with increasing B. Grower pigs demonstrated a linear increase ($P = 0.05$) in Mg content of the metacarpals with supplemental B; subsequently, this response was later found to be quadratic ($P = 0.02$) in finishing pigs. There was a greater ($P = 0.05$) percentage of marrow found in the metacarpals of finishing pigs supplemented with 5 ppm B. In conclusion, B supplementation at levels of 5 and 25 ppm B fed to grower and finishing pigs resulted in minor changes in Mg concentrations of select tissues and metacarpals without affecting Ca

and P concentration. The lack of consistency in observations between grower and finishing pigs may be a product of the absence of a nutritional deficiency.

Keywords: boron, pig, tissue, carcass

6.2 Introduction

Although boron (B) has been fully accepted as an essential trace element required by plants, the full understanding of the essentiality of B in animals is still unclear. Some of the first work evaluating B in animal nutrition found that supplemental B improved growth and a partial correction of leg abnormalities present in cholecalciferol-deficient chicks (Hunt and Nielsen, 19871). Since then, there have been numerous reports suggesting B supplementation may improve mechanical properties and chemical composition of bone in several different animal models including rats, poultry, rabbits, and pigs (Chapin et al., 1998; Wilson and Rutzler, 1997; Rossi et al., 1993; Hakki et al., 2013; Armstrong and Spears, 2001; Armstrong et al., 2002). However, interpreting reports regarding B supplementation can be somewhat difficult as there are often differences between either the source of supplemental B (boric acid vs. sodium borate) or the quantity of B supplied. Nonetheless, there has been accumulating evidence suggesting that B is an indispensable mineral in bone development, cell proliferation, and mineralization in bones and tissues (Chen et al., 2011). Therefore, the purpose of this experiment was to evaluate the effects of supplemental B on grower and market pig tissue, bone, and carcass composition.

6.3 Experimental procedures

The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky. The animal slaughter was performed at the University of Kentucky Meat Laboratory in the Department of Animal and Food Sciences.

6.3.1 *Animals, housing, management, and experimental design*

Crossbred pigs [n = 42; 27 barrows and 15 gilts; (Yorkshire x Landrace) x Large White] from sows that were supplemented with one of three experimental diets which contained different levels of B were assigned to the same level of supplemental B from weaning until slaughter as their dam had received. Experimental diets consisted of a common corn-SBM based basal diet that was formulated to meet or exceed NRC (2012) requirement estimates for the respective production phase with B supplemented at either 0, 5, or 25 ppm B as sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc. Boron, CA). To minimize differences in non-treatment components of the diets, a large amount of the corn-SBM basal diet was firstly mixed (Table 6.1). Then, an aliquot of the basal diet was mixed with B to create the high inclusion level (i.e., 25 ppm) diet. Following the creation of the high-level diet (25 ppm B), the high-level B diet was blended with the control diet in an appropriate portion to create the intermediate level of B (i.e., 5 ppm B).

The pigs were provided with ad libitum access to feed and water during the entire experimental period. A total of 18 (6 pigs per treatment; 4 barrows and 2 gilts per treatment) of the 42 pigs were randomly selected and humanely slaughtered at approximately 70 kg BW. A total of 22 (8, 7, and 7, respective to dietary treatment) of the remaining 24 pigs were later humanely slaughtered at approximately 124 kg. The market slaughter consisted of 5 barrows and 3 gilts per treatment with the exception that 1 barrow from the 5 ppm B treatment and 1 gilt from the 25 ppm B treatment had been earlier removed from the study and euthanized due to rectal prolapses. All pigs were humanely slaughtered by conventional methods and in accordance with University of

Kentucky Meat Lab procedures. Pigs were electrically stunned, hung by one leg by wrapping a chain around the hock, and then exsanguinated. Carcasses were scalded, mechanically dehaired, and then hung on the rail by the digital flexor tendons of both hind legs. All bleeding and evisceration procedures were conducted with a 15-cm boning knife.

Table 6.1 Formulation and calculated composition of experimental basal diets (as-fed basis).

Items	7-11 kg	11-25 kg	25-50 kg	50-75 kg	75-100 kg
Ingredient, %					
Corn	52.32	59.68	69.86	75.09	79.36
Soybean meal, 48% CP	28.50	35.00	25.00	20.00	16.00
Grease, choice white	2.00	2.00	2.00	2.00	2.00
Fish meal (Menhaden)	5.00	-	-	-	-
Spray-dried animal plasma	2.00	-	-	-	-
Whey dried	8.00	-	-	-	-
L-Lysine•HCl	0.13	0.26	0.25	0.25	0.23
DL-Methionine	0.14	0.18	0.10	0.07	0.04
L-Threonine	0.07	0.11	0.08	0.08	0.06
Dicalcium phosphate	0.20	1.10	1.08	0.98	0.90
Limestone	0.92	0.95	0.91	0.82	0.70
Salt	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[1]	0.10	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10
Santoquin[3]	0.02	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
Metabolizable energy, kcal/kg	3,391	3,363	3,374	3,385	3,395
Crude protein, %	23.85	22.06	18.05	16.07	14.46
SID Lysine, %[4]	1.36	1.14	0.99	0.86	0.75
Ca, %	0.80	0.71	0.66	0.59	0.52
Total Phosphorus, %	0.61	0.60	0.56	0.52	0.49
STTD P, %[5]	0.40	0.40	0.35	0.32	0.29
Analyzed composition, %					
Ca	-	-	-	0.83	0.83
P	-	-	-	0.59	0.58
Mg	-	-	-	0.13	0.12

[1]Supplied the following per kilogram of total diet: 50 mg of Mn as manganous sulfate, 100 mg of Fe as ferrous sulfate, 125 mg of Zn as zinc sulfate, 18 mg of Cu as copper sulfate, 0.35 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.
[2]Supplied the following per kilogram of total diet: 4,245 IU of vitamin A; 1,062 IU of vitamin D3; 28.3 IU of vitamin E; 3.2 IU of vitamin K; 0.012 mg of vitamin B12; 9.45 mg of pantothenic acid; 0.104 mg of biotin; 0.076 mg of folic acid; 18.81 mg of niacin; 8.27 mg of riboflavin; 1.89 mg of vitamin B6; and 0.52 mg of thiamin.
[3]Santoquin (Novus International, Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diet.
[4]Calculated composition of lysine is presented on a standard ileal digestible (SID) basis.
[5]Calculated composition of phosphorus is presented on a standard total tract digestible (STTD) basis.

6.3.2 *Sample collection and processing*

Representative samples of corn, soybean meal, and mixed feed were collected at the feed mill for all experimental diets. Feed samples were stored at -20°C until analyzed. During the evisceration process, heart, kidneys, liver, ovaries (for gilts), and both front feet separated at the knee joint and 3rd and 4th metacarpals were extracted, and their weights recorded. Once tissue weights were recorded, tissues samples were stored at -20°C until further analysis.

In addition to tissue and bone samples, carcass traits were measured and obtained for the market pigs. Carcass trait measurements consisted of hot carcass weight (HCW), cold carcass weight (CWC), backfat thickness at the first rib, last rib, 10th rib, and last lumbar vertebrate, as well as determination of the longissimus dorsi muscle area (LMA) using methods described by NPPC (2000). Briefly, HCW was recorded immediately after harvest to calculate dressing percentage [(HCW/BW) × 100]. Following a 24-h chill (4 °C), CCW, backfat depth at the 10th rib, 1st rib, last rib, and last lumbar were measured. Shrink loss (%) was calculated using HCW and CCW, with the equation {[1 − (CCW/HCW)] × 100}. Carcass length was measured from the anterior edge of the symphysis pubic to the recess of the first rib.

Tissue samples were placed through a kitchen grade meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ) to provide a homogenous tissue sample. After samples were ground and mixed, 1-2 g of tissue was digested with nitric acid in a pressurized microwave digester (MARS 6 CEM, Matthews, NC) according to the recommendations by the manufacture, and appropriately diluted. The third and 4th metacarpal samples were carefully cleaned by removing any excess cartilage or

connective tissue. Collected bone samples were stored at -20 °C in plastic bags until further analyzed.

6.3.3 *Laboratory analysis*

Feed samples for the grower and market period were analyzed for Ca, P, and Mg composition at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO) and are presented in Table 6.1. Diet mineral analysis (Ca, P, and Mg) was analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) under methods described by AOAC (method 985.01; AOAC, 2006). Diets were also analyzed for B composition. The B analysis of the diet samples confirmed the rank of the treatments but variability between duplicate samples precluded acceptance of the absolute values.

To assess bone strength, bones were brought to room temperature and subjected to breaking strength determinations using an Instron Materials tester (Model TM 1123; Instron Corp., Canton, MA, USA). Bone breaking strength was determined by obtaining the peak amount of force, before fracture, applied by a wedge mounted on a pressure-sensitive compression cell at a loading rate of 40 mm/min at the center of the fresh bone when placed horizontally on two supports 3.2 cm apart. Subsequently, the metacarpals were cut in half to remove the marrow. After drying in an oven, they were wrapped in cheesecloth and extracted with fresh petroleum ether three times at 24-h intervals or until the solvent was clear. They were then air-dried at room temperature under a chemical hood for 24 h and, dried in an oven overnight. The percentage ash content of the dry, fat-free bone was determined by weighing the bone into a dried and preweighed porcelain crucible. Samples were then placed into a muffle furnace and heated to 600°C overnight

(method 942.05; AOAC, 2003). Ash weight was recorded and the ash percent in dry, fat-free bone was determined. The ash from the metacarpal samples was then acid-digested and diluted to 250 mL for mineral analysis.

Tissue samples were placed through a kitchen-grade meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ) to provide a homogenous tissue sample. After samples were ground and mixed, 1-2 g of tissue was digested with nitric acid in a pressurized microwave digester (MARS 6 CEM, Matthews, NC) according to the recommendations by the manufacturer, and appropriately diluted.

All tissue and bone samples were analyzed for Ca and Mg concentration by flame atomic absorption spectrophotometry (AAnalyst 200, PerkinElmer, Waltham, MA, USA). Before Ca analysis, samples were prepared in a 0.1 % NaCl solution (to help capture free Ca ions) and ran with a Ca reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves. Calcium determination was performed using a nitrous oxide-acetylene gas mixture and a wavelength of 422.7 nm (modification of method 927.02; AOAC, 2003). Magnesium determination was performed using acetylene gas, at a wavelength of 285.21 nm (modification of method 927.02; AOAC, 2003) and ran with a Mg reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves. Phosphorus was analyzed using a gravimetric method (method 968.08; AOAC, 1990), where 20 mL of digested sample was mixed with Quimociac solution, filtered, and the precipitate obtained was weighed to calculate the P concentration (Appendix 6). Dry matter (DM) was determined for all tissue samples by placing around 2-3 g of the ground sample into a gravity convection drying oven (Precision Scientific Co., Chicago, IL) at 107°C for

approximately 16 hours and weighing the sample again to observe the moisture content lost (AOAC International, 2006). The ash content of tissues was determined as previously described for bone.

6.3.4 *Statistical analysis*

All data were subjected to an outlier analysis prior to the final statistical analyses. The process involved identifying potential outliers that were 3 standard deviations from the mean of the response measure, as well as reviewing study notes, before a final decision was made to exclude that value. All data were subjected to ANOVA using the GLM procedure of SAS (SAS 9.4, Cary, NC). The individual pig served as the experimental unit and the statistical model included the effects of treatment, sex, and the interaction between treatment and sex. The original model also evaluated the effect of BW as a covariate on both grower and finisher metacarpal composition, however, there were no significant observations for BW, thus it was subsequently dropped from the final model. The final model for grower and finisher tissue and metacarpal data were analyzed by the model:

$$Y_{ij} = \mu + \text{Boron}_i + \text{Sex}_j + (\text{Boron} \times \text{Sex})_{ij} + e_{ij}$$, where

Y = response variable (absolute and relative organ size, tissue, and metacarpal composition)

μ = a constant common to all observations

boron_i = supplemental boron level

sex_j = sex of pig

$(\text{boron} \times \text{sex})_{ij}$ = supplemental boron level × sex interaction

e_{ij} = error term of the model

Carcass characteristic data were analyzed by the same model above with the addition of slaughter BW as a covariate:

$Y_{ijk} = \mu + \text{Boron}_i + \text{Sex}_j + (\text{Boron} \times \text{Sex})_{ij} + \text{SlaughterBW}_k + e_{ijk}$, where

Y_{ijk} = response variable (carcass characteristic measures)

μ = a constant common to all observations

boron_i = supplemental boron level

sex_j = sex of pig

$(\text{boron} \times \text{sex})_{ij}$ = supplemental boron level × sex interaction

SlaughterBW_k = Bodyweight at slaughter

e_{ij} = error term of the model

Orthogonal polynomial contrasts were performed to evaluate the linear and quadratic effects of increasing supplemental B levels. Linear and quadratic coefficients for unequal spacing of treatments were obtained using the PROC IML function in SAS. Results are reported as least squares means by using the LSmeans function in SAS. The α level for determination of statistical significance was set at 0.05, with ≤ 0.10 used to declare a tendency for significance.

6.4 Results

6.4.1 *Tissue composition*

Bodyweight (BW) and organ weight (absolute and relative) from grower and market pigs fed diets supplemented with 5 and 25 ppm B are reported in Table 6.2. There was a linear increase ($P = 0.01$) observed for grower pig BW as pigs supplemented with 25 ppm B were approximately 5% heavier at the time of slaughter compared to the other treatments. Although there was a linear effect observed in grower pig BW, it is worth noting that this should be considered an artifact of the study as pigs were randomly selected and not selected for slaughter based on BW. Furthermore, there was a tendency for a sex effect followed by a more pronounced sex by treatment interaction for grower pig BW ($P = 0.09$ and $P = 0.01$, respectively) where gilts, on average, were about 3% heavier (72.79 vs. 70.86 kg, respectively) at the time of slaughter compared to barrows. It should further be noted that gilts from the 25 ppm B treatment were ~ 11% heavier than gilts from the other two treatments, likely explaining the sex by treatment interaction present for BW. There were no treatment effects observed for absolute and relative weight of the liver, kidney, and heart for grower pigs. However, there was a sex affect present for both grower pig absolute and relative liver weight in which barrows had a larger liver compared to the gilts. There were no sex by treatment interactions observed for grower pig organ size.

In market pigs, there were linear tendencies present for both absolute and relative kidney weights to decrease with increasing supplemental B ($P = 0.07$ and $P = 0.08$, respectively). There were no treatment effects observed for liver and heart size in market pigs. There was a sex effect ($P = 0.01$) present for market pig BW where barrows on

average were about 7% heavier at the time of slaughter compared to the gilts (127.26 vs. 118.67 kg, respectively). This sex effect was further present in the absolute and relative weight of the liver ($P < 0.001$ and $P < 0.01$, respectively) where barrows had a larger liver. Furthermore, there was a tendency for a sex affect along with a treatment by sex interaction observed for relative heart size ($P = 0.08$ and $P = 0.07$ respectively) where gilts from the B treatment had a larger heart relative to BW compared to barrows.

Although not significant, ovaries from gilts supplemented 25 ppm B were about 23 and 20 % smaller than control gilts during the grower and market slaughter respectively.

Liver and kidney composition of grower and market pigs are displayed in Table 6.3. Grower pigs had a tendency ($P = 0.07$) for a linear increase in liver Mg content alongside a linear increase ($P = 0.03$) in kidney ash percentage with increasing dietary B concentration. There was a quadratic effect ($P = 0.03$) for liver DM content for the market pigs. There were no other effects observed for liver content of market pigs. Like the grower pigs, kidney ash content of the market pigs tended to increase with increasing B level ($P = 0.08$). There were no other effects observed on kidney content with regards to B supplementation.

Table 6.2 Effects of dietary boron (B) supplementation on absolute and relative organ weights of grower and market pigs.

	Supplemental B, ppm				*P*-value[3]	
Items	0	5	25	SEM	L	Q
Grower pigs, n[1]	6	6	6			
Pig BW, kg[X]	70.69	70.60	74.18	0.91	0.01	-
Pig Age, d	98.88	99.50	98.00	0.42	0.06	-
Organ absolute weight, g						
Liver[S]	1,273.4	1,197.2	1,329.0	59.55	-	-
Kidney	270.3	281.2	260.3	15.79	-	-
Heart	280.8	274.0	276.2	12.27	-	-
Ovaries	8.80	7.50	8.05	1.20	-	-
Organ relative weight, %[4]						
Liver[S]	1.80	1.70	1.80	0.08	-	-
Kidney	0.38	0.40	0.35	0.02	-	-
Heart	0.40	0.39	0.37	0.02	-	-
Ovaries	0.013	0.011	0.010	0.002	-	-
Market pigs[2], n	8	7	7			
Pig BW, kg[S]	124.4	122.2	122.4	2.84	-	-
Pig Age, d[S]	155.7	154.5	153.2	1.62	-	-
Organ absolute weight, g						
Liver[S]	1,756.4	1,775.3	1,762.1	68.92	-	-
Kidney	366.2	372.8	330.7	16.38	0.07	-
Heart	394.0	409.7	397.7	14.84	-	-
Ovaries	12.60	10.47	9.10	3.62	-	-
Organ relative weight, %[4]						
Liver[S]	1.41	1.45	1.44	0.04	-	-
Kidney	0.29	0.30	0.27	0.01	0.08	-
Heart	0.32	0.34	0.33	0.01	-	-
Ovaries	0.010	0.009	0.008	0.002	-	-

[1]Treatment means represent 4 barrows and 2 gilts each.
[2]Treatment means represent 5 barrows and 3 gilts. One barrow (5 ppm B) and one gilt (25 ppm B) were removed earlier due to rectal prolapses.
[3]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[4]Relative weight is calculated as a percentage of bodyweight (BW).
[S]Sex effect present ($P \leq 0.05$).
[X]Sex by treatment interaction present ($P \leq 0.05$).

Table 6.3 Effects of dietary boron (B) supplementation on liver and kidney composition of grower and market pigs[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Grower pigs, n	6	6	6			
Liver						
DM, %	29.00	29.32	28.55	0.23	0.08	-
Ash, %	1.59	1.65	1.61	0.06	-	-
Ca[S]	239.4	236.6	236.4	8.73	-	-
P	12,668.9	13,180.4	13,294.0	280.8	-	-
Mg	350.3	373.7	368.4	7.78	-	0.07
Kidney						
DM, %	19.79	19.95	20.78	0.45	-	-
Ash, %[X]	1.17	1.18	1.22	0.02	0.02	-
Ca	757.6	692.0	684.8	55.28	-	-
P	10,986.0	11,323.3	10,726.3	223.6	-	-
Mg	487.6	503.5	483.2	15.15	-	-
Market pigs, n	8	7	7			
Liver						
DM, %	28.58	27.55	28.76	0.37	-	0.03
Ash, %	1.50	1.51	1.52	0.04	-	-
Ca	214.3	209.8	201.0	10.71	-	-
P	12,574.0	12,847.7	12,023.9	377.9	-	-
Mg[S]	352.1	362.9	344.3	9.00	-	-
Kidney						
DM, %	19.55	19.43	19.77	0.29	-	-
Ash, %[S]	1.13	1.10	1.16	0.02	0.08	-
Ca	616.4	597.8	573.1	45.84	-	-
P[S]	10,245.4	10,413.1	10,201.2	148.0	-	-
Mg	462.0	458.0	453.3	8.75	-	-

[1]Calcium, P, and Mg concentrations are reported as mg/kg DM.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.
[X]Sex by treatment interaction present, $P \leq 0.05$.

6.4.2 *Metacarpal composition*

Effects of dietary B supplementation on metacarpal composition of grower and market pigs are reported in Tables 6.4 and 6.5. The metacarpals from grower pigs fed diets supplemented with increasing B resulted in a linear increase in the Mg concentration ($P = 0.05$). There were no sex by treatment interactions present for grower pig metacarpal composition. However, there was a sex effect present for Mg concentration in which barrows had a greater Mg concentration in the metacarpal. There were no other effects observed for metacarpal content of growing pigs.

Metacarpals from market pigs supplemented with increasing B demonstrated a quadratic increase ($P = 0.03$ and $P = 0.05$, respectively) in absolute marrow weight and marrow percent where the pigs that were fed the diet supplemented with the 5 ppm B having the greatest marrow content (Table 6.5). Furthermore, there was a quadratic ($P = 0.02$) response regarding bone-breaking strength where pigs supplemented with 5 ppm B having the lowest force. Interestingly, there was a sex by treatment interaction present for absolute and relative marrow and lipid content in the metacarpals of market pigs. These findings are explained by barrows receiving the supplemented B diets having more marrow and lipid content of the bone compared to barrows on the control diet whereas gilts expressed the opposite, in which gilts receiving the supplemented B diets having less marrow and lipid content compared to that of control gilts. Lastly, there was a quadratic response ($P = 0.02$) for Mg content of the metacarpal where Mg concentration was lowest for the 5 ppm B treatment.

Table 6.4 Effects of dietary boron (B) supplementation on grower pig metacarpal composition[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
n	6	6	6			
Bone weight, g						
Fresh	19.97	18.87	20.80	0.74	-	-
Dry Fat free	9.09	8.52	9.35	0.32	-	-
Length, cm	6.64	6.65	6.72	0.10	-	-
Breaking strength, kg	127.64	112.49	118.86	6.36	-	-
Bone composition						
Marrow weight, g	2.45	2.46	2.48	0.14	-	-
Marrow, %	12.22	13.00	11.94	0.46	-	-
Lipid weight, g	0.58	0.55	0.60	0.03	-	-
Lipid, %	3.32	3.31	3.28	0.10	-	-
Ash, %	55.98	56.43	55.88	0.33	-	-
Ca, %	18.85	18.90	18.50	0.42	-	-
P, %	10.16	10.22	10.11	0.07	-	-
Mg, %[S]	0.183	0.189	0.196	0.004	0.05	-

[1]Third and 4th metacarpals from growing pigs (mean BW: 71.5 ± 0.9 kg).
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.

Table 6.5 Effects of dietary boron (B) supplementation on market pig metacarpal composition[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
n	8	7	7			
Bone weight, g						
Fresh[S]	27.44	28.80	26.19	0.99	-	-
Dry fat free[S]	14.43	14.58	13.69	0.44	-	-
Length, cm[S]	7.29	7.54	7.14	0.11	0.09	0.05
Breaking strength, kg	239.04	204.26	229.42	9.96	-	0.02
Bone composition						
Marrow weight, g[X]	2.92	3.40	2.76	0.18	-	0.03
Marrow, %	10.61	11.82	10.48	0.50	-	0.05
Lipid weight, g[S]	0.81	0.85	0.77	0.03	-	-
Lipid, %[X]	3.31	3.34	3.29	0.06	-	-
Ash, %	59.51	58.67	59.06	0.40	-	-
Ca, %[S]	23.96	23.71	23.98	0.37	-	-
P, %	10.66	10.61	10.64	0.12	-	-
Mg, %[X]	0.188	0.179	0.186	0.003	-	0.02

[1]Third and 4th metacarpals from market pigs (mean BW: 124.4 ± 2.8 kg).
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.
[X]Sex by treatment interaction present, $P \leq 0.05$.

6.4.3 *Carcass composition*

Carcass composition of market pigs supplemented dietary B are reported in Table 6.6. There were no effects observed for carcass composition regarding dietary B supplementation. Furthermore, there were no sex by treatment interactions present for any carcass response measures. However, there were sex effects ($P < 0.05$) observed for 10^{th} rib back fat depth and loin muscle area where barrows had about 16% more back fat at the 10^{th} rib coupled with about 11% smaller LMA.

Table 6.6 Effects of dietary boron (B) supplementation on market pig carcass measures.

Items	Supplemental B, ppm			SEM	*P*-value[1]	
	0	5	25		L	Q
n	8	7	7			
Age, d[S]	155.70	154.50	153.24	1.62	-	-
Slaughter weight, kg	124.39	122.15	122.35	2.84	-	-
Hot carcass weight, kg	92.59	93.18	93.65	0.62	-	-
Cold carcass weight, kg	90.31	90.90	91.38	0.58	-	-
Dressing, %	74.46	74.91	75.31	0.48	-	-
Shrink loss, %	2.46	2.44	2.43	0.15	-	-
Carcass length, cm	82.07	82.66	82.32	0.92	-	-
Back fat depth, cm						
1st rib	4.19	4.04	4.13	0.33	-	-
Last rib	2.45	2.30	2.06	0.24	-	-
10th rib[S]	2.50	2.47	2.53	0.13	-	-
Last lumbar	2.07	2.02	1.90	0.15	-	-
Loin muscle dimension[2], cm						
Vertical	6.55	6.18	6.58	0.24	-	-
Horizontal	8.71	9.17	9.06	0.29	-	-
Area[3,S], cm^2	38.97	36.89	37.62	1.02	-	-

[1]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[2]Vertical distance refers to depth vertical to the 10th rib; horizontal distance refers to width horizontal to the 10th ribs.
[3]Area was measured directly with a plastic standard grid as described by NPPC (2000).
[S]Sex effect present, $P \leq 0.05$.

6.5 Discussion

Tissues and metacarpals were evaluated from grower (70 kg) and market pigs that were fed diets supplemented with 0, 5, and 25 ppm B from sodium borate. Pigs on experiment originated from sows which had received the same levels of dietary B during gestation and lactation (Chapter 5). Boron has been found to accumulate in both bone and soft tissue in the form of boric acid (Sutherland et al., 1998). However, some early work performed in rats reported that increasing B supplementation (2, 12.5, and 25 mg B/rat/d as boric acid) resulted in increased B concentration in tissues and, among the organs examined, the kidney showed the highest concentration of B (Naghii and Samman, 1996). Interestingly, kidney ash percentage increased linearly with increasing B supplementation in grower pigs. Ash percentage can be indicative of the amount of inorganic material present. The increase in kidney ash percentage observed with increasing supplemental B in grower pigs was less pronounced in market pigs. Nonetheless, these findings coupled with the lack of change in kidney Ca, P, and Mg content may suggest that either B and/or other minerals appear in the kidney at greater concentrations with increasing supplemental B. However, in the current study, neither B nor any other minerals apart from Ca, P, and Mg were analyzed in the soft-tissue, thus, the above speculation cannot be confirmed.

Armstrong and Spears (2001) reported that barrows fed diets supplemented with 15 ppm B (as sodium borate) had about a 15 % increase in ultimate sheer force of the fibula compared to barrows fed diets supplemented with 5 ppm B. Interestingly, these findings are somewhat consistent with the current findings herein where grower pigs fed diets supplemented with 25 ppm B had about a 6% increase in bone breaking strength for

the metacarpals compared to pigs fed diets supplemented with 5 ppm B. The difference in the magnitude of response between the two studies may be due to the difference in the type of bone evaluated. However, a remarkable difference between the studies that should be noted is that although the strength of the bone measured was lower for the intermediate treatment in comparison to the high treatment, Armstrong and Spears (2001) reported that the bone strength for the high B treatment was numerically greater than that of the control group whereas in the current study, the high B treatment was numerically lower than the control group.

Other work in rabbits reported that supplementing B at 50 mg B/kg BW (as sodium borate) to mature rabbits resulted in a 27% increase in femur break force compared to control rabbits (Hakki et al., 2013). Although the previously mentioned work disagrees with the findings herein, it agrees with other work done in swine evaluating supplemental B (5 ppm B as sodium borate) to a semi-purified basal diet that was fed to pigs resulted in an increase in maximum bending moment of the femur (Armstrong et al., 2000) compared to the control pigs. In addition, the same lab also reported that long-term B supplementation (5 ppm B as sodium borate) to reproductive gilts tended to increased mechanical properties of the femur including force at yield, bending moment at yield point, yield stress, and deformation all of which have been considered relevant measures in assessing bone quality properties (Crenshaw et al., 1991a,b).

The differences between the above studies and the present study are that the above studies evaluated femur bones whereas the current study evaluated metacarpals and thus may account for difference in observations. Furthermore, the age of the bone is

an important factor regarding its physical and chemical characteristics. It has been reported in older work (1981) in young pigs (2-month-old) that the femur, humerus, and ribs elicit a better response compared to meta- carpals and tarsals regarding Ca and P levels in young pigs (2-month-old), indicating that these should be the bones that are selected to assess bone mineralization (Crenshaw et al., 1981). Whereas older pigs (3, 5, and 7 mo of age) have the best response to mineralization in the metacarpals and metatarsals (Crenshaw et al., 1981). The pigs in the present study would have been approximately 3 and 5 months of age at bone collection.

Hunt et al. (1994) suggested that B influences bone development through enhancing major mineral content of the bone. In ostriches, supplementing B in the water at 200 ppm B resulted in increased bone ash content (Cheng et al., 2011). Supplementing dietary B as boric acid to broiler chickens for 42 days led to increased femur bone ash, Ca, and P content (Pradhan et al., 2021). In the current study, Ca, and P content of the metacarpals from grower and market pigs were not affected by B supplementation. However, Mg content increased linearly with increasing B supplementation in grower pigs. Yet, in market pigs, Mg content quadratically decreased in the metacarpals.

6.6 Conclusion

In the current study, major minerals associated with bone development (Ca, P, and Mg) were analyzed for metacarpals of grower and market pigs. In both grower and market pigs, there were no effects observed for metacarpal ash, Ca, and P content. However, there were effects observed on metacarpal Mg content for both grower and market pigs. Effects observed at both collection points were not consistent with each

other; grower pigs demonstrated a linear increase in Mg concentration in the metacarpal whereas market pigs illustrated a quadratic decrease with the 5 ppm B treatment having the lowest Mg concentration. Interestingly, grower pigs also had a quadratic increase in liver Mg concentration with increasing supplemental B indicating a possible interaction between B supplementation and Mg utilization. However, the lack of response regarding supplemental B observed in market pigs may be a result of the length of time from the grower slaughter compared to the market slaughter. For example, market pigs remained on full-feed (ad-libitum access) for an additional 56 days following the slaughter of grower pigs. In this time, market pigs may have had enough time to adjust and compensate for any of the differences previously observed at the grower slaughter.

CHAPTER 7. Effects of dietary boron supplementation on apparent digestibility and retention of macro minerals in growing barrows.

7.1 Abstract

An experiment was conducted to evaluate the effects of boron (B) supplemented to a corn-soybean meal-based diet on apparent digestibility and retention of nutrients in growing barrows. A total of 24 barrows (mean initial BW = 61.15 ± 2.45 kg) were allotted to 3 dietary treatments with 8 replicates. Dietary treatments consisted of 0, 5, and 25 mg B/kg diet as sodium tetraborate decahydrate ($Na_2 B_4 O_7 \cdot 10 H_2O$; 11.34% B) which were fed from weaning onward. Barrows underwent a 5-7 d adaptation period to the meal allowance and metabolism crate which was followed by a 5-d total fecal and urine collection using the marker-to-marker method. Barrows were fed at 2.7% of their metabolic BW (2.7 x maintenance ME needs [2.7 × 106 Kcal ME/kg $BW^{0.75}$]) during the adaptation and collection periods in a gruel form (feed:water, 1:1 wt/vol). Supplemental B did not affect (P > 0.05) energy, lipid, nitrogen, calcium, or phosphorus apparent digestibility and retention. However, there was a linear increase observed in both magnesium digestibility (*P* = 0.04; 22.35, 23.56, and 28.49 %, respectively) and absorption (*P* = 0.01; 0.24, 0.26, and 0.32 g/d, respectively) with increasing B supplementation. Retnention of magnesium did not differ (*P* > 0.05; 0.13, 0.14, and 0.17 g/d, respectively). In conclusion, supplementing B at 5 and 25 mg B/kg diet resulted in an increase in magnesium digestibility and retention with no adverse effects observed on apparent digestibility and retention of other nutrients.

Keywords: boron, digestibility, pigs

7.2 Introduction

Currently, boron (B) is only identified as a "probably essential" nutrient for higher-order animals and humans (WHO, 1996). Early work by Hunt and Nielsen (1981) initiated the now-growing interest of dietary B as they found B to stimulate growth and partially prevent leg abnormalities that were present in vitamin D-deficient chicks. Since then, there has been a great amount of evidence from a variety of experimental models showing that B is a bioactive and beneficial (perhaps essential) element for animals and humans (Nielsen and Meacham, 2011). Despite that, there has been limited research focused on dietary B supplementation in swine and more specifically, the effects of B on metabolism of other nutrients in swine. Thus, the current experiment was designed to evaluate the apparent digestibility, absorption, and retention of macro minerals (Ca, P, and Mg), nitrogen, fat, and energy in growing barrows.

7.3 Experimental procedures

The experiment was conducted under protocols approved by the University of Kentucky Institutional Animal Care and Use Committee.

7.3.1 *Animals, housing, management, and experimental design*

A total of 24 crossbred barrows (Yorkshire x Landrace x Large White) were used in the experiment. The experiment was split into 2 separate trials (Trial 1, 12 barrows; Trial 2, 12 barrows; mean initial BW = 49.65 kg and 72.65 kg, respectively). In each trial, pigs were individually housed in stainless steel metabolism crates in an environmentally controlled room in an animal research facility at the University of Kentucky. Metabolism crates were equipped with a sliding aluminum screen that rested underneath the floor of

the metabolism crate to separate the feces and urine. There was also a stainless steel funneled-pan underneath the crate used to direct urine into a 10 L plastic bucket. The interior space of the crates was adjusted to restrain pigs from turning around, thereby preventing defecation into the feeder but leaving enough room for the pig to stand up and lie down. Room temperature was always kept in the thermo-neutral range of the pigs. Pigs used in both trials were selected from a larger animal pool where they were being fed diets that were supplemented with 0, 5, or 25 ppm B starting at weaning. Barrows were selected and enrolled on the study based on BW, genetics, and previous dietary treatment.

The experimental diets were formulated to meet or exceed the NRC (2012) nutrient requirement estimates of growing pigs relative to BW. To minimize differences in non-treatment components of the diets, a large amount of the corn-SBM basal diet was firstly mixed (Table 7.1). Then, an aliquot of the basal diet was mixed with B to create the high inclusion level (i.e., 25 ppm) diet. Following the creation of the high-level diet (25 ppm B), the high-level B diet was blended with the control diet in an appropriate portion to create the intermediate level of B (i.e., 5 ppm B). Boron was supplemented to the diets as sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc., Boron, CA).

All animals underwent a 5-7 d adaptation period to the crate and feeding schedule, followed by a 5-d total feces and urine collection period. Barrows were fed at 2.7% of their metabolic BW (2.7 x maintenance ME needs [2.7×106 Kcal ME/kg $BW^{0.75}$]) during the adaptation and total collection periods in a gruel form (feed:water, 1:1 wt/vol), with the feed divided into 2 meals/d. The beginning and end of the total collection

periods were marked by the addition of 0.5% indigo carmine (Aldrich Chemical Company Inc, Milwaukee, WI) to the morning meal. After consumption of each meal, water was added to the metabolism crate feeder to allow ad libitum access between meals. Rejected feed was dried in a forced-air oven at 55ºC, air-equilibrated, weighed, and discounted from the amount initially offered. Feed intake during the 5-day collection periods was calculated as the feed allowance minus feed rejection. The total amount of feces was collected daily, weighed, stored in plastic bags, and frozen at -20 °C until analysis. Urine was collected simultaneous with feces collections. For each pig, the daily amount of urine excreted was measured and individual urine samples obtained. Urine collection started at 9:00 am after pigs were fed the indigo dye and finished when five 24-h collections were completed. Urine was collected in 10 L plastic buckets containing 150 mL of 3 N HCl to limit microbial growth and reduce loss of ammonia. Every day, the total volume of urine produced was mixed, filtered with cheese cloth to remove any solid contaminates (feed, feces, hair, etc.), and a 100 mL urine sample was taken and stored frozen in labeled, capped, plastic containers, while the rest of the collected urine was discarded.

Table 7.1 Formulation and calculated and analyzed nutrient composition of basal diet (as-fed basis).

Item	%
Ingredient	
Corn	75.09
Soybean meal, 48% CP	20.00
Grease, choice white	2.00
L-Lysine•HCl	0.25
DL-Methionine	0.07
L-Threonine	0.08
Dicalcium phosphate	0.98
Limestone	0.82
Salt	0.50
Trace mineral premix[1]	0.10
Vitamin premix[2]	0.10
Santoquin[3]	0.02
Total	100.00
Calculated composition	
Metabolizable energy, kcal/kg	3,384.5
Crude protein, %	16.07
SID Lysine, %[4]	0.86
Calcium, %	0.59
STTD Phosphorus, %[5]	0.32
Analyzed composition	
Gross energy, kcal/kg	3,979.6
DM, %	88.33
CP, %	15.02
N, %	2.40
Ca, %	0.63
P, %	0.53
Mg, %	0.07

[1]Supplied the following per kilogram of all diets: 50 mg of Mn as manganous sulfate, 100 mg of Fe as ferrous sulfate, 125 mg of Zn as zinc sulfate, 18 mg of Cu as copper sulfate, 0.35 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.
[2]Supplied the following per kilogram of diet: 4,245 IU of vitamin A; 1,062 IU of vitamin D3; 28.3 IU of vitamin E; 3.2 IU of vitamin K; 0.012 mg of vitamin B12; 9.45 mg of pantothenic acid; 0.104 mg of biotin; 0.076 mg of folic acid; 18.81 mg of niacin; 8.27 mg of riboflavin; 1.89 mg of vitamin B6; 0.52 mg of thiamin; and 8.27 mg of riboflavin.
[3]Santoquin (Novus International Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diet.
[4]Calculated composition of lysine is presented on a standard ileal digestible (SID) basis.
[5]Calculated composition of phosphorus is presented on a standard total tract digestible (STTD) basis.

7.3.2 *Sample processing*

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55°C for one week, then air-equilibrated, weighed, and ground through a 1-mm screen using a Wiley Laboratory Mill (model 3, Arthur H. Thomas Co., Philadelphia, PA). All ground feces from each collection period were thoroughly mixed in a single bag for each pig. After being composited and mixed thoroughly, a subsample was taken from the larger sample and kept in a cold room at 4 to 8°C until chemical analyses were performed.

To obtain a representative sample of urine for nutrient analysis, the daily collected samples were thawed at room temperature and proportionally composited for each pig, mixed thoroughly, and a subsample of the composited sample collected. Composited samples remained frozen until analysis.

7.3.3 *Laboratory analysis*

Feces and feed were analyzed for DM and lipid content. Feces, feed, and urine were analyzed for energy, N, P, Ca, and Mg. Total contents of nutrients in feces, urine, and feed were calculated as the product of nutrient concentration and the total amount of material. Samples were analyzed at least in duplicate, and analysis were repeated when the coefficient of variation was greater than 5%. Dry matter (DM) contents of feed and feces were determined by drying the samples at 105°C for ~ 16 h in a convection oven (Precision Scientific Co., Chicago, IL) and then calculating moisture contents as the difference between the initial and final weighing (AOAC International, 2006). The crude

fat content of the feed and feces were determined in duplicates using the ether extraction method. Briefly, 1 gram of diet or excreta samples was weighed into a filter paper, which was placed into a cotton thimble and placed in the fat extraction machine (Velp Scientifica, Bohemia, NY, USA). The fat was extracted using petroleum ether. The weight of the resulting fat was expressed as a percentage of the starting sample weight.

Gross energy contents of the feed and feces were assessed by bomb calorimetry (a detailed method for determining gross energy can be found in Appendix 5). Benzoic acid pellets with a known combustion heat were ignited at the beginning and end of each set of samples to verify calorimeter calibration. To measure gross energy of the urine, samples were oven dried for 2 d at 55 C into polyethylene flat bags (Jeb Plastics Inc., Wilmington, DE) before combustion. To obtain urinary energy contents, the known heat of combustion per gram of bag material was subtracted from the total heat measured (Agudelo-Trujillo, 2005).

Nitrogen was measured using Dumas methodology in an automatic N analyzer (model FP-2000, LECO Corp., Saint Joseph, MI). Ignition of blanks and EDTA samples with known N contents was conducted daily to calibrate the equipment and to check for drift in the readings.

Phosphorus in feed and feces was assessed by a gravimetric method (method 968.08; AOAC, 1990), in which samples were weighed, ashed, acid-digested, diluted to 250 mL and then reacting 20 mL of the resultant liquid with Quimociac solution, filtered, and the precipitate weighed to calculate the P concentration (Appendix 6). Phosphorus concentration in urine was assessed as inorganic P by a colorimetric procedure (procedure number 360-UVP, Sigma Diagnostics, St. Louis MO) using a

spectrophotometer (model Ultrospec IIE, 4057 UV/visible, LKB biochrom Ltd., Cambridge, UK). The concentration was measured under UV light at 340 nm. A commercial reagent was used (ammonium molybdate, 0.40 mmol/L in sulfuric acid with surfactant; catalog number 360-3, Sigma Diagnostics) along with a set of 3 standards containing 1, 5, and 15 mg of P/dL (Ca/P Standard, catalog number 360-5, Sigma Diagnostics). Calcium and Mg contents were assessed by flame atomic absorption spectrophotometry (AAnalyst 200, PerkinElmer, Waltham, MA, USA). Before Ca analysis, samples were prepared in a 0.1 % NaCl solution (to help capture free Ca ions) and ran with a Ca reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves. Calcium determination was performed using a nitrous oxide-acetylene gas mixture and a wavelength of 422.7 nm (modification of method 927.02; AOAC, 2003). Magnesium determination was performed using acetylene gas, at a wavelength of 285.21 nm (modification of method 927.02; AOAC, 2003) and ran with a Mg reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves.

Diets were also analyzed for B composition. The B analysis of the diet samples confirmed the rank of the treatments but variability between duplicate samples precluded acceptance of the absolute values.

7.3.4 *Calculations and statistical analysis*

Apparent digestibility coefficients were calculated on a DM basis. Additionally, nutrient retention as well as excretion via feces and urine were calculated. Apparent nutrient digestibility coefficients (DM basis) were calculated using the formula below:

Apparent total tract digestibility (ATTD), % =

$$\left[\frac{Nutrient\ Consumed - Nutrient\ Feces}{Nutrient\ Consumed}\right] \times 100$$

Where *Nutrient Consumed* is the amount of nutrient consumed by the pig and *Nutrient Feces* is the amount of nutrient excreted in the feces.

Apparent retention per day, g/d =

$$daily\ nutrient\ intake - daily\ nutrient\ excreted\ (fecal + urine)$$

Retention as percent of absorption, % =

$$\left[\frac{daily\ nutrient\ retention}{daily\ nutrient\ intake - daily\ nutrient\ in\ feces}\right] \times 100$$

Prior to final analysis, all data were initially evaluated for any potential statistical outliers by identifying any values that were 3 standard deviations from the mean of the response measure of interest. After reviewing experimental notes, a final decision whether to exclude the value was made. However, there were no statistical outliers determined for this experiment.

All data were subjected to analysis of variance using the GLM procedure of SAS (SAS 9.4, Cary, NC). The individual pig served as the experimental unit and the statistical model included the effects of treatment, trial group, and the interaction between treatment and trial group. Orthogonal polynomial coefficients for treatments were obtained using the IML procedure of SAS. The coefficients were used to partition

treatments into linear and quadratic components. Results are reported as least squares means by using the LSmeans function in SAS. All data were analyzed by the model:

$$Y_{ijk} = \mu + boron_i + trial_j + (boron \times trial)_{ij} + e_{ij}$$, where

Y = the response variables (ATTD and retention of nutrients)

μ = a constant common to all observations

$boron_i$ = supplemental boron level

$trial_j$ = trial group

$(boron \times trial)_{ij}$ = supplemental boron level × trial group interaction

e_{ij} = error term of the model

There were no treatment by trial interactions observed for any response measures. The α level for determination of statistical significance was set at 0.05, with ≤ 0.10 used to declare a tendency for significance.

7.4 Results

All pigs were in good health and condition during the experimental periods. All pigs gained weight (Table 7.2) during the experimental periods indicating that pigs were in a positive nutrient balance. Dietary B supplementation resulted in no differences observed in DM digestibility (Table 7.2).

Table 7.2 Effects of dietary boron (B) supplementation on growth during the collection period and digestibility (apparent) of dry matter (DM) in growing barrows.[1]

	Supplemental B, ppm				*P*-value[2]	
Item	0	5	25	SEM	L	Q
BW, kg						
Initial	60.80	61.20	61.45	0.96	-	-
Final	65.83	66.52	65.74	1.04	-	-
ADG	0.63	0.67	0.54	0.03	0.02	-
Feed allowance, g/d	1,645.5	1,656.3	1,663.0	25.93	-	-
DM						
Intake, g/d	1359.7	1367.8	1375.6	21.15	-	-
Total excreted, g/d	143.6	151.7	149.1	4.99	-	-
Absorption, g/d	1216.1	1216.1	1226.4	21.60	-	-
Digestibility, %	89.40	89.02	89.21	0.367	-	-

[1]Treatment means represent 8 observations.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Effects of B supplementation on apparent digestibility and retention of nitrogen, energy, and lipid are reported in Table 7.3. There were no differences observed in energy and lipid digestibility and retention with dietary treatment, however, a linear tendency for N digestibility to increase with increasing dietary B supplementation ($P = 0.09$) was observed.

Table 7.3 Effects of dietary boron (B) supplementation on digestibility (apparent), and retention (as % of absorption) of nitrogen, energy, and lipid in grower barrows.[1]

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Nitrogen						
Intake, g/d	36.98	37.2	37.41	0.575	-	-
Fecal excreted, g/d	4.48	4.62	4.30	0.115	-	-
Urinary excreted, g/d	8.86	9.06	11.5	1.403	-	-
Absorption, g/d	32.5	32.58	33.11	0.579	-	-
Digestibility, %	87.85	87.68	88.53	0.329	0.09	-
Retention, % of absorbed	73.94	72.55	66.2	3.734	-	-
Energy						
Intake, kcal/d	6125.6	6162.2	6197.2	95.29	-	-
Fecal excreted, kcal/d	717.8	738.9	723.1	20.76	-	-
Urinary excreted, kcal/d	168.9	179.8	202.1	19.21	-	-
Absorption, kcal/d	5407.8	5423.2	5474.1	95.78	-	-
Retention, kcal/d	5238.9	5243.4	5272	91.52	-	-
Digestibility, %	88.15	88.04	88.31	0.344	-	-
Retention, % of absorbed	96.9	96.7	96.3	0.31	-	-
Lipid						
Intake, g/d	60.2	60.6	60.9	0.94	-	-
Total excreted, g/d	12.7	13.3	12.2	0.67	-	-
Absorption, g/d	47.5	47.3	48.7	1.09	-	-
Digestibility, %	78.79	78.42	80.02	1.046	-	-

[1]Treatment means represent 8 observations.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

The effects of dietary B supplementation on ATTD and retention of Ca, P, and Mg are reported in Table 7.4. There was a numerical decrease (~7 to 9 % lower) in Ca digestibility for pigs supplemented 25 ppm B compared to the other treatments. However, there were no differences observed in Ca retention between treatments. There was a tendency for a quadratic response ($P = 0.06$) for urinary excretion of P where the 5 ppm B group had the lowest amount excreted. Subsequently, a quadratic response ($P = 0.05$) was observed for P absorption followed by a tendency for a quadratic response for P digestibility. Although not significant, the 5 ppm B group had approximately 5 to 6 % greater P retention compared to the 0 and 25 ppm B groups, respectively. A linear increase ($P = 0.03$) was observed for urinary excretion of Mg with regards to increasing dietary B supplementation. Conversely, there was an even more noticeable linear increase ($P = 0.01$) in Mg absorption. Lastly, there was approximately a 27 % increase in Mg digestibility for barrows supplemented 25 ppm B compared to barrows supplemented with no B which is a product of the linear increase ($P = 0.04$) in Mg digestibility observed with dietary B supplementation.

Table 7.4 Effects of dietary boron (B) supplementation on digestibility (apparent) and retention (as % of absorption) of calcium, phosphorus, and magnesium in growing barrows[1].

	Supplemental B, ppm				*P*-value[2]	
Item	0	5	25	SEM	L	Q
Calcium						
Intake, g/d	9.73	9.79	9.84	0.15	-	-
Fecal excreted, g/d	4.12	4.10	4.57	0.27	-	-
Urinary excreted, g/d	0.16	0.15	0.12	0.02	-	-
Absorption, g/d	5.61	5.69	5.27	0.21	-	-
Retention, g/d	5.45	5.55	5.15	0.21	-	-
Digestibility, %	58.03	58.55	53.47	2.32	-	-
Retention, %	97.30	97.52	97.76	0.40	-	-
Phosphorus						
Intake, g/d	8.10	8.15	8.20	0.13	-	-
Fecal excreted, g/d	4.00	4.32	4.16	0.14	-	-
Urinary excreted, g/d	0.59	0.34	0.60	0.10	-	0.06
Absorption, g/d	4.10	3.84	4.04	0.10	-	0.05
Retention, g/d	3.52	3.50	3.44	0.15	-	-
Digestibility, %	50.96	47.26	49.34	1.29	-	0.06
Retention, %	86.24	90.76	85.30	2.38	-	-
Magnesium						
Intake, g/d	1.08	1.09	1.10	0.02	-	-
Fecal excreted, g/d	0.84	0.83	0.78	0.03	-	-
Urinary excreted, g/d	0.11	0.12	0.15	0.01	0.03	-
Absorption, g/d	0.24	0.26	0.32	0.02	0.01	-
Retention, g/d	0.13	0.14	0.17	0.02	-	-
Digestibility, %	22.35	23.56	28.49	2.09	0.04	-
Retention, %	52.72	50.30	50.19	6.26	-	-

[1]Treatment means represent 8 observations.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

7.5 Discussion

Nielsen et al. (1987), first reported that 3 mg of B per day (as sodium borate) decreased urinary Ca and P excretion in humans. A later study that fed diets containing supplemental B (as sodium borate) at 75 and 200 mg B per day to wethers, resulted in an increase in apparent absorption of Ca (Brown et al., 1989). Furthermore, in rats, an increase in apparent absorption of Ca and P was observed after consuming a diet supplemented with 2.72 ppm B (as sodium borate) for twelve weeks (Hegsted et al., 1991). Other work reported a 56 and 59% decrease in urinary phosphate excretion in heifers that consumed drinking water supplemented with 150 and 300 ppm B (as sodium borate), respectively (Green and Weeth, 1997).

Unlike the previously mentioned work, in the present study, feeding growing pigs diets supplemented with 5 and 25 ppm B resulted in no direct effects on Ca and P metabolism. The lack of response for Ca and P ATTD in the current study, are like those of Armstrong and Spears (2001), where authors also reported that supplemental B had no effects on apparent Ca and P absorption and retention in grower pigs fed diets supplemented with 5 and 15 ppm B (as sodium borate). Similar across both studies, pigs were provided a diet that was adequate in all nutrients whereas the other work that reported observed effects of B supplementation on Ca and P metabolism in other non-ruminant species (Nielsen et al., 1987; Hegsted et al., 1991) utilized experimental subjects that were also under nutritional stressors (Mg or vitamin D deficiency). Nielsen (1991) proposed that B may only have a pronounced influence on mineral metabolism when animals are subjected to nutritional or other stressors such as a nutrient deficiency. Furthermore, Ca and P metabolism is tightly regulated within the body through hormonal

regulation of parathyroid hormone, metabolites of vitamin D, and calcitonin (DeLuca, 1979). Calcium metabolism is ultimately a function of the rate of absorption, deposition, and excretion of both Ca and P, and is regulated by balancing and maintaining circulating concentrations that permit bone mineralization to occur (DeLuca, 1979; Mahan and Vallet, 1997).

It may be appropriate to speculate that the differences observed between the study herein and the report by Green and Weeth (1997) regarding P metabolism may simply be due to the large difference in the level of supplemental B. For example, Green and Weeth (1997) supplied B at rates of 6 and 12 times (150 and 300 ppm B, respectively) that of the highest supplemental level for the current study (25 ppm B). Furthermore, authors reported that heifers supplemented with B also had decreased plasma P concentrations compared to heifers not supplemented with B. The decreased P content in the plasma coupled with a reduction in the urinary P excretion is suggestive that at high levels, B may play an inhibitory role with P and thus, B supplemented subjects are excreting less P to manage the lower circulating P concentration in the plasma.

In the present study, increasing B supplementation to the diets of pigs resulted in an increase in absolute absorption and apparent digestibility of Mg. This positive interaction between B supplementation and Mg digestibility and absorption in the current study agrees with recent work conducted in pregnant and lactating Murrah buffaloes (Sharma et al., 2022), where it was reported that supplementation of 200 and 400 ppm B in the form of boric acid resulted in pregnant and lactating animals both having an increased apparent absorption of Mg compared to control animals. An earlier report by Nielsen et al. (1987) suggested that B supplementation (sodium borate) in women

resulted in decreased urinary Mg excretion. Interestingly, the decrease in urinary Mg excretion was irrespective of whether the women received an inadequate or adequate amounts of Mg in the diet. In tandem, Hunt (1989) reported an increase in Mg retention in chicks, however, chicks were in a vitamin D deficient state unlike the work herein where the pigs were receiving diets formulated to supply adequate levels of nutrients (NRC, 2012). Thus, some of the inconsistency in the results reported herein and in previously reported literature may be a product of not only the difference in species but more importantly the biological and physiological state that the subjects are in which may alter the magnitude of response for certain response measures.

7.6 Conclusion

Currently, there is limited information regarding dietary supplementation of B in swine. However, there has been accumulating evidence dating back several decades in support that B may be essential to certain biological functions in animals. The current experiment was conducted to evaluate the effects of supplemental B on apparent digestibility and retention of nutrients in growing barrows. Boron supplemented to growing barrows did result in increased digestibility and absorption of Mg. Furthermore, B supplementation at levels up to 25 ppm B resulted in no adverse effects observed on apparent digestibility and retention of other nutrients.

CHAPTER 8. Effects of dietary boron supplementation on broiler chick performance, apparent total tract retention, and tibia content

8.1 Abstract

The objective of this study was to evaluate the effects of boron (B) supplementation on the performance, tibia mineral content, and apparent total tract retention (ATTR) of nutrients in 22-day-old broiler chickens. The experiment used 448 day-old male by-product Cobb chicks in a randomized complete block design with 7 treatments consisting of 8 replicates of 8 birds per cage. The 7 dietary treatments consisted of a semi-purified basal diet with graded levels of added B (0, 1, 2, 5, 10, 25, 50 mg B/kg of diet) from sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B) that was fed for 22 days. Three birds/cage were euthanized on d 22 to determine tibia bone breaking strength (BBS) and mineral composition. No effects ($P > 0.05$) of increasing B supplementation were observed for BW, ADG, ADFI, FCR, or ATTR of N, energy, or P. Calcium ATTR tended to decrease linearly ($P = 0.09$) with increasing dietary B levels. However, although not significant, tibia BBS numerically increased when B was supplemented at levels ≥ 5 ppm B. Additionally, there was a tendency for a linear increase in tibia ash percentage ($P = 0.09$) with increasing B supplementation and, as B supplementation increased, tibia Mg content had a tendency for a quadratic increase ($P = 0.08$). Furthermore, birds fed diets supplemented with B had a greater tibia Mg concentration ($P < 0.01$) compared to birds fed the control diet. In conclusion, increasing dietary B supplementation to a semi-purified diet resulted in no adverse effects. The inverse effects of B supplementation on selected mineral ATTR observed while bone measures improved suggest that B supplementation may alter the efficiency of Ca and Mg utilization. However, further research is needed to better understand the relationship between B, Ca, and Mg.

Keywords: Boron, broiler, chick, mineral

8.2 Introduction

Boron (B) was identified as an essential nutrient for plants in the early 1900s, establishing that it was indispensable for the life and growth of plants (Sommer and Lipman, 1926). However, B is a rather novel concept to higher-order organisms (humans and animals) nutrition, thus the importance of B as a nutrient has not clearly been established and has only been identified as a "probably essential" nutrient (Institute of Medicine, 2001). Despite the lack of understanding of B as an essential nutrient for humans and animals, there have been numerous studies conducted over the last several decades that show potential for B to play a vital role in animal nutrition (Nielsen, 1996; Nielsen, 2017). For example, dietary B supplementation (3 ppm B as boric acid) to day-old cockerels ameliorated signs of vitamin-D deficiency (Hunt and Nielsen, 1981). Furthermore, Hunt (1989) later reported that dietary B supplementation in chicks resulted in effects observed in relation to calcium (Ca), magnesium (Mg), and vitamin-D metabolism. Cockerels that were fed diets supplemented with 5 ppm B for 21 days had a heavier BW and increased tibia load stress compared to the control birds (Rossi et al., 1993). However, a subsequent study that fed diets that were supplemented with 300 ppm B as boric acid to day-old chicks observed a reduction in final BW but still observed an increase in tibia ash percentage (Rossi et al., 1993). Ash percentage of the bone is representative of the inorganic residue remaining following the vaporization and combustion of organic materials. Thus, bone ash percentage is primarily representative of Ca and phosphorus (P) as they make up the majority of the minerals found in the body (Crenshaw, 2001). Furthermore, Pradhan et al. (2021) reported an increase in femur ash, Ca, and P content in broiler chicks that were fed a diet supplemented with B (0, 25, 50, 75, and 100 ppm B as boric acid). Moreover, the same lab also reported that B

supplemented birds had an increased retention of Ca and P that was accompanied with a reduction in the retention of Mn and Fe (Pradhan et al., 2020). These reports all agree with an earlier hypothesis that B influences bone development by enhancing the macro-mineral content (Ca, P, and Mg) of the bone which may be a function of B interacting with the metabolism of Ca, P, and Mg (Hunt et al., 1994). Thus, the objective of this experiment was to further evaluate the relationship that dietary B supplementation has on macro-mineral metabolism (Ca, P, and Mg), bone quality characteristics, and growth performance in broiler chickens.

8.3 Experimental procedures

This experiment was conducted at the University of Kentucky under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

8.3.1 *Animals, housing, and management*

A total of 500 day-old male broiler chicks (Cobb by-product breeder chicks) were obtained locally from a commercial hatchery (d 0 of the study) out of which 448 birds were selected and housed in metal battery cages with grid floors (0.61 × 0.51 × 0.36 m) in an environmentally controlled room. All birds had free access to feed and water through metal gutter feeders and waterers external to the cage throughout the experimental period.

The lighting was provided by 60W incandescent bulbs and fluorescent lights and was controlled by a timer during the entire study. The lighting program consisted of 22 hours of light and 2 hours of darkness. During the study, the room temperature was

maintained at 30℃ for the first week and gradually decreased to 25℃ by the end of the experimental period. All birds had unrestricted access to feed and water throughout the experiment.

8.3.2 *Experimental design and diets*

The experiment employed a randomized complete block design with 7 dietary treatments and 8 replicates was used, totaling 56 experimental units, in which each battery cage represented an experimental unit consisting of 8 and 5 birds from d 0 to 11 and from d 11 to 22, respectively. The initial chick bodyweight (BW) was used as the blocking factor.

The diets were formulated to meet or exceed the nutrient requirements of broiler chickens according to the NRC (1994). Semi-purified diets based on ground corn, casein, corn starch, and dextrose containing graded levels of added B (0, 1, 2, 5, 10, 25, 50 mg B/kg of diet) from sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc. Boron, CA) were fed for the 22-d experimental period (Table 8.1).

To minimize differences in non-treatment components of the diets, a large amount of the control diet was firstly mixed. Then, an aliquot of that control diet was mixed with B to create the high inclusion level (i.e., 50 ppm) diet. Following the creation of the high-level diet, the high-level B diet was blended with the control diet in appropriate portions to create the intermediate levels of B (i.e., 1, 2, 5, 10, 25 ppm). The basal diet contained 3g/kg of titanium dioxide as an index marker for energy and nutrient retention

calculations following excreta collection. Analyzed composition of the experimental basal diet is presented in Table 8.2.

Table 8.1 Ingredient composition of the experimental diet (as-fed basis).

Items	Basal diet
Ingredient, g/kg	
Corn starch	284.6
Corn	280.0
Casein	190.0
Dextrose	120.0
Corn oil	30.0
Dicalcium phosphate	17.5
Limestone	12.5
Solkafloc[1]	30.0
Salt (NaCl)	5.0
Copper sulfate, $CuSO_4$ $5H_2O$	0.030
Zinc oxide (ZnO)	0.1
Manganous oxide, MnO	0.1
Calcium iodate, $Ca(IO_3)_2$	0.0012
Sodium selenite, Na_2SeO_3	0.0006
Potassium chloride	5.0
DL-methionine	3.0
L-arginine	5.3
Choline chloride, 60%	2.6
Vitamin premix[2]	1.3
Sand	10.0
Santoquin[3]	0.2
Titanium dioxide	3.0
Total	1,000

[1]Purified cellulose, International Fiber Corp., North Tonawanda, NY.
[2]Supplied the following per kilogram of diet: 29,252 IU of vitamin A; 7,318 IU of vitamin D3; 183.7 IU of vitamin E; 21.7 IU of vitamin K; 0.08 mg of vitamin B12; 65.2 mg of pantothenic acid; 0.72 mg of biotin; 0.52 mg of folic acid; 129.6 mg of niacin; 13.0 mg of vitamin B6; 3.58 mg of thiamin and 22.9 mg of riboflavin.
[3]Santoquin (Novus International Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diets.

Table 8.2 Calculated and analyzed nutrient composition of the experimental basal diet (as-fed basis).

Items	Basal diet
Calculated nutrients and energy, g/kg	
Crude protein	191.5
SID lysine[1]	10.4
SID methionine[1]	5.28
Metabolizable energy, Kcal/kg	3,402.5
Ca	10.0
Total P	5.66
STTD P[2]	4.50
Mg	0.70
Analyzed nutrients and energy, g/kg	
Dry matter	899.1
Gross energy, kcal/kg	3,960.86
N	32.8
Ca	9.80
P	6.50
Mg	0.30

[1]Calculated composition of lysine and methionine are presented on a standardized ileal digestible (SID) basis.
[2]Calculated composition of phosphorus is presented on a standardized total tract digestible (STTD) basis.

8.3.3 *Measurements and chemical analysis*

On d 11 and d 22, feed intake and average daily gain (ADG) were determined (performance: d 0-11, d 11-21, and d 0 to 21) by weighing individual birds and feeders. Furthermore, on d 11 and d 22, 3 birds per cage were selected based on bodyweight (heaviest, lightest, and median bird in the cage) and sacrificed for tibia mineral determination. Birds were humanely euthanized by CO_2 asphyxiation for harvesting of tibias. Tibias were harvested through manual displacement of the condylus and malleolus joints, cutting the muscle at the connection with femur, and pressing the tibia towards that incision. Harvested tibias were placed in labeled plastic bags, frozen at -20ºC and later thawed under refrigeration for 48 hours prior to bone measurements.

Once the frozen tibias were thawed, the surrounding soft tissues, flesh, and bone cap were removed manually. Bone breaking strength was subsequently measured on tibia samples using an Instron Materials tester (model 4301, Instron Corp., Canton, MA). Breaking strength was determined by obtaining the peak amount of force, before fracture, applied by a wedge mounted on a pressure-sensitive compression cell at a loading rate of 40 mm/min at the center of the fresh bone when placed horizontally on two supports 3.2 cm apart. Before ash content determination, the bones were first dried at 105°C for 24 hours, after which they were weighed. The lipid content of the bones was then determined via lipid extraction with petroleum ether for 3 extraction periods that lasted 72 h. Bones were completely soaked in a glass jar containing petroleum ether. The ether was drained and replaced with fresh ether every 24 hours until no change in the color of the ether after 24 h was observed. After the final extraction, the bones were dried under a vacuum hood at room temperature for 6 h before further drying at 105°C to remove all

remaining moisture. After drying, the bones were weighed individually for lipid content loss determination and then placed in a porcelain crucible for ashing in a muffle furnace overnight at 600 ℃ (method 942.05; AOAC, 2003). The bone weight was taken again following the ashing procedures to determine the percentage of the remaining bone (i.e., ash) relative to the dry weight of the bones before ashing. Following the ash determination, tibia ash contents were acid-digested (HCl) into a solution and diluted to 250 mL for mineral analysis.

Excreta samples from each cage were collected on day 20, 21, and 22, and the contents pooled together and weighed before drying at 55 ℃ in a forced-air oven for 5 days. Once dried, the excreta samples were ground using a Wiley Mill Laboratory Standard (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA) fitted with a 1 mm screen and then stored in airtight plastic bags before being analyzed for titanium, N, dry matter (DM), gross energy, Ca, P, and Mg.

All diet and excreta samples were analyzed in duplicates. Dry matter content of the diets and excreta samples were determined by placing around 2-3 g of ground sample into a gravity convection drying oven (Precision Scientific Co., Chicago, IL) at 107°C for approximately 24 hours and weighing the sample again to observe the moisture content lost (AOAC International, 2006). The gross energy (GE) of the diets and excreta samples was analyzed using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr Instruments, Moline, IL, USA) with benzoic acid as a calibration standard.

The titanium, N, Ca, P, and Mg contents of the diets and excreta samples were analyzed at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Titanium determination was performed under

methods listed by Myers et al. (2004). Nitrogen contents were determined by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC International, 2006; method 990.03), with EDTA as the internal standard. Calcium, P, and Mg contents were determined by inductively coupled plasma - optical emission spectroscopy (ICP-OES) under methods described by AOAC (method 985.01; AOAC, 2006) and reported in Table 8.2.

8.3.4 *Calculations and statistical analysis*

Apparent total tract retention (ATTR) of DM, GE, N, Ca, P, and Mg were calculated using the following equation:

$$ATTR\ (\%) = 100 - \left[100 \times \left(\frac{T_i}{T_o}\right) \times \left(\frac{N_o}{N_i}\right)\right]$$

where T_i and T_o are the titanium concentration (%) in the diet or excreta, respectively; and N_O and N_I are the concentrations (%) of nutrient or energy in excreta and the diet, respectively (Kong and Adeola, 2014).

Prior to the final statistical analysis, all data were subjected to an outlier analysis. Outlier analysis involved identifying potential outliers that were 3 standard deviations from the mean of the response measure, as well as reviewing experimental notes, before a final decision was made whether to exclude that value.

All data were subjected to ANOVA using the GLM procedure of SAS (SAS 9.4, Cary, NC). The cage served as the experimental unit and the statistical model included the effects of treatment and replicate. The statistical model used was:

$Y_{ij} = \mu + Boron_i + Replicate_j + e_{ij}$, where

Y = response variable (growth performance and apparent total tract retention)

μ = a constant common to all observations

$boron_i$ = supplemental boron level

$Replicate_j$ = cage replicate

e_{ij} = error term of the model

For tibia measures, day 22 BW was used as a covariate. The statistical model is listed below:

$Y_{ijk} = \mu + Boron_i + Replicate_j + BW_k + e_{ijk}$, where

Y = response variable (tibia composition)

μ = a constant common to all observations

$boron_i$ = supplemental boron level

$Replicate_j$ = cage replicate

BW_k = day 22 bodyweight (BW)

e_{ij} = error term of the model

Orthogonal polynomial contrasts were performed to evaluate the linear and quadratic effects of increasing supplemental B levels. Linear and quadratic coefficients for unequal spacing of treatments were obtained using the PROC IML function in SAS. Results are reported as least squares means by using the LSmeans function in SAS. The α

level for determination of statistical significance was set at 0.05, with ≤ 0.10 used to declare a tendency for significance.

8.4 Results

There were no effects regarding supplemental B on BW, ADG, and ADFI (Table 8.3). Birds that received 1 ppm B had the greatest numerical BW, ADG, and ADFI during the 22-day experiment. There was a linear increase observed for FCR during d 0-11 with regards to increasing supplemental B ($P = 0.02$), but no other effects observed thereafter.

Apparent total tract retention of N, Ca, P, Mg, GE, and AME are presented in Table 8.4. There was a linear tendency for Ca ATTR to decrease with increasing supplemental B ($P < 0.09$). Although not significant, P ATTR was lower for birds fed diets supplemented with dietary B. Magnesium ATTR for all treatments were negative which is likely a result of Mg contents in both the diet and excreta being very low. Furthermore, there were no other effects observed for N, Mg GE, and AME, with increasing dietary B supplementation.

Dietary B supplementation did not affect tibia BBS (Table 8.5), however, there were numerical increases in BBS when B levels ≥ 5 ppm B. There was also a linear tendency observed for tibia ash content to increase with increasing dietary B supplementation ($P = 0.10$). Furthermore, a quadratic tendency for tibia Mg content to increase with increasing dietary B supplementation was observed ($P = 0.08$). Moreover, a single degree of freedom comparison between birds fed diets supplemented with B (1, 2, 5, 10, 25, and 50 ppm B) compared to control birds (0 ppm B) resulted in B

supplemented birds having a greater tibia Mg content ($P < 0.01$) compared to the birds receiving the control diet.

Table 8.3 Effects of dietary boron (B) supplementation on growth performance in broiler chicks[1].

	Supplemental B, ppm								*P*-value[3]	
Items	0	1	2	5	10	25	50	SEM	L	Q
BW, g										
d 0	42.2	42.2	42.4	42.2	42.2	42.0	42.1	0.09	-	-
d 11	231.7	244.1	233.3	237.1	230.8	239.1	233.4	4.71	-	-
d 22[2]	704.0	725.2	701.5	712.8	700.1	714.9	722.0	15.79	-	-
ADG, g										
d 0-11	17.2	18.4	17.4	17.7	17.1	17.9	17.3	0.44	-	-
d 11-22[2]	42.8	43.7	42.4	43.2	42.8	43.3	44.3	1.20	-	-
d 0 -22[2]	30.1	31.0	30.0	30.5	29.9	30.6	30.9	0.72	-	-
ADFI, g									-	
d 0-11	23.6	25.9	24.5	24.8	24.7	25.2	25.4	0.49	-	-
d 11-22[2]	68.4	69.7	67.9	69.1	68.2	69.2	70.3	1.56	-	-
d 0 -22[2]	41.0	42.8	41.2	41.9	41.4	42.1	42.7	0.77	-	-
FCR										
d 0-11	1.38	1.41	1.42	1.40	1.44	1.41	1.49	0.03	0.02	-
d 11-22[2]	1.60	1.60	1.61	1.60	1.60	1.60	1.59	0.02	-	-
d 0 -22[2]	1.37	1.38	1.38	1.38	1.39	1.38	1.38	0.01	-	-

[1]Total of 448 male broiler chicks were assigned to dietary treatments on d 0. Treatment means represent 8 cages consisting of 8 birds/cage. Bodyweight = BW; average daily gain = ADG; average daily feed intake = ADFI; feed conversion ratio = FCR.
[2]On d 11, 3 birds/cage were sacrificed resulting in 5 birds/cage following d 11.
[3]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 8.4 Effects of dietary boron (B) supplementation on apparent total tract retention (ATTR) of energy and nutrients in broiler chicks[1].

	Supplemental B, ppm								*P*-value[2]	
Items	0	1	2	5	10	25	50	SEM	L	Q
ATTR, %										
Nitrogen	66.18	67.83	66.99	66.28	67.26	64.77	66.34	0.85	-	-
Calcium	51.46	50.19	48.05	46.06	46.81	46.25	44.25	2.70	0.09	-
Phosphorus	58.76	56.58	55.69	54.87	55.15	54.72	53.67	1.95	-	-
Magnesium	-20.31	-12.41	-30.10	-26.52	-13.68	-21.57	-23.71	5.53	-	-
Energy	79.14	79.43	79.54	79.81	79.51	78.91	78.98	0.35	-	-
AME, kcal/kg	3,486.52	3,499.24	3,504.15	3,516.29	3,502.98	3,476.39	3,479.60	15.43	-	-

[1]Treatment means represent 8 cages of 5 birds/cage.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Table 8.5 Effects of dietary boron (B) supplementation on tibia bone breaking strength (BBS) and mineral content at d 22.

	Supplemental B, ppm								*P*-value[2]	
Items	0	1	2	5	10	25	50	SEM	L	Q
BW, g										
d 0	42.12	42.15	42.16	42.10	42.33	41.74	42.16	0.16	-	-
d 11	237.52	243.84	232.14	236.41	232.10	233.83	229.63	4.96	-	-
d 22	703.75	723.21	707.75	705.13	690.00	715.63	713.17	17.17	-	-
BBS, kg	17.85	17.30	17.62	18.28	18.12	18.46	17.93	0.54	-	-
Tibia weight, g										
Fresh	6.95	6.95	6.98	6.93	6.99	6.94	6.90	0.11	-	-
Dry fat-free	2.21	2.21	2.24	2.23	2.24	2.23	2.20	0.03	-	-
Tibia mineral content, %										
Ash	44.32	44.52	44.15	44.58	44.62	44.82	44.86	0.29	0.10	-
Ca	16.76	17.17	17.00	16.76	16.69	17.11	17.16	0.22	-	-
P	8.31	8.67	8.07	8.61	8.50	8.53	8.25	0.19	-	-
Mg[3]	0.21	0.22	0.22	0.22	0.22	0.23	0.22	0.001	-	0.08

[1]Treatment means are represented by 8 cages of 3 birds/cage. Bodyweight at d 22 was used as a covariate in the final statistical model as birds were selected for sacrifice based on bodyweight (BW) at d 22 (heavy, light, and median bird per cage).
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[3]Preplanned single degree of freedom comparison between B supplementation and no B supplementation ($P = 0.003$).

8.5 Discussion

In the current study, a linear increase in FCR was observed for d 0-11 with increasing B supplementation. However, this observation was absent during d 11-22 and d 0-22. In addition, there were no other affects observed for any growth performance measures with increasing supplemental B. Thus, although there was a linear increase in FCR at d 0-11, this observation should be considered no more than an artifact.

Numerous studies have reported conflicting findings regarding dietary B supplementation to broiler chicks. In some instances, dietary B supplementation yielded greater final BW and increased FCR whereas others, as this one, have yielded either no effects or reduced final BW and decreased feed intake. For example, Elliot and Edwards (1992) reported no effects observed on BW when B was supplemented at levels of 0, 20, 40, and 80 ppm B (boric acid) to the diets fed to cockerel chicks for 16 days. The same authors also conducted a subsequent study that fed diets supplemented with lower supplemental levels of dietary B (0, 5, 10, and 20 ppm B as boric acid) to cockerel chicks for 16 days also found no effects on BW. Conversely, Rossi et al. (1993) reported that male birds fed a corn-soybean meal basal diet (9.4 ppm B) supplemented with 5 ppm B (as boric acid; total B = ~14.4 ppm) resulted in a 10% heavier BW at 21 days compared to male birds fed only the basal diet that was not supplemented B.

Like the report by Elliot and Edwards (1992), the current study found no effects on growth performance with increasing dietary B supplementation although the current study supplemented B in the form of sodium borate rather than boric acid. Furthermore, the current study lasted for 22 days whereas Elliot and Edwards (1992) experiments

lasted only 16 days. The length of B supplementation likely impacted the magnitude of response as Rossi et al. (1993) reported differences in final BW by the end of 21 days, but it is worth noting that the effects observed in final BW were only noticeable in the faster-growing (male) birds compared to female birds that grew slower. Interestingly, the study herein utilized cockerel chicks but found no response in final BW which may suggest that the form of supplemental B could impact the response due to the current study supplementing sodium borate versus boric acid.

The lack of response observed for growth performance in the current study may be associated with either the form of B that was supplemented or the amount of B present in the basal diet. Rossi et al. (1993) reported a basal diet B concentration of 9.4 ppm B. In the present study, the basal diet was composed of semi-purified ingredients attempting to keep B concentrations low. However, in the present study, B analysis of the diets yielded results with great variation and thus, not reported. Furthermore, Rossi et al. (1993) supplemented boric acid rather than sodium borate which was the form of B supplemented for the current experiment.

In the present study, there was a linear tendency for Ca ATTR to decrease with increasing dietary B supplementation. Furthermore, P ATTR was numerically lower for birds fed diets that were supplemented with increasing levels of B. These data disagree with those of Pradhan et al. (2020), where authors reported that broiler chicks supplemented with B (25, 50, 75, and 100 ppm B as boric acid) resulted in increases observed in Ca and P retention. However, in the current study, excreta were collected on d 20, 21, and 22 whereas Pradhan et al. (2020) collected excreta on d 36 to 40. The differences observed between studies may be due to the differences in Ca and P

metabolism of an older versus younger bird. Moreover, the study herein fed a semi-purified diet compared to a practical corn-soybean meal diet utilized by Pradhan et al. (2020). Additionally, while B supplementation lowered the ATTR of Ca and P in this study, bone ash was increased which reveals a disconnect in the results as bone would be the principal site of Ca and P deposition.

Hunt et al. (1994) suggested that B influences bone development by enhancing the macro-mineral content of the bone. Work that supplemented B (0, 30, and 60 ppm as boric acid) to the diets that were fed to broiler chicks for 42 days resulted in an increase in tibia ash percentage (Bozkurt et al., 2012). More recent work that also supplemented B (0, 25, 50, 75, and 100 ppm B) as boric acid to the diets of broiler chicks resulted in an increase in femur ash percentage (Pradhan et al., 2021). Similarly, in the current study birds receiving diets supplemented with increasing levels of dietary B also demonstrated a linear tendency for tibia ash percentage to increase. However, Hunt et al. (1994) did not measure ATTR of Ca and P. Conversely, Bozkurt et al. (2012) did measure Ca and P excretion; and reported that Ca and P content in the excreta were lower for B-supplemented birds. Similarly, a continuation of the work by Pradhan et al. (2021), reported an increase in Ca and P retention with increasing B levels (Pradhan et al., 2020). Moreover, both Bozkurt et al. (2012) and Pradhan et al. (2021) reported that Ca and P content of the bone was also increased along with the ash percentage of bone. In the present study, despite the increase in tibia ash, Ca and P content of the tibia was not affected. The differences observed in Ca and P content in bone may be due to the age of bird, as an older bird has had a longer time to accumulate and deposit minerals into bone compared to a younger bird. In the present study, birds were slaughtered at d 22

compared to an older slaughter age of 42 d used in the other two reports. Moreover, an older bird is more likely to have a greater efficiency to metabolize and deposit minerals into bone compared to a younger bird that is undergoing rapid growth and development.

In the current study, tibia Mg content was greater for birds receiving diets supplemented with B compared to birds that received no supplemental B. There are few reports that evaluate bone Mg content in poultry species supplemented with dietary B. Nonetheless, work that fed diets supplemented with B (0, 5, 10, 50, 100, 200, and 400 ppm B as boric acid) to laying hens resulted in an increase in serum Mg concentration at 24 and 26 weeks of production in birds supplemented with B at levels ≥ 10 ppm B.

Lastly, Rossi et al (1993) reported that birds fed a diet that was supplemented with 5 ppm B (~ 14.4 ppm total B) resulted in an 23 % increase in tibia breaking load compared to birds fed the basal diet containing only 9.4 ppm B. Similarly, in other poultry species (Wilson and Ruszler, 1998), rats (Chapin et al., 1997), and pigs (Armstrong et al., 2000; Armstrong and Spears, 2001; Armstrong et al., 2002), B supplementation has demonstrated a potential to increase bone mechanical properties like bone breaking strength, ultimate sheer force, bending moment, etc. However, in the current study, there were only numerical improvements in BBS that were found in birds supplemented with B at levels ≥ 5 ppm B. Although there were only numerical increases observed for BBS in the study herein, these findings agree with findings by Armstrong et al. (2000) where authors reported that barrows supplemented with 5 ppm B as sodium borate had an increased bending moment of the femur. Nevertheless, it should be noted that the vast differences between species, type of bone, and source and level of

supplemental B makes it difficult to consider and speculate on the differences in responses observed between studies.

8.6 Conclusion

In summation, feeding semi-purified diets that were supplemented with increasing levels of dietary B in the form of sodium borate resulted in no adverse effects observed on broiler chick health. Furthermore, supplementing B at levels upwards of 50 ppm B did not suppress growth, nor B affected growth performance overall. The lack of consistency observed between findings in the current study and previous reported literature suggests that source of dietary B plays an important role on the magnitude of response for measures. The majority of previous literature regarding dietary B supplementation to poultry species has used boric acid whereas the present study used sodium borate. Furthermore, these differences between studies suggest that there is a different bioavailability between boric acid and sodium borate. Thus, further research regarding the source of supplemental B is warranted to evaluate bioavailability and utilization of either boric acid or sodium borate and their potential differences in responses regarding growth performance, nutrient utilization, and bone composition.

CHAPTER 9. General discussion

Improving the productivity of sows is a primary approach to increasing pork production because sow productivity ultimately determines the number of available pigs for finishing and slaughter. The swine industry continues to improve genetic selection, nutrition, and management in order to fulfill the continuous increase in consumer's demand for pork. Yet, a conflict exists between the genetic selections for greater productivity and the target of a prolonged productive lifetime of the sow. In the US, a continuous high culling rate (40 to 50%) and increasing sow death rate (6.5 to 10.0%) is common in commercial breeding herds during the past 15 years (PigChamp, 2022). Currently, the short productive life of a sow is considered harmful to swine production because of more unproductive days, less acquired immunity to herd disease, and greater female/gilt replacement cost (Lucia et al., 2000b; Hoge and Bates, 2011), which all would be considered economic losses. The length of the productive life of sows is influenced by many factors, including genetics, management, housing, disease, and nutrition (Farmer, 2015). Modern sows are producing larger and heavier litters, and consequently, sows must mobilize greater amounts of body nutrient reserves to sustain the increase in fetal tissue development during gestation and the increase needed in milk yield to support a larger litter during lactation. Severe nutrient mobilization of body reserves to meet the demand of increased production ultimately impacts sow longevity, in turn, limiting the economic potential due to the cost associated with replacement females. Thus, a nutritional approach to increase sow longevity by possibly influencing mineral metabolism may elicit greater tissue mineral reserves and as a result, reduce the rate of tissue mineral depletion in older parity sows.

It has been demonstrated in a variety of different species that B supplementation has a positive influence on mineral metabolism with the most noticeable relationships regarding Ca, P, and Mg metabolism (Nielsen et al., 1987; Brown et al., 1989; Hegsted et al., 1991; Green and Weeth, 1997; Sharma et al., 2020; Sharma et al., 2022). In Chapter 7, growing barrows fed diets supplemented with 5 and 25 ppm B had an improved absolute absorption of Mg which was also accompanied by an increase in Mg digestibility. Moreso, results from Chapter 5 indicated that sows supplemented with 5 ppm B for 1 reproductive cycle (breeding to weaning) had greater kidney P accompanied by a tendency to have greater kidney Ca concentrations compared to sows supplemented with either no B or 25 ppm B. However, the different observations in tissue mineral concentration between piglets at birth and weaning from sows fed diets supplemented with 5 and 25 ppm B can likely be attributed to the differences in physiological state, as these are two critical time periods during a pig's life. Ultimately, there are differences in mineral metabolism and homeostasis that impact mineral deposition in certain tissues of a newborn piglet and that of a piglet at weaning. Even more so, the general differences in nutrient sourcing by newborn piglets versus weaning pigs. For example, newborn piglets received all their nutrient supply through placental transfer whereas weaning piglets were receiving nutrient supply through maternal milk supply. Thus, further research evaluating supplemental B on placental and mammary nutrient transfer may provide better understanding of the differences found in the current work.

The effects of dietary B supplementation on grower and market pig tissue, bone, and carcass characteristics were limited (Chapter 6). Nonetheless, grower pigs had a quadratic tendency for liver Mg concentration to increase in pigs supplemented with 5

ppm B which, subsequently, was numerically present in market pigs. The effects of B supplementation in the studies herein appear to be more noticeable in the kidney composition rather than the liver. Between the swine models in these studies, B supplementation had minimal impact on tissue mineral concentration. However, there were several instances herein where B supplementation resulted in effects observed for Mg concentration in selective tissues, which accompanied by the improvements in Mg digestibility and absorption observed in Chapter 7, suggests a relationship between B supplementation and Mg status.

There have been reports where B supplementation has positively impacted bone mechanical properties in pigs (Armstrong and Spears, 2001; Armstrong et al., 2002) as well as bone ash content in poultry (Qin and Klandorf, 1991; Elliot and Edwards, 1992; Rossi et al., 1993; Wilson and Ruszler, 1997). In Chapter 8 herein, B was supplemented to a semi-purified diet to evaluate the effects on tibia bone-breaking strength (BBS) and composition. Although not significant, broiler chicks that received supplemental B at levels equal to or greater than 5 ppm B had numerically greater tibia bone-breaking strength compared to broiler chicks fed a diet without B supplementation. Furthermore, there was a linear tendency for tibia ash content to increase with increasing supplemental B. These findings were also accompanied by a quadratic tendency for tibia Mg concentration to increase with increasing supplemental B. However, in sows (Chapter 5), the 3rd and 4th metacarpal bone-breaking strength was linearly decreased with increasing B supplementation. Moreover, femur bone breaking strength in piglets at birth and weaning from sows fed diets supplemented with B were found to be conflicting as a numerical increase in the femur BBS of piglets at birth was observed yet, weaning pigs

had a numerical decrease in femur BBS with maternal B supplementation. Boron supplementation resulted in no effects observed in the 3rd and 4th metacarpal BBS for grower pigs (Chapter 6). Nonetheless, in market pigs, a quadratic decrease in 3rd and 4th metacarpal BBS was observed with increasing supplemental B. In both instances, grower and market pigs supplemented with B had a lower BBS when compared to grower and market pigs supplemented without B.

Overall, the effects of B supplementation on bone composition were evaluated in a multitude of pigs at different physiological production stages. Thus, the inconsistency in observations regarding B supplementation may partially be contributed to these physiological differences in the model evaluated. For example, a multiparous sow is not experiencing the same bone growth and development as a weaning or growing pig is. Furthermore, the differences in production phase of the animal models used herein likely influenced other contributing aspects to bone mineral composition and deposition such as basal nutrient requirement and metabolism efficiency.

It has been hypothesized that B may reduce the stress that pancreatic β-cells experience during the overproduction of insulin (Hunt, 1996). This is of great interest regarding the sow as there have been reports suggesting that sows become less tolerant to glucose and have diabetic tendencies during pregnancy (George et al., 1978; Bouillon-Hausman et al., 1986; Scheafer et al, 1991).

In the present work, serum insulin and glucose concentrations were evaluated during a fasting and postprandial state. In Appendix 1, grower pigs were used to evaluate the change in serum insulin and glucose concentrations following access to feed to determine if 60 or 90 minutes postprandial differed in response. In this study, the

response in serum insulin and glucose concentrations at 60 minutes postprandial were more similar with previous literature. Thus, in Chapters 3 and 4, postprandial sampling time was targeted for around 50 minutes after pigs and sows, respectively had access to feed. In Chapter 3, on d 20, pigs supplemented with B exhibited numerically lower fasting serum insulin concentrations compared to pigs not receiving supplemental B. This was more noticeable in a postprandial state where a linear decrease in serum insulin concentration was observed with increasing supplemental B. Conversely, the findings observed on d 20 were not consistent with findings observed on d 41. Nonetheless, on d 41, pigs receiving B-supplemented diets exhibited numerically lower fasting serum insulin/glucose ratios which were also observed on d 20. Evock-Clover et al. (1993) suggest that the insulin/glucose ratio can be used as a crude index for tissue sensitivity to insulin. Moreover, the lower fasting insulin/glucose ratio observed at fasting may be illustrative of a lower maintenance level of insulin needed to maintain basal glucose levels in a fasting period.

Insulin plays a vital role in metabolism due to its role in decreasing lipolysis and lipogenesis (Hadley, 1988). Insulin also reduces the activity of carnitine palmitoyltransferase I and therefore may reduce the oxidation of non-esterified fatty acids (NEFA) to produce energy (Gamble and Cook, 1985). Maintenance of adequate, long-term intracellular stores of insulin relies on transcriptional and translational regulation of insulin biosynthesis (Poitout et al., 2006). Moreover, glucose is considered as the main regulator responsible for the expression of transcriptional factors of the insulin gene in the liver and other non-beta cells (Andrali et all., 2008). In addition, glucose is the major nutrient that regulates all processes of insulin gene expression including transcription,

pre-RNA splicing, and mRNA stability (Poitout et al., 2006). However, in response to a meal (short-term response), the regulation of insulin secretion is mainly dependent upon exocytosis (Poitout et al., 2006). Insulin is secreted mainly from the islet beta cells of the pancreas and is a vital component in the maintenance of energy homeostasis (Poitout et al., 2006).

The process of insulin secretion in order to maintain blood glucose levels is a very tightly regulated process (Poitout et al., 2006). Although glucose is the main physiological regulator of the insulin gene, over-exposure of glucose on beta cells for prolonged periods of time becomes toxic and is referred to as glucotoxicity (Robertson et al., 1992). Furthermore, pancreatic beta cells are also negatively affected by high levels of fatty acids through a process described as lipotoxicity (Unger, 1995). Both glucotoxicity and lipotoxicity play a major role in nutrient-induced beta cell dysfunction and deterioration, ultimately contributing to insulin insensitivity.

Insulin insensitivity in sows is often defined by a gradual increase in circulating insulin levels rather than a decline in insulin concentration and thus resulting in a decrease in insulin efficiency (Anderson, 1992). As insulin efficiency declines the body responds by producing more insulin to control blood glucose concentrations.

In sows, late gestation has been considered a critical time point in which sows become less sensitive to insulin (Pére et al., 2000). Further, insulin insensitivity becomes more pronounced until parturition. In Chapter 4, late gestation serum insulin and glucose concentrations during a fasting and postprandial state were evaluated in both primiparous and multiparous sows receiving diets with increasing supplemental B. In this study, fasting serum insulin concentration was quadratically decreased with increasing

supplemental B. Conversely, there were no effects on fasting serum glucose concentrations, which as a result, led to a quadratic decrease observed in fasting serum insulin/glucose ratio. Differently, postprandial insulin concentration was numerically increased in sows fed diets supplemented with B resulting in a linear increase in postprandial insulin/glucose ratio. However, sows fed diets supplemented with 5 ppm B had a numerically lower insulin/glucose ratio compared to sows either fed diets without supplemental B or supplemented with 25 ppm B. Earlier work demonstrated that serum insulin levels in sows elicit a peak response around 60 minutes after consumption of a meal on d 109 of pregnancy (Kemp et al., 1995). In comparison, data from Pére et al. (2000), indicated that plasma insulin concentration seems to peak at around 40 minutes after receiving a meal where it then slowly declines thereafter. More recently, the same lab demonstrated plasma insulin peaks at approximately 30 to 60 minutes after a meal test (Pére et al., 2007). In addition, the same author reported that sow plasma glucose peaks around 45 minutes after a meal test. Earlier work performed by Kemp et al. (1996), showed that peak glucose concentration was at 36 minutes while the end of peak glucose response was found to be around 58 minutes, after an oral glucose test performed during pregnancy (d 104 ± 4). In Chapter 4, the postprandial sample was collected between 40- and 55-minutes following access to the feed. Thus, the timing of the postprandial blood sampling to evaluate serum insulin and glucose concentrations in sows is consistent with previous literature. Overall, sows fed diets supplemented with 5 ppm B numerically had the lowest fasting and postprandial insulin/glucose ratio compared to sows fed either diets without B supplementation or diets supplemented with 25 ppm B. Irrespective of B supplementation, primi- and multiparous sows are subject to become resistant to insulin,

which can be exacerbated by certain feeding management strategies during gestation, ultimately, posing a great risk of gestational induced diabetes. This, coupled with the known relationship between glucose metabolism and its effects on progeny warrants further investigation. Further understanding the relationship between glucose metabolism in the sow and fetal nutrient supply relative to maternal energy and substrate metabolism may help discover ways to improve piglet birth weight while maintaining larger litter size.

In summation, there were many instances where dietary B supplementation exhibited positive responses across various swine models including sows, piglets, and grower and market pigs as well as broiler chicks. However, the inconsistencies that occurred between studies, which perhaps, may partly be due to the physiological differences that existed between the animal models used in the studies indicate that further research is needed to better understand the potential benefits of B supplementation. In particular, creating a B-deficient basal diet to better understand the optimum level of B needed for biological systems should take priority. Following this, evaluating the bioavailability of both sodium borate and boric acid may illustrate differences in biological mechanisms that can further explain the inconsistencies observed in the current literature.

Further work focused on evaluating and estimating the basal maintenance as well as the optimal production range of dietary B required by both swine and poultry species will contribute and aid in understanding the essentiality of B but also provide a clearer understanding to whether B needs to be supplemented in the diet. Following this, improved analytical methods that permit easier analysis of B concentrations in feedstuffs

would greatly benefit any potential future research but also provide a baseline understanding of the B content present in common feedstuffs. This ability would influence the decision on what level of B needs to be supplemented, or perhaps not supplemented. However, current methods regarding B analysis require additional steps to avoid potential sample contamination. Due to the reactivity of B, samples can often become compromised due to environmental elements. For example, most laboratory glassware is composed of borosilicate which will cause interference in the analytical determination of B concentration in the sample. Furthermore, the B supplemental level used in the studies herein was very low, which in some instances, may result in concentrations below the lower detection limit of the measuring equipment. Thus, the combination of the above interferences caused variation between sample duplicates and for these reasons B concentrations of the diets were not reported in the studies herein. Nonetheless, the B analysis did provide confirmation to the rank of treatments and respective treatment spacing was achieved. Overall, without knowing with certainty the B concentrations of the diets that were fed to the animals in the studies herein, it is difficult to associate accurate biological responses. Without knowing the biological responses associated with B concentration, it is also difficult to calculate a potential ROI for the use of supplemental B. Thus, further research identifying and evaluating the biologically relevant dietary range of B in swine and poultry species is warranted. This information should then assist in understating potential responses which will be insightful for swine and poultry producers to calculate the economic impact and potential ROI, ultimately permitting an intuitive decision.

APPENDICES

APPENDIX 1. Pilot study – Effects of dietary boron (B) supplementation to grower pigs on growth performance, serum insulin and glucose concentration, and metacarpal composition

Objective

Previous work conducted by Kucukkurt et al. (2015) showed that dietary boron (B) supplementation (100 ppm B) in the form of sodium borate to rats resulted in an 83% and 49% decrease observed for plasma insulin and glucose respectively, compared to rats that received a low-B diet (6.4 ppm B). Thus, the objectives of this pilot study were to (1) evaluate increasing supplemental levels of dietary B on growth performance, serum insulin and glucose concentration, and metacarpal composition in growing pigs, and (2) to assess serum insulin and glucose concentrations in response to an overnight fast, and 60- or 90-minutes post prandial at 3 and 6 weeks of the study to determine the most appropriate sampling time.

Materials and methods

A total of 64 pigs (32 barrows and 32 gilts) with a mean initial bodyweight (BW) 13.55 ± 0.43 kg) were allotted to pens based on initial BW, sex, and litter of origin. Pens were randomly assigned to receive one of four dietary treatments as follows: 0, 25, 50, and 100 ppm B. All pigs were housed by sex and 8 per pen, with 2 pens per treatment (8 barrows and 8 gilts per treatment). Boron was supplemented as sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$; 11.34% B; Borax Decahydrate, U.S. Borax Inc. Boron, CA).

All experimental diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates for growing swine with respect to BW (Table A.1.1). Pigs were maintained on their respective experimental diets throughout a 42-d grower period and averaged a final BW of 45.02 ± 1.22 kg.

Table A.1.1 Formulation and calculated composition of basal diet (as-fed basis).

Items	Basal diet
Ingredient, %	
Corn	60.76
Soybean meal, 48% CP	33.40
Grease, choice white	2.60
L-Lysine•HCl	0.30
DL-Methionine	0.16
L-Threonine	0.12
Dicalcium phosphate	0.88
Limestone	1.07
Salt	0.50
Vitamin premix[1]	0.04
Trace mineral premix[2]	0.15
Santoquin[3]	0.02
Total	100.00
Calculated composition	
Metabolizable energy, kcal/kg	3,396.10
Crude protein, %	21.42
SID Lysine, %[4]	1.23
Ca, %	0.70
Total P, %	0.56
STTD P, %[5]	0.33

[1]Vitamin inclusion per kg of total diet: 9,361 IU of vitamin A; 2,342 IU of vitamin D3; 62 IU of vitamin E; 6.9 IU of vitamin K; 0.03 mg of vitamin B12; 0.23 mg of biotin; 0.17 mg of folic acid; 41.5 mg of niacin; 20.85 mg of pantothenic acid; 4.16 mg of riboflavin; 0.23 mg of biotin; 0.17 mg of folic acid; 41.5 mg of niacin; 4.16 mg of vitamin B6; and 1.15 mg thiamin.
[2]Trace mineral inclusion per kg of total diet: 50 mg Mn as manganese sulfate monohydrate; 100 mg of Fe as ferrous sulfate monohydrate; 125 mg of Zn as zinc sulfate monohydrate; 20 mg of Cu as copper sulfate; 0.35 mg of I as calcium iodate; and 0.30 mg of Se as sodium selenite.
[3]Santoquin (Novus International, Inc., St. Louis, MO) supplied 130 ppm ethoxyquin to the diets.
[4]Calculated composition of lysine is presented on a standard ileal digestible (SID) basis.
[5]Calculated composition of phosphorus is presented on a standard total tract digestible (STTD) basis.

At the end of week 3 and 6, all pigs underwent a 6 h fasting period where feeders were removed at approximately 0600 h. At 1200 h, all pigs were bled for a fasting sample.

Once the entire pen had been collected, the pigs were allowed access to feed for 30 minutes. Following the 30 minutes, the feed was removed again. Sixty minutes after the feed offering, 4 pigs/pen were bled again. Then 90 minutes after the feed offering the other 4 pigs/pen were bled. This method provided 32 blood samples at 60 and 90 minutes. After all blood samples were collected, feed was provided to all pens at ad libitum access.

At 6 weeks, the blood collection process above was repeated including the same pigs used for 60- and 90-minute samples. Venous blood samples were obtained from the jugular vein and collected using a needle holder and vacutainer tube with polymer gel (Becton, Dickson and Company, Franklin Lakes, NJ) for serum separation. Serum was obtained by centrifugation of the blood at 2500×g and 4°C for 20 minutes. Serum samples were stored at -80 °C until the chemical assays were performed. Following the blood collection, a subset of barrows (n = 12; 3 barrows/treatment) were euthanized to obtain tissue (liver, kidney, and heart) and 3rd and 4th metacarpal samples. Tissues were harvested and weighed to determine absolute and relative organ weight. The third and 4th metacarpal samples were carefully cleaned by removing any excess cartilage or connective tissue. Collected bone samples were stored at -20 °C in plastic bags until further analyzed.

Laboratory analysis

Serum glucose concentration was determined using an automated enzymatic assay using a YSI 2700 biochemical analyzer (YSI Incorporated, Yellow Springs, OH). Insulin concentration was determined using a commercial porcine insulin radioimmunoassay (RIA) kit (EDM Millipore Corporation, Billerica, MA). Standards and samples were analyzed in duplicates.

To assess bone strength, bones were brought to room temperature and subjected to breaking strength determinations using an Instron Materials tester (Model TM 1123; Instron Corp., Canton, MA, USA) at a loading rate of 40 mm/min. Breaking strength was determined by obtaining the peak amount of force, before fracture, applied by a wedge mounted on a pressure-sensitive compression cell at the center of the fresh bone when placed horizontally on two supports 3.2 cm apart. Subsequently, the metacarpals were cut in half to remove the marrow. After drying in an oven, they were wrapped in cheesecloth and extracted with fresh petroleum ether three times at 24-h intervals. They were then air-dried at room temperature under a chemical hood for 24 h, dried in an oven overnight. The percentage ash content of fat-free bone was determined by weighing the bone into a dried and preweighed porcelain crucible. Samples were then placed into a muffle furnace and heated to 600°C overnight (method 942.05; AOAC, 2003). Ash weight was recorded and the ash percent in dry, fat-free bone was determined. The ash from the metacarpal samples was then acid-digested and diluted to 250 mL.

Digested bone samples were analyzed for Ca and Mg concentration by flame atomic absorption spectrophotometry (AAnalyst 200, PerkinElmer, Waltham, MA, USA). Before Ca analysis, samples were prepared in a 0.1 % NaCl solution (help capture

free Ca ions) and ran with a Ca reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves. Calcium determination was performed using a nitrous oxide-acetylene gas mixture and a wavelength of 422.7 nm (modification of method 927.02; AOAC, 2003). Magnesium determination was performed using acetylene gas, at a wavelength of 285.21 nm (modification of method 927.02; AOAC, 2003) and ran with a Mg reference solution (1,000 ppm; Fisher Scientific, Fair Lawn, NJ) for development of the standard curves. Phosphorus was analyzed using a gravimetric method (method 968.08; AOAC, 1990), where 20 mL of digested sample was mixed with Quimociac solution, filtered, and the precipitate obtained was weighed to calculate the P concentration (Appendix X).

Statistical analysis

The experimental data were subjected to ANOVA using Generalized Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Data regarding growth performance and serum insulin and glucose were analyzed by a model that included terms for treatment, sex, replicate, and sex by treatment interaction with the individual pig serving as the experimental unit. Data regarding tissue and bone composition were analyzed by a model that included terms for only treatment. All data are presented as least squares means. Orthogonal polynomial contrasts were performed to determine linear and quadratic effects of dietary B supplementation. Coefficients used in the polynomial contrast were obtained using PROC IML procedure in SAS. Differences were considered significant when $P < 0.05$ and tendencies were detected at $P \leq 0.10$.

Results

There was no treatment by sex interactions present for any of the response measures. However, there were expected sex effects present for select response measures indicated by a superscript in the tables. There were no treatment effects present for BW and ADG (Table A.1.2.). However, it is worth noting that increasing dietary B supplementation up to 100 ppm B did not result in any detrimental effects on growth performance. Serum insulin and glucose concentration at fasting, 60- and 90-minutes following feed offering at the end of week 3 are presented in Table A.1.3. Although not significant, there were numerical decreases in 60-minute insulin concentration with regard to increasing B supplementation. Furthermore, there was a linear decrease present for 60-minute glucose concentration ($P = 0.02$), which was less pronounced at the 90-minute sampling ($P = 0.07$). Again, not significant but 60-minute insulin/glucose ratio numerically decreases with increasing B supplementation ($P = 0.18$).

Serum insulin and glucose concentrations at the end of Week 6 are presented in Table A.1.4. Unlike the previous sampling at the end of Week 3, there was a linear increase in fasting insulin concentration with increasing supplemental B ($P = 0.04$). Like Week 3, 60-minute insulin concentration decreased numerically with increasing supplemental B ($P = 0.64$). At the end of both Week 3 and Week 6, the response in insulin concentration was greater at the 60-minute sampling compared to the 90-minute sampling whereas serum glucose was greater at the 90-minute sampling. Nonetheless, it was observed that serum insulin may have a greater magnitude of response compared to serum glucose.

Organ and metacarpal composition of barrows are presented in Table A.1.5. Supplementing B to the diet of barrows did not result in any effects present on absolute and relative organ weight. However, metacarpal bone breaking strength was numerically increased in barrows receiving diets supplemented with increasing B ($P = 0.14$). Furthermore, lipid percent of the metacarpal was also numerically increased with increasing B supplementation. Lastly, Mg content of the metacarpals appeared marginally greater in barrows supplemented with 50 or 100 ppm B compared to barrows supplemented with 0 and 25 ppm B.

Table A.1.2 Effects of dietary boron (B) supplementation on individual pig bodyweight (BW) and average daily gain (ADG)[1].

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
BW, kg							
d 0	13.53	13.53	13.57	13.58	0.43	-	-
d 20	28.16	28.44	28.32	28.40	0.76	-	-
d 41[S]	44.90	45.32	44.87	44.98	1.22	-	-
ADG, kg							
d 0-20	0.73	0.75	0.74	0.74	0.02	-	-
d 20-41[S]	0.80	0.80	0.82	0.82	0.03	-	-
d 0-41[S]	0.76	0.78	0.78	0.78	0.02	-	-

[1]Means represent a total of 64 pigs, 16 pigs per treatment (8 barrows and 8 gilts per treatment).
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.

Table A.1.3 Effects of dietary boron (B) supplementation on serum insulin and glucose concentration at the end of Week 3[1].

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
Insulin, μU/mL							
Fasting	4.90	4.74	4.77	4.19	0.85	-	-
60 minutes	23.19	20.52	17.67	14.50	3.80	-	-
90 minutes[S]	12.85	8.6	16.93	9.28	1.44	-	0.06
Glucose, mmol/L							
Fasting[S]	5.68	5.68	5.72	5.55	0.19	-	-
60 minutes	6.57	6.14	6.55	5.75	0.20	0.02	-
90 minutes	6.68	6.16	6.56	6.11	0.16	0.07	-
Insulin/glucose							
Fasting	0.81	0.79	0.82	0.72	0.13	-	-
60 minutes	3.50	3.36	2.73	2.50	0.58	-	-
90 minutes[S]	1.98	1.41	2.59	1.57	0.25	-	-

[1]All pigs underwent a fasting period of ~ 6 h. Following the last blood sample, pigs were given access to feed for 30 minutes then feeders were removed. At 60- and 90- minutes after the feed offering, 4 pigs/pen were bled. Fasting means represent 16 pigs/treatment whereas 60- and 90- minute means represent 8 pigs/treatment.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.

Table A.1.4. Effects of dietary boron (B) supplementation on serum insulin and glucose concentration at the end of Week 6.

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
Insulin, μU/mL							
Fasting	3.95	4.56	4.86	5.28	0.46	0.04	-
60 minutes	25.50	19.90	20.00	21.54	4.40	-	-
90 minutes	18.46	14.37	16.74	12.28	2.80	-	-
Glucose, mmol/L							
Fasting[S]	5.11	5.12	5.20	5.36	0.12	0.10	-
60 minutes	5.74	5.39	6.02	5.84	0.27	-	-
90 minutes[S]	6.04	5.77	5.81	5.91	0.16	-	-
Insulin/glucose							
Fasting	0.77	0.91	0.93	0.98	0.09	-	-
60 minutes	4.56	3.69	3.42	3.85	0.83	-	-
90 minutes	3.10	2.43	2.88	2.06	0.48	-	-

[1]All pigs underwent a fasting period of ~ 6 h. Following the last blood sample, pigs were given access to feed for 30 minutes then feeders were removed. At 60- and 90- minutes after the feed offering, 4 pigs/pen were bled. Fasting means represent 16 pigs/treatment whereas 60- and 90- minute means represent 8 pigs/treatment.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".
[S]Sex effect present, $P \leq 0.05$.

Table A.1.5Effects of dietary boron (B) supplementation on organ size and 3rd and 4th metacarpal composition of barrows[1].

	Supplemental B, ppm					*P*-value[2]	
Items	0	25	50	100	SEM	L	Q
BW, kg	47.62	46.86	49.89	47.16	2.83	-	-
Absolute organ weight, g							
Liver	1751.73	1748.77	1705.20	1683.93	168.85	-	-
Kidney	289.77	317.33	296.97	310.37	35.14	-	-
Heart	219.43	230.93	248.13	229.27	19.62	-	-
Relative organ weight, %							
Liver	3.67	3.71	3.41	3.56	0.18	-	-
Kidney	0.61	0.67	0.60	0.65	0.04	-	-
Heart	0.46	0.49	0.50	0.48	0.02	-	-
Metacarpal composition							
Bone breaking strength, kg	75.60	72.11	85.68	81.97	4.01	-	-
Fresh weight, g	16.40	16.36	16.39	15.59	1.20	-	-
Dry fat-free weight, g	6.74	6.55	6.71	6.36	0.52	-	-
Marrow, %	9.05	8.91	8.37	8.40	0.36	-	-
Lipid, %	20.00	21.12	23.12	22.95	1.27	-	-
Ash, %	48.98	49.90	47.40	47.52	1.08	-	-
Ca, %	15.06	16.29	14.61	15.06	0.79	-	-
P, %	9.15	8.80	9.36	9.38	0.20	-	-
Mg, %	0.368	0.357	0.386	0.379	0.009	-	-

[1]Means represent a total of 12 barrows, 3 barrows per treatment.

[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B. *P*-values greater than 0.10 are replaced with "-".

Conclusion

Overall, this pilot study provided several observations that permitted further research to be conducted in part of this work. Firstly, supplementing B upwards of 100 ppm did not result in any adverse effects on growth, serum insulin and glucose concentrations, organ size, and metacarpal composition. Secondly, it appeared that the 60-minute sampling time elicited a more representative response in peak insulin concentration compared to 90 minutes whereas glucose concentration appeared to only change slightly between fasting, 60- and 90- minutes. Furthermore, increasing B supplementation resulted in marginal decreases in 60-minute serum insulin concentration at the end of Week 3 but this observation was less pronounced at the end of Week 6. Metacarpal bone breaking strength and lipid content was numerically increased with increasing B supplementation. However, the lack of statistical significance present may be a result of the small sample size. Lastly, the observations on 60-minute serum insulin concentration and previous literature led to the decision to use a 50-minute postprandial sampling time in Chapters 3 and 4.

APPENDIX 2. Excluded observations in Chapter 4

Table A.2.1 Excluded observations for sow and litter performance in Chapter 4[1].

Treatment, mg/kg B	Sow ID	No. of litters	Reason for removal
0	2470	3	Low pig numbers (< 7BA/litter)
	5224	2	Crushed > 6 pigs/litter
	3684	3	Low pig numbers (< 7BA/litter)
	7020	3	Missed breeding; only 7 pig BA
5	5204	3	Low pig numbers < 7BA; parity 5
	4110	1	Farrowed only 1 litter
	5861	1	Farrowed only 1 litter
	6870	1	Farrowed only 1 litter
25	7142	2	Low pig numbers (< 8BA/litter)
	7486	1	Farrowed only 1 litter
	5203	3	Missed breeding; 9 stillborns; parity 5

[1]Data of the entire litter was excluded because these litters were considered to be qualitatively different from other litters.

APPENDIX 3. Effects of test parity on reproductive performance and late gestation serum insulin and glucose concentration of sows.

Table A.3.1 Effects of sow test parity on sow performance[1].

	Test parity				
Items	1	2	3	SEM	*P*-value
No. Obs.	61	60	35		
Avg. parity[2]	1.28	2.28	3.03		
Gestation length, d	115.4	116.1	115.7	0.27	0.04
Lactation length, d	20.5	20.0	20.4	0.30	0.28
Sow BW, kg					
Breeding	170.23	172.80	179.50	3.63	0.13
Pre-farrow	216.01	212.44	201.54	3.94	0.01
Post-farrow	199.74	198.31	197.21	3.72	0.86
Weaning	186.51	197.39	205.59	3.90	< 0.01
BW Change, kg					
Gestation	45.78	39.64	22.04	3.07	<.0001
Lactation	-13.23	-0.92	8.38	2.73	<.0001
Lactation ADFI, kg/d	4.69	5.56	5.66	0.13	<.0001
Days to estrus, d	4.86	5.23	4.91	0.15	0.05

[1]Test parity represents the number of reproductive cycles completed while being on the experimental diets. Pre-farrow BW was recorded on d 109-112 of gestation whereas post-farrow BW was recorded within 24 h following parturition.

[2]Average parity is representative of the real parity of the sow.

Table A.3. 2 Effects of sow test parity on litter size[1].

	Test parity				
Items	1	2	3	SEM	*P*-value
No. Obs.	61	60	35		
Litter Size, No.					
Total born	14.17	14.22	14.84	0.48	0.49
Born alive	13.30	13.09	13.52	0.48	0.78
Adjusted live born	13.06	12.88	13.52	0.46	0.54
Stillborn	0.85	1.11	1.32	0.24	0.30
Mummified	0.36	0.07	0.06	0.11	0.03
Weaning	11.86	11.38	11.62	0.37	0.48
Preweaning mortality	1.20	1.50	1.89	0.25	0.09

[1]Test parity represents the number of reproductive cycles completed while being on the experimental diets.

Table A.3. 3 Effects of sow test parity on litter performance[1].

Items	Test parity			SEM	*P*-value
	1	2	3		
Litter weight, kg					
Total born	18.90	19.84	19.56	0.69	0.44
Born alive	17.94	18.45	18.27	0.69	0.78
Adjusted live born	17.83	18.48	18.27	0.60	0.60
Weaning	69.14	64.70	63.78	2.28	0.10
Gain	51.21	46.25	45.50	2.01	0.03
Adjusted litter weight, kg					
Weaning	70.38	66.94	65.23	2.19	0.14
Gain	52.55	48.46	46.96	1.86	0.03
Piglet weight, kg					
Total born	1.38	1.44	1.35	0.04	0.15
Born alive	1.40	1.45	1.38	0.04	0.26
Adjusted live born	1.39	1.45	1.38	0.04	0.20
Weaning	5.89	5.74	5.58	0.17	0.34
Gain	4.51	4.29	4.20	0.15	0.23
Adjusted piglet weight, kg					
Weaning	4.51	4.29	4.20	0.15	0.28
Gain	4.62	4.51	4.31	0.14	0.23

[1]Test parity represents the number of reproductive cycles completed while being on the experimental diets.

Table A.3. 4 Effects of sow test parity on late gestation (d 80-90) serum insulin and glucose concentration[1].

	Test parity				
Items	1	2	3	SEM	*P*-value
n	61	60	35		
Insulin, μU/mL					
Fasting	5.77	6.69	5.08	0.57	0.06
Postmeal	39.67	37.32	40.19	3.95	0.78
Difference	33.97	30.72	35.11	3.99	0.60
Glucose, mmol/L					
Fasting	3.85	3.53	3.57	0.07	<.0001
Postmeal	4.05	3.56	3.71	0.10	<.0001
Difference	0.20	0.02	0.14	0.11	0.26
Insulin/glucose					
Fasting	1.49	1.90	1.42	0.14	0.01
Postmeal	9.80	10.56	11.10	1.05	0.57

[1]Test parity represents the number of reproductive cycles completed while being on the experimental diets. Fasting is defined as an overnight fast (~ 13 h) whereas postprandial is defined as ~ 50 minutes after access to their morning meal.

APPENDIX 4. Statistical analysis of sow and litter performance from Chapter 5

In Chapter 5, two piglets from each litter were sacrificed at both birth and weaning to determine litter tissue and bone composition, ultimately affecting the litter performance data. The following Tables A.4.1 to A.4.3 present linear and quadratic effects that were intentionally removed from the chapter due to the interference caused by piglet tissue and bone collection.

Table A.4. 1 Effects of dietary boron (B) supplementation on sow bodyweight (BW) and lactation feed intake.

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
No. of sows	5	5	5			
Mean parity	3.8	3.6	4.0			
Gestation length, d	115.8	114.6	116.2	0.42	0.16	0.04
Lactation length, d	22.8	23.8	22.2	0.49	0.15	0.10
Sow BW, kg						
Breeding	197.5	206.3	216.3	8.38	0.15	0.66
Pre-farrow	237.2	251.9	249.3	7.34	0.45	0.22
Farrowing	229.8	236.8	239.8	8.33	0.47	0.65
Weaning	231.4	226.4	240.0	10.97	0.47	0.65
Sow weight changes, kg						
Gestation gain	39.6	45.6	33.0	9.26	0.47	0.55
Lactation loss	1.6	-10.4	0.2	8.85	0.82	0.33
Lactation daily feed intake, kg	4.7	4.4	5.0	0.38	0.39	0.50

[1]Pre-farrow BW was recorded on d 109-112 of gestation whereas post-farrow BW was recorded within 24 h following parturition.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B.

Table A.4. 2 Effects of dietary boron (B) supplementation on litter size of slaughter sows[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
No. of litters	5	5	5			
Litter size, n						
Total born	13.0	13.6	14.6	1.23	0.38	0.86
Born alive	10.6	11.6	14.2	1.07	0.03	0.84
Adjusted born alive[3]	9.6	10.4	12.4	1.14	0.10	0.87
Stillborn	2.4	2.0	0.4	0.69	0.05	1.00
Mummified	0.2	0.4	0.4	0.35	0.76	0.73
Weaning	8.4	9.6	10.4	1.09	0.26	0.58
Preweaning mortality	1.2	0.8	2.0	0.73	0.32	0.56

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B.
[3]Adjusted born alive represents number of piglets alive after cross-fostering.

Table A.4. 3 Effects of dietary boron (B) supplementation on litter performance of slaughter sows[1].

	Supplemental B, ppm				*P*-value[2]	
Items	0	5	25	SEM	L	Q
Litter weight, kg						
Total born	14.66	18.85	19.75	1.80	0.13	0.20
Born alive	12.58	16.73	19.41	1.75	0.03	0.24
Adjusted born alive[3]	11.44	15.31	17.65	1.71	0.04	0.26
Weaning	49.10	66.22	61.91	8.02	0.49	0.19
Gain	36.52	49.48	42.50	6.65	0.84	0.20
Individual piglet weight, kg						
Total born	1.12	1.38	1.37	0.09	0.18	0.12
Born alive	1.17	1.42	1.39	0.09	0.25	0.08
Adjusted born alive[3]	1.17	1.45	1.45	0.08	0.11	0.06
Weaning	5.99	6.87	5.88	0.42	0.45	0.12
Gain	5.99	6.87	5.88	0.42	0.26	0.21
Adjusted weight, kg[4]						
Litter weight at weaning	45.99	60.10	59.14	7.08	0.36	0.23
Litter weight gain	34.55	44.79	41.48	5.81	0.64	0.26
Piglet weight at weaning	5.61	6.23	5.64	0.35	0.69	0.20
Piglet weight gain	4.44	4.78	4.19	0.33	0.40	0.38

[1]Sows were allotted to study following estrus detection and 2 subsequent artificial inseminations. Sows remained on their respective dietary treatments until removed from the study.
[2]Orthogonal polynomial contrast performed for linear (L) and quadratic (Q) effects of supplemental levels of B.
[3]Adjusted born alive represents number of piglets alive after cross-fostering.
[4]Adjusted for a 21 d lactation period.

APPENDIX 5. Gross energy determination

Feed, feces, excreta, and urine gross energy (cal/g) were assessed by bomb calorimetry, consisting of the ignition of samples in a pressurized-O_2 environment, and measuring the heat of combustion as the amount of energy transferred to a known mass of water contained in the calorimeter (model 1261 isoperibol bomb calorimeter, Parr Instruments Co., Moline, IL). This is an adaptation of the method by the AOAC (1995). It includes running several benzoic acid pellets (6318 cal/g) at the beginning and at the end of every set of samples to test for precision and accuracy of the equipment. The calorimeter used has two bombs and two metal buckets. Each bomb has been calibrated to be used with a particular bucket.

Feed, feces, and excreta samples

Feed, feces, and excreta samples were ground, weighed (1 g), and pelleted in duplicate prior to combustion in the calorimeter.

Urine samples

Prior to assessing urine gross energy, samples were dried into plastic bags. The bags used were flat 1.5” x 3” clear 2 mil product No. 01-0103-2, 47.60 for 1000 (Jeb Plastics, Inc., Wilmington, DE). The average bag energy content (cal/g) was established by conducting 20 individual bag combustions. Urine samples were centrifuged at 1,500 rpm for 10 minutes prior to gross energy assessment. Each bag was cut and sealed to generate 3 smaller bags able to hold about 3 g of urine each. Bags were weighed, opened, and placed in tared metal crucibles over a scale. Then the urine sample was pipetted into the bag, and the bag plus urine weight was recorded. Then bags were placed in a draft

oven at 40 ºC until dry - 48 to 72 hours. Once bags were dry, they were combusted in the bomb calorimeter. Urine energy was calculated as:

Gross energy, cal/ml =

$$\frac{(Total\ energy\ released - (bag\ weight\ x\ plastic\ energy\ factor))}{wet\ urine\ weight}$$

APPENDIX 6. Phosphorous determination in feed, tissue, bone, and fecal samples

This gravimetric procedure is a modification of method 968.08 from AOAC, 1990.

Feed (4.0 g), feces (1.0 g), and bone (tibia, femur, and metacarpals) were weighed in quartz crucibles and dry-ashed overnight in a muffle furnace at 600°C. After cooling down in a desiccator, samples were digested with 40 mL 3N HCl on a hot plate at a high temperature and left boiling for approximately 15 minutes. Digested solutions were then quantitatively transferred into 250 mL volumetric flasks and diluted to volume with DD water. Flasks were shaken, sealed with parafilm paper, and left overnight to settle. Tissue samples (liver and kidney; 1-2 g) were digested with nitric acid in a pressurized microwave digester (MARS 6 CEM, Matthews, NC) according to the recommendations by the manufacture, and appropriately diluted. Then, 20 mL aliquots of the solutions were transferred into Erlenmeyer flasks, heated to a boil using a hot plate, 50 mL of Quimociac reagent added, and left on the hot plate until the color changed from 'milky' yellow to clear yellow. Then solutions were vacuum filtered in pre-weighed porcelain gooch crucibles using fiberglass filter paper circles. Crucibles with the filtered precipitate were then oven-dried overnight at 105° C, then cooled down in a desiccator and weighed.

Total phosphorus concentration was calculated as:

$$Total\ P, \% = \left[\frac{(Precipitate\ weight\ \times\ V_1}{V_2} \times \frac{(0.013997\ \times 100}{Sample\ Weight}\right]$$

V_1: Initial volume after quantitative transferring HCl-digested sample (250 cc).

V_2: Aliquot of V_1 to be reacted with Quimociac (50 cc).

APPENDIX 6. (Continued) Quimociac reagent preparation procedure

To prepare 1 L of Quimociac reagent:

1. Dissolve 70.0 g sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$) into 150 mL DD water.
2. Dissolve 63.8 g citric acid dehydrate [$HOCCOOH(CH_2COOH)_2 \cdot H_20$] into 150 mL deionized (DD) water, add 85 mL concentrated nitric acid (HNO_3) and allow to cool.
3. Add the molybdate solution to the citric-nitric solution while stirring.
4. Add 5 mL synthetic quinoline (C_6H_4N:CHCH:CH) to a mixture of 100 mL DD water and 35 mL concentrated nitric acid.
5. Slowly add the quinoline mixture to the molybdate-citric-nitric solution, while stirring.
6. Let solution stand overnight.
7. Filter solution through a No. 2 Whatman filter.
8. Add 280 mL of acetone (CH_3COCH_3) and dilute to 1 L with DD water.

References

Adarsh, V., P. Dintaran, G. N. Shivakumar, E. A. Vijayarangam, D. D. Kumar, K. Nagaraj, and J. S. Eknath. 2021. Effect of boron supplementation on laying performance of White Leghorn hens fed diet with and without adequate level of calcium. Trop. Anim. Health Pro. 53(4): 1-9. doi: 10.1007/s11250-021-02878-x.

Agudelo-Trujillo, Jorge Hernan. 2005. An examination of dietary amendments to affect phosphorus utilization in growing pigs. University of Kentucky Doctoral Dissertations. 242. https://uknowledge.uky.edu/gradschool_diss/242

Anderson, D. M., F.W.H. Elsley, I. McDonald, and R. M. MacPherson. 1971. A study of the relationship between glucose tolerance of sows and the mean birth weight of their offspring. J. Agric. Sci. 76:179-182. doi:10.1017/S0021859600015732.

Andrali, S. S., M. L. Sampley, N. L. Vanderford, and S. Ozcan. 2008. Glucose regulation of insulin gene expression in pancreatic beta-cells. Biochem. J. 415:1–10. doi: 10.1042/BJ20081029.

Anil, S. S., L. Anil, and J. Deen. 2009. Effect of lameness on sow longevity. J. Am. Vet. Med. Assoc. 235: 734-738. doi:10.2460/javma.235.6.734.

AOAC. 2006. Official methods of analysis of AOAC international (18th Ed.). AOAC Int., Gaithersburg, MD.

Armstrong T. A., J. W. Spears, T. D. Crenshaw, and F. H. Nielsen. 2000. Boron supplementation of a diet for weanling pigs improves feed efficiency and bone

strength characteristics and alters plasma lipid metabolites. J. Nutr. 130: 2575-2581. doi: 10.1093/jn/130.10.2575.

Armstrong, T. A., and J. W. Spears. 2001. Effect of dietary boron on growth performance, calcium and phosphorus, and bone mechanical properties in growing barrows. J. Anim. Sci. 79:3120-3127. doi 10.2527/2001.79123120x.

Armstrong, T. A., J. W. Spears, and K. E. Lloyd. 2001. Inflammatory response, growth, and thyroid hormone concentrations are affected by long-term boron supplementation in gilts. J Anim. Sci. 79(6):1549-1556. doi: 10.2527/2001.7961549x.

Armstrong, T. A., W. L. Flowers, J. W. Spears, and F. H. Nielsen. 2002. Long-term effects of boron supplementation on reproductive characteristics and bone mechanical properties in gilts. J. Anim. Sci. 80: 154-161. doi: 10.2527/2002.801154x.

Armstrong, T. A., and J. W. Spears. 2003. Effect of boron supplementation of pig diets on the production of tumor necrosis factor and interferon. J. Anim. Sci. 81: 2552 – 2561. doi: 10.2527/2003.81102552x.

ATSDR (Agency for Toxic Substances and Disease Registry). 2010. Toxicological Profile for Boron. US department of health and human services public health service, Atlanta, GA.

Aydın, S., S. Demirci, A. Doğan, D. Sağraç, E. Kaşıkcı, and F. Şahin. 2019. Boron containing compounds promote the survival and the maintenance of pancreatic β-cells. Mol. Biol. Rep. 46(5): 5465-5478. doi: 10.1007/s11033-019-05002-3.

Bai, Y. and C.D. Hunt. 1996. Absorption and distribution of boron in rats following a single oral administration of boron. Proc. ND Acad. Sci. 50:53.

Bakken, N. A., and C. D. Hunt. 2003. Dietary boron decreases peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. J Nutr. 133:3577–3583. doi: 10.1093/jn/133.11.3577.

Ball, R. O., R. Samuel, and S. Moehn. 2008. Nutrient requirements of prolific sows. Adv. Pork Prod. 19: 223-236. doi: 10.1093/jn/133.11.3577.

Baspinar, N., A. Basoglu, O. Ozdemir, C. Ozel, F. Terzi, and O. Yaman. 2015. Effects of boron compounds in rabbits fed high protein and energy diet: a metabolomic and transcriptomic approach. Int. J. Vet. Sci. 9(5): 570-575. doi: 10.5281/zenodo.1107147.

Berger, K. C. 1949. Boron in soils and crops. Adv. Agron. 1: 321-351. doi: 10.1016/S0065-2113(08)60752-X.

Berridge, M. J. 2017. Vitamin D deficiency and diabetes. Biochem J. 474(8):1321-1332. doi: 10.1042/BCJ20170042.

Bhasker, T.V., N.K.S. Gowda, S. Mondal, P. Krishnamoorthy, D.T. Pal, A. Mor, S.K. Bhat, and A.K. Pattanaik. 2016. Boron influences immune and antioxidant responses by modulating hepatic superoxide dismutase activity under calcium deficit abiotic stress in Wistar rats. J. Trace. Elem. Med. Bio. 36: 73-79. doi: 10.1016/j.jtemb.2016.04.007.

Bhasker, T. V., N. Gowda, D. T. Pal, S. K. Bhat, P. Krishnamoorthy, S. Mondal, and A. K. Verma. 2017. Influence of boron supplementation on performance, immunity and antioxidant status of lambs fed diets with or without adequate level of calcium. Plos one, 12(11): e0187203. doi: 10.1371/journal.pone.0187203.

Blevins, D.G., and K.M. Lukaszewski. 1994. Proposed physiologic functions of boron in plants pertinent to animal and human metabolism. Envro. Health. Perspect. 102:31-33. doi: 10.1289/ehp.94102s731.

Bökönyi, S. 1974. History of domestic mammals in central and eastern Europe. Akadémiai Kiadó, Budapest.

Bouillon-Hausman, D., T. R. Kasser, R. W. Seerly, and R. J. Martin. 1986. Studies of gestational diabetes using the pig as a model. In: M. E. Tumbleson (Ed.) Swine in Biomedical Research. Vol. 1. Plenum Press, New York. p 561.

Boyd, R. D., K. J. Touchette, G. C. Castro, M. E. Johnston, K. U. Lee, and I. K. Han. 2000. Recent advances in amino acid and energy nutrition of prolific sows - Invited Review. Asian-Aus. J. Anim. Sci. 13:1638-1652. doi: 10.5713/ajas.2000.1638.

Bozkurt, M., K., Küçükyılmaz, A. U. Çatlı, M. Çınar, M. Çabuk, and E. Bintaş. 2012. Effects of boron supplementation to diets deficient in calcium and phosphorus on performance with some serum, bone and fecal characteristics of broiler chickens. Asian-Aus. J. Anim. Sci. 25(2): 248-255. doi: 10.5713/ajas.2011.11211.

Bozkurt, Y., İ. Yavaş, A. Gül, M. N. Bucak, D. Yeni, and F. Avdatek. 2019. Effect of extender supplemented with boron on post-thaw motility, viability, DNA damage

and fertilization ability of cryopreserved brown trout (Salmo trutta macrostigma) spermatozoa. Cryo-Lett. 40(5): 275-283.

Brown, T. F., M. E. McCormick, D. R. Morris, and L. K. Zeringue. 1989. Effects of dietary boron on mineral balance in sheep. Nutr. Res. 9:503–512. doi: 10.1016/S0271-5317(89)80175-7.

Buffler, M., C. Becker, and W. M. Windisch. 2017. Effects of different iron supply to pregnant sows (Sus scrofa domestica L.) on reproductive performance as well as iron status of new-born piglets. Arch. Anim. Nutr. 71: 219-230. doi:10.1080/ 1745039x.2017.1301059.

Cakmak, I., and V. Römheld. 1997. Boron deficiency — induced impairments of cellular functions in plants. Plant and Soil. 193:71-83. doi: 10.1023/A:1004259808322.

Cakmak, I., H. Kurz, and H. Marschner. 1995. Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. Physiol. Plantarum. 95(1): 11-18. doi: 10.1111/j.1399-3054.1995.tb00801.x

Carney-Hinkle, E. E., H. Tran, J.W. Bundy, R. Moreno, P.S. Miller, and T.E. Burkey. 2013. Effect of dam parity on litter performance, transfer of passive immunity, and progeny microbial ecology. J. Anim. Sci. 91(6): 2885-2893. doi: 10.2527/jas.2011-4874.

Castevens, K., J. B. Ferreira, T. Gillespie, C. Olsen, J. P. Nielsen, and G. Almond. 2020. Assessment of hemoglobin concentration in relation to sow reproductive stage and parity. J. Swine Health Prod. 28(5): 254-257.

Chapin, R. E., W. W. Ku, M. A. Kenney, and H. McCoy. 1998. The effects of dietary boric acid on bone strength in rats. Biol. Tr. Elem. Res. 66:395-399. doi: 10.1006/faat.1996.2275.

Chen, J., J. H. Han, W. T. Guan, F. Chen, C. X. Wang, Y. Z. Zhang, Y. T. Lv, and G. Lin. 2016a. Selenium and vitamin E in sow diets: I. Effect on antioxidant status and reproductive performance in multiparous sows. Anim. Feed Sci. Technol. 221, Part A: 111-123. doi: 10.1016/j.anifeedsci.2016.08.022.

Chen, J., J. H. Han, W. T. Guan, F. Chen, C. X. Wang, Y. Z. Zhang, Y. T. Lv, and G. Lin. 2016b. Selenium and vitamin E in sow diets: II. Effect on selenium status and 236 antioxidant status of the progeny. Anim. Feed Sci. Technol. 221:101-110. doi: 10.1016/j.anifeedsci.2016.08.021.

Cheng, J., K. Peng, E. Jin, Y. Zhang, Y. Liu, N. Zhang, and Z. Tang. 2011. Effect of additional boron on tibias of African ostrich chicks. Biol. Trace. Elem. Res. 144(1): 538-549. doi: 10.1007/s12011-011-9024-y.

Cho, H. M., S. P. Macelline, S. S. Wickramasuriya, T. K. Shin, E. Kim, H. C. Son, and J. M. Heo. 2022. Moderate dietary boron supplementation improved growth performance, crude protein digestibility and diarrhea index in weaner pigs regardless of the sanitary condition. Animal Bioscience, 35(3): 434-443. doi: 10.5713/ab.21.0110.

Clowes, E. J., F. X. Aherne, G. R. Foxcroft, and V. E. Baracos. 2003. Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function. J. Anim. Sci. 81(3): 753-764. doi: 10.2527/2003.813753x.

Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis, and B. D. Moser. 1981a. Bone strength as a trait for assessing mineralization in swine: a critical review of techniques involved. J. Anim. Sci. 53:827-835. doi: 10.2527/jas1981.533827x.

Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis, B. D. Moser, and D.Olsen. 1981b. Influence of age, sex and calcium and phosphorus levels on the mechanical properties of various bones in swine. J. Anim. Sci. 52:1319–1329. doi: 10.2527/jas1981.5261319x.

Crenshaw, T. D. 2000. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. J. Lewis and L. L. Southern, editors, Swine Nutrition. 2nd ed. CRC Press, New York, NY. p. 207-232.

Crenshaw, T. D., J. R. Danielson, L. E. Hoffman, and D. K. Schneider. 2009. Femurs are more accurate than fibulas as predictors of whole-body bone mineral content in growing pigs. J. Anim. Sci. 87(Suppl. 2):510. (Abstr.).

Cromwell, G. L., and C. K. Baer. 2005. Setting the NRC standards for minerals - were we right? In: J. A. Taylor-Pickard, and L. Tucker, editors, Redefining Mineral Nutrition. Nottingham University Press. Nottingham, UK. p. 1-19.

Cromwell, G. L., H. J. Monegue, and T. S. Stahly. 1993. Long-term effects of feeding a high copper diet to sows during gestation and lactation. J. Anim. Sci. 71: 2996-3002. doi:10.2527/1993.71112996x.

Dell, B., and L. Huang. 1997. Physiological response of plants to low boron. Plant and Soil. 193:103-120.

DeLuca, H.F., 1979. The vitamin D system in the regulation of calcium and phosphorus metabolism. Nutr. Rev. 37: 161-193. doi: 10.1111/j.1753-4887.1979.tb06660.x.

Demirci, S., A. Doğan, S. Aydın, E.Ç. Dülger, and F. Şahin. 2016. Boron promotes streptozotocin-induced diabetic wound healing: roles in cell proliferation and migration, growth factor expression, and inflammation. Mol. Cell. Biochem. 417: doi: 119-133. 10.1007/s11010-016-2719-9.

Díaz, J. A. C., M. T. Nikkilä, and K. Stalder. 2015. Sow longevity. In: C. Farmer, editor, The gestating and lactating sow. Wageningen Academic Publishers, Wageningen, Netherlands. p. 423-452.

Downing, R.G., and P.L. Strong. 1999. Analytical challenges of low level boron analysis in biological matrices. J. Trace Elem. Exp. Med. 12: 205-212. doi: 10.1002/(SICI)1520-670X(1999)12:3<205::AID-JTRA4>3.0.CO;2-4.

Eckhert, C. D. 1998. Boron stimulates embryonic trout growth. J. Nutr. 128: 2488–2493. doi: 10.1093/jn/128.12.2488.

Edmunds, C. E., A. S. Cornelison, C. Farmer, C. Rapp, V. E. Ryman, W. P. Schweer, M. E. Wilson, C. R. Dove, 2022. PSVII-9 The Impact of Increasing Dietary Manganese on the Reproductive Performance of Sows. J. Anim. Sci. 100(Supplement_2): 172-173. doi: 10.1093/jas/skac064.294

EFSA. 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Boron (Sodium Borate and Boric Acid). The EFSA Journal. 80:1-22.

Elkomy, A. E., A. M. Abd El-hady, and O. A. Elghalid. 2015. Dietary boron supplementation and its impact on semen characteristics and physiological status of adult male rabbits. Asian J. Poult. Sci. 9:85-96. doi:10.3923/ajpsaj.2015.85.96.

Elliot, M. A., and H. M. Edwards, Jr. 1992. Studies to determine whether an interaction exists among boron, calcium, and cholecalciferol on the skeletal development of broiler chickens. Poult. Sci. 71:677-690. doi: 10.3382/ps.0710677.

Eren, M., F. Uyanik, and S. Küçükersan. 2004. The influence of dietary boron supplementation on egg quality and serum calcium, inorganic phosphorus, magnesium levels and alkaline phosphate activity in laying hens. Res.Vet. Sci. 76:203-210. doi: 10.1016/j.rvsc.2003.11.004.

Everts, H., A. W. Jongbloed, and R. A. Dekker. 1998. Calcium, magnesium and phosphorus balance of sows during lactation for three parities. Livest. Prod. Sci. 55:109-115. doi:10.1016/S0301-6226(98) 00133-X.

Evock-Clover, C. M., M. M. Polansky, R. A. Anderson, and N. C. Steele. 1993. Dietary chromium supplementation with or without somatotropin treatment alters serum hormones and metabolites in growing pigs without affecting growth performance. J. Nutr. 123(9): 1504-1512. doi: 10.1093/jn/123.9.1504

FAO. 2022. Food and agriculture organization of the United Nations statistical databases. https://www.fao.org/faostat/en (Accessed on 02/27/2023)

Farmer, C., and M. T. Sørensen. 2001. Factors affecting mammary development in gilts. Livest. Prod. Sci. 70(1-2): 141-148. doi: 10.1016/S0301-6226(01)00207-X.

Feldpausch, J. A., J. Jourquin, J. R. Bergstrom, J.L. Bargen, C. D. Bokenkroger, D. L. Davis, J. M. Gonzalez, J. L. Nelssen, C.L. Puls, W.E. Trout, and M.J. Ritter. 2019. Birth weight threshold for identifying piglets at risk for preweaning mortality. Transl. Anim. Sci. *3*(2): 633-640. doi: 10.1093/tas/txz076

Ferreira, S. V., L. A. Rodrigues, M. A. Ferreira, D. V. Alkmin, J. M. Dementshuk, F. R. C. L. Almeida, and D. O. Fontes. 2021. Plane of nutrition during gestation affects reproductive performance and retention rate of hyperprolific sows under commercial conditions. Animal. 15(3):100153. doi: 10.1016/j.animal.2020.100153

Feyera, T., and P. K. Theil. 2017. Energy and lysine requirements and balances of sows during transition and lactation: A factorial approach. Livest. Sci. 201: 50–57. doi: 10.1016/j.livsci.2017.05.001.

Feyera, T., T. F. Pedersen, U. Krogh, L. Foldager, and P. K. Theil. 2018. Impact of sow energy status during farrowing on farrowing kinetics, frequency of stillborn piglets, and farrowing assistance. J. Anim. Sci. 96(6): 2320-2331. doi: 10.1093/jas/sky14.

Fix, J. S., J. P. Cassady, J. W. Holl, W. O. Herring, M. S. Culbertson, and M. T. See. 2010. Effect of piglet birth weight on survival and quality of commercial market swine. Livest. Sci. 132:98–106. doi:10.1016/j.livsci.2010.05.007.

Follis Jr., R.H., 1947. Effect of adding boron to a potassium-deficient diet in rats. Am. J. Physiol. 150: 520-522. doi: 10.1152/ajplegacy.1947.150.3.520.

Food and Drug Administration (FDA). 1987. Food additives permitted in feed and drinking water of animals: Selenium. Federal register. 52:21001.

Food and Nutrition Board. 2002. Standing committee on the Scientific Evaluation of Dietary Reference Intake, Institute of Medicine, Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Natl. Acad. Press, Washington, DC.

Foreign Agricultural Service (FAS), U. F. A. 2023. Livestock and Poultry: World Markets and Trade.

Fort, D. J., T. L. Propst, E. L. Stover, F. J. Murray, and P. L. Strong. 1999a. Adverse effects from low dietary and environmental boron exposure on reproduction, development, and maturation in Xenopus laevis. J. Trace Elem. Exp. Med. 12:175–185. doi: 10.1002/(SICI)1520-670X(1999)12:3<175::AID-JTRA2>3.0.CO;2-R.

Fort, D. J., E. L. Stover, P. L. Strong, F. J. Murray, and C. L. Keen. 1999b. Chronic feeding of a low boron diet adversely affects reproduction and development in Xenopus laevis. J. Nutr. 129:2055–2060. doi: 10.1093/jn/129.11.2055.

Fraser, D., P.A. Phillips, and B.K. Thompson, 1997. Farrowing behaviour and stillbirth in two environments: an evaluation of the restraint-stillbirth hypothesis. Appl. Anim. Behav. Sci. 55(1-2): 51-66. doi: 10.1016/S0168-1591(97)00007-5.

Gamble, M. S., and G. A. Cook. 1985. Alteration of the apparent Ki of carnitine palmitoyltransferase for malonyl-CoA by the diabetic state and reversal by insulin. J. Biol. Chem. 260:9516. doi: 10.1016/S0021-9258(17)39262-1.

George, P. B., D. C. England, D. G. Siers, and H. C. Stanton. 1978. Diabetogenic effects of pregnancy in sows on plasma glucose and insulin release. J. Anim. Sci. 46:1694. doi: 10.2527/jas1978.4661694x.

Gill, P. 2007. Managing reproduction-critical control points in exceeding 30 pigs per sow per year. In: Proc 7th London Swine Conference. “Today's challenges... tomorrow's opportunities”. London, Ontario, Canada, p. 171-184.

Giuffra, E., J. M. Kijas, V. Amarger, O. Carlborg, J. T. Jeon, and L. Andersson. 2000. The origin of the domestic pig: independent domestication and subsequent introgression. Genetics. 154(4):1785-1791. doi: 10.1093/genetics/154.4.1785.

Gondret, F., L. Lefaucheur, I. Louveau, B. Lebret, X. Pichodo, and Y. Le Cozler. 2005. Influence of piglet birth weight on postnatal growth performance, tissue lipogenic capacity and muscle histological traits at market weight. Livest. Prod. Sci. 93(2): 137-146. doi: 10.1016/j.livprodsci.2004.09.009.

Gordon V. 1987. The case of the toxic life-preserver. Borax Rev. 2:10-12.

Green, G. H., and H. J. Weeth. 1977. Response of heifers ingesting boron in water. J. Anim. Sci. 46:812–818. doi:10.2527/jas1977.454812x.

Hadley, M. E. 1988. Pancreatic hormones and metabolic regulation. In: M. E. Hadley (Ed.) Endocrinology. p 237. Prentice-Hall, Englewood Cliffs, NJ.

Hagen, C. D., M. D. Lindemann, and K. W. Purser. 2000. Effect of dietary chromium tripicolinate on productivity of sows under commercial conditions. J. Swine Health Prod. 8(2): 59-63.

Hakki, S. S., B. S. Bozkurt, and E. E. Hakki. 2010. Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). J. Trace Elem. Med. Bio. 24(4): 243-250. doi: 10.1016/j.jtemb.2010.03.003.

Hakki, S. S., N. Dundar, S. A. Kayis, E. E. Hakki, M. Hamurcu, U. Kerimoglu, and F. H Nielsen. 2013. Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. J. Trace Elem. Med. Bio 27(2): 148-153. doi: 10.1016/j.jtemb.2012.07.001.

Hegsted, M., M. J. Keenan, F. Siver, and P. Wozniak. 1991. Effect of boron on vitamin D deficient rats. Biol. Trace Elem. Res. 28:243–255. doi: 10.1007/BF02990471.

Hill, G. M. 2013. Minerals and mineral utilization. In: L. I. Chiba, editor, Sustainable Swine Nutrition. Blackwell Publishing Ltd., Ames, IA. p. 173-195.

Hill, G. M., E. R. Miller, and H. D. Stowe. 1983. Effect of dietary zinc levels on health and productivity of gilts and sows through two parities. J. Anim. Sci. 57: 114-122. doi:10.2527/jas1983.571114x.

Hoge, M. D., and R. O. Bates. 2011. Developmental factors that influence sow longevity. J. Anim. Sci. 89: 1238-1245. doi:10.2527/jas.2010-3175.

Hou, W. X., S. Y. Cheng, S. T. Liu, B. M. Shi, and A. S. Shan. 2014. Dietary supplementation of magnesium sulfate during late gestation and lactation affects

the milk composition and immunoglobulin levels in sows. Asian Austral. J. Anim. 27: 1469-1477. doi:10.5713/ajas.2014.14190

Hoving, L. L., N. M. Soede, C. M. C. Van der Peet-Schwering, E. A. M. Graat, H. Feitsma, and B. Kemp. 2011. An increased feed intake during early pregnancy improves sow body weight recovery and increases litter size in young sows. J. Anim. Sci. 89(11): 3542-3550. doi: 10.2527/jas.2011-3954.

Hu, Q., S. Li, E. Qiao, Z. Tang, E. Jin, G. Jin, and Y. Gu. 2014. Effects of boron on structure and antioxidative activities of spleen in rats. Biol. Trace Elem. Res. 158:73-80. doi: 10.1007/s12011-014-9899-5.

Hunt, C. D. 1989. Dietary boron modified the effects of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. Biol. Trace. Elem. Res. 22:201-220. doi: 10.1007/BF02916650.

Hunt, C. D. 1994. The biochemical effects of physiologic amounts of dietary boron in animal nutrition models. Environ Health Perspect. 102(Suppl 7):35-43. doi: 10.1289/ehp.94102s735.

Hunt, C. D. 1997. Dietary boron and vitamin D affect hepatic glycolytic metabolite concentrations in the chick. In: P. W. F. Fischer, et al. (ed.). Trace Elements in Man and Animals, (TEMA-9). NRC Research Press: Ottawa, Canada. p 599–601.

Hunt, C D. 2008. Dietary boron: possible roles in human and animal physiology. Biomed. Res. Trace Elem. 19(3): 243-253.

Hunt C. D. and F. H. Nielsen: Interaction between boron and cholecalciferol in the chick. In McC Howell J, Gawthorne J. M. White C. L. (eds): Trace Element Metabolism in Man and Animals, (TEMA-4). Canberra, Australia: Australian Academy of Science, 1981. p 597-600.

Hunt, C. D., and F.H. Nielsen. Dietary boron affects bone calcification in magnesium- and cholecalciferol deficient chicks. In: Underwood, E.J., (eds): Trace Elements in Human and Animal Nutrition, (TEMA-5). New York: Academic Press, Inc., 1986. p 275–277.

Hunt C. D., and F. H. Nielsen. 1987. Interactions among dietary boron, magnesium, and cholecalciferol in the chick. Proc ND Acad. Sci. 41:50.

Hunt, C. D., and J. L. Herbel. 1991. Boron affects energy metabolism in the streptozotocin-injected, vitamin D3-deprived rat. Magnes. Trace Elem. 10(5-6): 374-386.

Hunt, C. D., and J. L. Herbel. 1993. Physiological amounts of dietary boron improve growth and indicators of physiological status over a 20-fold range in the vitamin D3-deficienct chick. In M. Anke, D. Meissner, C. Mills (eds), Trace Element Metabolism in Man and Animals: TEMA-8: Proc. 8th Int. Symp. Trace. Elem. Man. Anim. Gersdorf, Germany. 714-718.

Hunt, C. D., and J. P. Idso. 1999. Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research progress. J. Trace Elem. Exp. Med. 12:221–233. doi: 10.1002/(SICI)1520-670X(1999)12:3<221::AID-JTRA6>3.0.CO;2-X.

Hunt, C. D., J. L. Herbel, and J.P. Idso. 1994. Dietary boron modifies the effects of vitamin D3 nutrition on indices of energy substrate utilization and mineral metabolism in the chick. J. Bone Miner. Res. 9(2): 171-182. doi: 10.1002/jbmr.5650090206.

Hunt, C. D., 1998. Regulation of enzymatic activity: one possible role of dietary boron in higher animals and humans. Biol. Trace Elem. Res. 66: 205-225. doi: 10.1007/BF02783139.

Ibrahim, T. B., A. Abdel-Wahab, R. L. Abdel Aziz, A. H. El-Anwar, and S. S. Ibrahim. 2019. Dietary boron supplementation and its impact on testicular function, thyroid activity and serum calcium in rams. Small Rumin. Res. 174:156–162. doi: 10.1016/j.smallrumres.2019.03.008.

Jang, Y. D., M. D. Lindemann, H. J. Monegue, and J. S. Monegue. 2017. The effect of coated sodium butyrate supplementation in sow and nursery diets on lactation 253 performance and nursery pig growth performance. Livest. Sci. 195: 13-20. doi:10.1016/j.livsci.2016.11.005.

Jansen, J. A., J. S. Schou, and B. Aggerbeck. 1984a. Gastro-intestinal absorption and in vitro release of boric acid from water-emulsifying ointments. Food Chem. Toxicol. 22:49-53. doi: 10.1016/0278-6915(84)90052-8.

Jansen, J.A., J. Anderson, and J. S. Schou. 1984b. Boric acid single dose pharmokinetics after intravenous administration to man. Arch. Toxicol. 55:64-67. doi: 10.1007/BF00316588.

Jindal, R., J. R. Cosgrove, F. X. Aherne, and G. R. Foxcroft. 1996. Effect of nutrition on embryonal mortality in gilts: association with progesterone. J. Anim. Sci. 74: 620-624. doi:10.2527/1996.743620x.

Kaneko, J. J. 1989. Reference values for blood gas and electrolyte determinations. Page 546 in Clinical Biochemistry of Domestic Animals. 4th ed. J. J. Kaneko, ed. Academic Press, San Diego, CA.

Kemp, B., N. M. Soede, P. C. Vesseur, F. A. Helmond., J. H. Spoorenberg, K. Frankena. 1996. Glucose tolerance of pregnant sows is related to postnatal pig mortality. J. Anim. Sci. 74: 879-885. doi: 10.2527/1996.744879x.

Kim, S. W., R. D. Mateo, Y. L. Yin, and G. Wu. 2007. Functional amino acids and fatty acids for enhancing production performance of sows and piglets. Asian Austral. J. Anim. 20: 295-306. doi: 10.5713/ajas.2007.295.

Kim, S. W., W. L. Hurley, G. Wu, and F. Ji. 2009. Ideal amino acid balance for sows during gestation and lactation. J. Anim. Sci. 87(E. Suppl.):E123-E132. doi: 10.2527/jas.2008-1452.

Kim, S. W., A. C. Weaver, Y. B. Shen, and Y. Zhao. 2013. Improving efficiency of sow productivity: nutrition and health. J. Anim. Sci. Biotechnol. 4: 26-26. doi:10.1186/ 2049-1891-4-26

Kim J. S., X. Yang, D. Pangeni, and S. K. Baidoo. 2015. Relationship between backfat thickness of sows during late gestation and reproductive efficiency at different parities. Acta. Agr. Scand. 65: 1-8. doi: 10.1080/09064702.2015.1045932.

King, R. H., and I. H. Williams. 1984. The effect of nutrition on the reproductive performance of first-litter sows. 1. Feeding level during lactation, and between weaning and mating. Anim. Prod. 38:241. doi:10.1017/S0003356100002233.

Kirkwood, R. N., E. S. Lythgoe, and F. X. Aherne. 1987. Effect of lactation feed intake and gonadotrophin-releasing hormone on the reproductive performance of sows. Can. J. Anim. Sci. 67: 715. doi: 10.4141/cjas87-074.

Koketsu, Y., G. D. Dial, J. E. Pettigrew, and V. L. King. 1996. Feed intake pattern during lactation and subsequent reproductive performance of sows. J. Anim. Sci. 74: 2875- 2884. doi: 10.2527/1996.74122875x.

Krishnan, B. B., S. Selvaraju, N. K. S. Gowda, K. B. Subramanya, D. Pal, S. S. Archana, and R. Bhatta. 2019. Dietary boron supplementation enhances sperm quality and immunity through influencing the associated biochemical parameters and modulating the genes expression at testicular tissue. J. Trace Elem. Med. Biol. 55:6–14. doi: 10.1016/j.jtemb.2019.05.004.

Kucukkurt, I., E. Akbel, F. Karabag, and S. Ince. 2015. The effects of dietary boron compounds in supplemented diet on hormonal activity and some biochemical parameters in rats. Toxicol. Ind. Health. 31(3):255-260. doi: 10.1177/0748233712469648.

Kucukyilmaz, K., M. Bozkurt, M. Cinar, and A. E. Tuzun. 2017. Evaluation of the boron and phytase, alone or in combination, in broiler diets. J. Poult. Sci. 54: 26-33. doi: 10.2141/jpsa.0150181.

Langendijk, P. 2015. Early gestation feeding and management for optimal reproductive performance. In: C. Farmer, editor, The gestating and lactating sow. Wageningen Academic Publishers, Wageningen, Netherlands. p 27-46.

Lanoue, L., M. W. Taubeneck, J. Muniz, L. A. Hanna, P. L. Strong, F. J. Murray, F. H. Nielsen, C. D. Hunt, and C. L. Keen. 1998. Assessing the effects of low boron diets on embryonic and fetal development in rodents using in vitro and in vivo model systems. Biological trace element research. 66:271-298. doi: 10.1007/BF02783143.

Larson, G., T. Cucchi, and K. Dobney. 2011. Genetic aspects of pig domestication. In: M. F. Rothschild and A. Ruvinsky, eds, The genetics of the pig: second edition. CABI, Cambridge, MA. p. 14-37.

Lee, I. P., R. J. Sherins, and R. L. Dixon. 1978. Evidence for induction of germinal aplasia in male rats by environmental exposure to boron. Tox. Appl. Pharmacol. 45: 577-590. doi: 10.1016/0041-008X(78)90119-9

Liao, S. F., J. S. Monegue, M. D. Lindemann, G. L. Cromwell, and J. C. Matthews, 2011. Dietary supplementation of boron differentially alters expression of borate transporter (NaBCl) mRNA by jejunum and kidney of growing pigs. Biol. Trace Elem. Res. 143(2): 901-912. doi: 10.1007/s12011-010-8936-2

Lindemann, M. D., C. M. Wood, A. F. Harper, E. T. Kornegay, and R. A. Anderson. 1995. Dietary chromium picolinate additions improve gain/feed and carcass characteristics in growing-finishing pigs and increase litter size in reproducing sows. J. Anim. Sci. 73: 457-465. doi:10.2527/1995.732457x.

Locatelli C., C. Minoia, M. Tonini, and L. Manzo. 1987. Human toxicology of boron with special reference to boric acid poisoning. G. Ital. Med. Lav. 9(3-4): 141-146.

Loomis, W. D., and R. W. Durst. 1992. Chemistry and biology of boron. BioFactors. Oxford, England. 3(4): 229-239.

Lovatt, C. J., and W. M. Dugger. 1984. Boron. In Biochemistry of the essential ultratrace elements. Ed. E. Frieden. Plenum Press, New York. p. 389-421.

Lu, N., 2018. Long-term effects of dietary copper source and level on performance and health of sows and piglets. Theses and Dissertations--Animal and Food Sciences. 85. https://uknowledge.uky.edu/animalsci_etds/85.

Lucia, T., G. D. Dial, and W. E. Marsh. 2000a. Lifetime reproductive and financial performance of female swine. J. Am. Vet. Med. Assoc. 216: 1802-1809. doi:10. 2460/javma.2000.216.1802.

Lucia, T., G. D. Dial, and W. E. Marsh. 2000b. Lifetime reproductive performance in female pigs having distinct reasons for removal. Livest. Prod. Sci. 63: 213-222. doi: 10.1016/S0301-6226(99)00142-6.

Mahan, D. C. 1990. Mineral nutrition of the sow: a review. J. Anim. Sci. 68: 573-582. doi: 10.2527/1990.682573x.

Mahan, D. C., and A. W. Fetter. 1982. Dietary calcium and phosphorus levels for reproducing sows. J. Anim. Sci. 54: 285-291. doi: 10.2527/jas1982.542285x.

Mahan, D. C., and E. A. Newton. 1995. Effect of initial breeding weight on macro- and micromineral composition over a three-parity period using a high-producing sow genotype. J. Anim. Sci. 73: 151-158. doi: 10.2527/1995.731151x.

Mahan D. C., J. L. Vallet.1997. Vitamin and mineral transfer during fetal development and the early postnatal period in pigs. J. Anim. Sci. 75(10): 2731–2738. doi: 10.2527/1997.75102731x.

Mallmann, A. L., G. S. Oliveira, R. R. Ulguim, A. Mellagi, M. L. Bernardi, U. Orlando, M. Gonçalves, R. J. Cogo, and F. P Bortolozzo. 2020. Impact of feed intake in early gestation on maternal growth and litter size according to body reserves at weaning of young parity sows. J. Anim. Sci. 98(3): skaa075. doi: 10.1093/jas/skaa075.

Matzat, P. D., M. G. Hogberg, R. L. Fogwell, and E. R. Miller. 1990. Lactation performance in high producing gilts fed in excess of ad libitum. In: Report of Swine Research, AS-SW-8904. p. 36−40. Michigan State University, East Lansing.

McDowell, L. R. 2003. Minerals in Animal and Human Nutrition. 2nd ed. Elsevier, Amsterdam, The Netherlands.

McPherson, R. L., F. Ji, G. Wu, J. R. Blanton Jr, and S. W. Kim. 2004. Growth and compositional changes of fetal tissues in pigs. J. Anim. Sci. 82(9):2534-2540. doi: 10.2527/2004.8292534x.

Meacham, S. L., L. J. Taper, and S. L. Volpe. 1994. Effects of boron supplementation on bone mineral density and dietary, blood, and urinary calcium, phosphorus,

magnesium, and boron in female athletes. Environ. Health Persp., 102(suppl 7): 79-82. doi: 10.1289/ehp.94102s779.

Merck. 2005. Reference guides, Table 7. Serum biochemical reference ranges. Pages 2586-2587 in The Merk Veterinary Manual. 9th ed. Merk & Co. Inc., Whitehouse Station, NJ.

Morgan, V. 1980. Boron geochemistry. In: Supplement to Mellon's Comprehensive Treatise on Inorganic and Theoretical Chemistry. Vol. V. Part A: Boron-Oxygen Compounds. Longman, New York, NY.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82: 179–18. doi: 10.2527/2004.821179x.

Naghii M. R., and S. Samman. 1993. The role of boron in nutrition and metabolism, Prog. Food Nutr. Sci. 17: 331-349.

Naghii M. R., and S. Samman. 1996. The effect of boron supplementation on the distribution of boron in selected tissues and on testosterone synthesis in rats. J. Nutr. Biochem. 7: 507-512.

Naghii M. R., and S. Samman. 1997. The effect of boron on plasma testosterone and plasma lipids in rats. Nutr. Res. 17: 523-532. doi: 10.1016/0955-2863(96)00102-7.

Newnham, R. E. 1991. Agriculture practices affect arthritis, Nutr. Health. 7: 89-100. doi: 10.1177/026010609100700204.

Nielsen, F.H., 2008. Is boron nutritionally relevant?. Nutrition reviews, 66(4):183-191. doi: 10.1111/j.1753-4887.2008.00023.x.

Nielsen, F. H. 1996. Evidence for the nutritional essentiality of boron. J. Trace Elem. Exp. Med. 9: 215-229. doi: 10.1002/(SICI)1520-670X(1996)9:4<215::AID-JTRA7>3.0.CO;2-P.

Nielsen, F. H. 2017. Historical and recent aspects of boron in human and animal health. Journal of Boron. 2(3): 153-160.

Nielsen, F. H., and S. L. Meacham. 2011. Growing Evidence for Human Health Benefits of Boron. J. Evid. Based Complementary Altern. Med. 16(3): 169–180. doi: 10.1177/2156587211407638.

Nielsen F. H., C. D. Hunt, L. M. Mullen, and J. R. Hunt. 1987 The effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. FASEB. J. 87:394-397.

Nimmo, R., E. Peo, B. Moser, and A. Lewis. 1981a. Effect of Level of Dietary Calcium-Phophorus during Growth and Gestation on Performance, Blood and Bone Parameters of Swine. J. Anim. Sci. 52: 1330-1342. doi:10.2527/jas1981.5261330x.

Nimmo, R. D., E. R. Peo, Jr., J. D. Crenshaw, B. D. Moser, and A. J. Lewis. 1981b. Effect of level of dietary calcium-phosphorus during growth and gestation on calcium-phosphorus balance and reproductive performance of first-litter sows. J. Anim. Sci. 52: 1343-1349. doi: 10.2527/jas1981.5261343x.

Noblet J., J. Y. Dourmad, and M. Etienne. 1990. Energy utilization in pregnant and lactating sows: Modeling of energy requirements. J. Anim. Sci. 68: 562–572. doi: 10.2527/1990.682562x.

National Research Committee (NRC). 1988. Nutrient requirements of swine. 9th ed. National Academy Press, Washington, DC.

National Research Committee (NRC). 1998. Nutrient requirements of swine. 10th ed. National Academy Press, Washington, DC.

National Research Committee (NRC). 2005. Mineral Tolerance of Animals: Second Revised Edition, 2005. The National Academies Press, Washington, DC.

National Research Committee (NRC). 2012. Nutrient requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Nzietchueng, R. M., B. Dousset, P. Franck, M. Benderdour, P. Nabet, and K. Hess. 2002. Mechanisms implicated in the effects of boron on wound healing. J. Trace Elem. Med. Biol. 16(4): 239-244. doi: 10.1016/S0946-672X(02)80051-7.

Ocepek, M., R. C. Newberry, and I. L. Andersen. 2017. Trade-offs between litter size and offspring fitness in domestic pigs subjected to different genetic selection pressures. Appl. Anim. Behav. Sci. 193: 7-14. doi: 10.1016/j.applanim.2017.03.008.

Park M, Q. Li, N. Shcheynikov, S. Muallem, and W. Zeng. 2005. Borate transport and cell growth and proliferation. Not only in plants. Cell Cycle. 4: 24-6. doi: 10.4161/cc.4.1.1394.

Patience, J., and R. Zijlstra. 2001. Sodium, potassium, chloride, magnesium, and sulfur in swine nutrition. In: A. J. Lewis and L. L. Southern, editors, Swine Nutrition. 2nd ed. CRC Press, New York, NY. p. 213-227.

Pére M. C., M. Etienne, and J. Y. Dourmad. 2000. Adaptations of glucose metabolism in multiparous sows: effects of pregnancy and feeding level. J. Anim. Sci. 78:2933-2941. doi: 10.2527/2000.78112933x.

Père, M. C., and M. Etienne. 2007. Insulin sensitivity during pregnancy, lactation, and postweaning in primiparous gilts. J. Anim. Sci. 85:101-110. doi: 10.2527/jas.2006-130.

Pettigrew, J. E., and R. L. Moser. 1991. Fat in Swine Nutrition. Pages 133-145 in Swine Nutrition. E. R. Miller, D. E. Ullrey, and A. J. Lewis, eds. Butterworth-Heinemann, Boston, MA.

Pig Champ. 2022. Benchmark Summaries. https://www.pigchamp.com/benchmarking/benchmarking-summaries. (Accessed Jan 15, 2023).

Pluske, J. R., I. H. Williams, L. J. Zak, E. J. Clowes, A. C. Cegielski, and F. X. Aherne. 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: III. Milk production and pig growth. J. Anim. Sci. 76:1165-1171. doi: 10.2527/1998.7641165x.

Poitout, V., Hagman, D., Stein, R., Artner, I., Robertson, R. P., & Harmon, J. S. 2006. Regulation of the insulin gene by glucose and fatty acids. J. Nutr. 136(4): 873–876. doi: 10.1093/jn/136.4.873.

Power, P. P., W. G. Woods. 1997. The chemistry of boron and its speciation in plants. Plant and soil. 1931: 1-13. doi: 10.1023/A:1004231922434.

Pradhan, S. K., B. Kumar, K. B. Banakara, V. R. Patel, H. R. Pandya, and R. R. Singh. 2020. Effect of boron supplementation on the performance and metabolism of minerals in broiler chicken. Anim. Nutr. Feed Technol. 20: 39-49. doi: 10.5958/0974-181X.2020.00004.9.

Pradhan, S. K., B. Kumar, K. B. Banakara, R. R. Singh, V. B. Kharadi, and S. S. Chaudhary. 2021. Effect of boron supplementation on bone mineralization and antioxidant status in broiler chicken. Asian J. Dairy Food Res. 40(3): 309-314. doi: 10.18805/ajdfr.DR-1646.

Prime, G., and H. Symonds. 1993. Influence of plane of nutrition on portal blood flow and the metabolic clearance rate of progesterone in ovariectomized gilts. J. Agric. Sci. 121: 389-397. doi:10.1017/S0021859600085580.

Quesnel, H., S. Boulot, S. Serriere, E. Venturi, and F. Martinat-Botte. 2010. Postinsemination level of feeding does not influence embryonic survival and growth in highly prolific gilts. Anim. Reprod. Sci. 120: 120-124. doi:10.1016/j.anireprosci.2010.04.006.

Quesnel, H., L. Brossard, A. Valancogne, and N. Quiniou. 2008a. Influence of some sow characteristics on within-litter variation of piglet birth weight. Anim. 2(12):1842-1849. doi: 10.1017/S175173110800308X.

Quesnel, H., A. Renaudin, N. Le Floc'h, C. Jondreville, M. C. Pere, J. A. Taylor-Pickard, and J. Le Dividich. 2008b. Effect of organic and inorganic selenium sources in

sow diets on colostrum production and piglet response to a poor sanitary environment after weaning. Anim. 2: 859-866. doi:10.1017/s1751731108001869.

Qin, X., and H. Klandorf. 1991. Effect of dietary boron supplementation on egg production, shell quality, and calcium metabolism in aged broiler breeder hens. Poult. Sci. 70(10): 2131-2138. doi: 10.3382/ps.0702131.

Quiniou, N., J. Dagorn and D. Gaudre´. 2002. Variation of piglets' birth weight and consequences on subsequent performance. Livest. Sci. 78: 63–70. doi: 10.1016/S0301-6226(02)00181-1.

Reese, D. E., B. D. Moser, E. R. Peo, Jr., A. J. Lewis, D. R. Zimmerman, J. E. Kinder, and W. W. Stroup. 1982. Influence of energy intake during lactation on the interval from weaning to first estrus in sows. J. Anim. Sci. 55:590. doi: 10.2527/jas1982.553590x.

Ritchie, H., P. Rosado, and M. Roser. 2017. Meat and Dairy Production. Retrieved from: 'https://ourworldindata.org/meat-production' (Accessed on 02/27/2023).

Rossi, A. F., R. D. Miles, B. L. Damron, and L. K. Flunker. 1993. Effects of dietary boron supplementation on broilers. Poult Sci. 72:2124-2130. doi: 10.3382/ps.0722124.

Rossi, A. F., G. D. Butcher, and R. D. Miles. 1994. The interaction o f boron with calcium, phosphorus, and cholecalciferol in broilers. J. Appl. Anim. Res. 6:151-160. doi: 10.1080/09712119.1994.9706037.

Rowe, R. I., and C. D. Eckhert. 1999. Boron is required for zebrafish embryogenesis. J. Exp. Biol. 202:1649–1654. doi: 10.1242/jeb.202.12.1649.

Samman, S., M. R. Naghii, P. M. Wall, and A. P. Verus. 1998. The nutritional and metabolic effects of boron in humans and animals. Biol. Trace Elem. Res. 66: 27-235. doi: 10.1007/BF02783140.

Scheafer, A. L., A.K.W. Tong, A. P. Sather, E. Beltranena, E. Pharazyn, and F. X. Aherne. 1991. Preparturient diabetogenesis in primiparous gilts. Can. J. Anim. Sci. 71:69. doi: 10.4141/cjas91-008.

Schenck, E. L., K. A. McMunn, D. S. Rosenstein, R. L. Stroshine, B. D. Nielsen, B. T. Richert, J. N. Marchant-Forde, and D. C. Lay, Jr. 2008. Exercising stall-housed gestating gilts: Effects on lameness, the musculo-skeletal system, production, and behavior, J. Anim. Sci. 86(11): 3166–3180. doi:10.2527/jas.2008-1046.

Seal, B. S., and H. J. Weeth. 1980. Effect of boron in drinking water on the male laboratory rat. Bull. Environ. Contam. Toxicol. 25:782-789. doi: 10.1007/BF01985608.

Serena, A., H. Jørgensen, and K. E. Bach Knudsen. 2009. Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber. J. Anim. Sci. 87: 136-147. doi:10.2527/jas.2007-0714.

Sharma, A., V. Mani, R. P. Pal, S. Sarkar, and C. Datt. 2020. Boron supplementation in peripartum Murrah buffaloes: The effect on calcium homeostasis, bone metabolism, endocrine and antioxidant status. J. Trace. Elem. Med. Biol. 62: 126623. doi: 10.1016/j.jtemb.2020.126623.

Sharma, A., V. Mani, R. P. Pal, S. Sarkar, H. Sharma, S. Yadav, and C. Datt. 2022. Effect of boron supplementation on nutrient utilization and productive performance of peripartum Murrah buffaloes. Biol. Trace. Elem. Res. 10:4303-4315. doi: 10.1007/s12011-021-03038-w.

Sheng, M. H. C., L. Janette Taper, H. Veit, H. Qian, S. J. Ritchey, and K. H. William Lau. 2001. Dietary boron supplementation enhanced the action of estrogen, but not that of parathyroid hormone, to improve trabecular bone quality in ovariectomized rats. Biol. Trace Elem. Res. 82(1-3):109-123. doi: 10.1385/bter:82:1-3:109.

Singh, A. K., N. Kewalramani, V. Mani, A. Sharma, P. Kumari, and R. P. Pal. 2021. Effects of boric acid supplementation on bone health in crossbred calves under tropical condition. J. Trace Elem. Med. Biol. 63: 126647. doi: 10.1016/j.jtemb.2020.126647.

Skinner, J. T. and J. S. McHargue. 1945. Response of rats to boron supplements when fed rations low in potassium. Am. J. Physiol. 143: 385-390. doi: 10.1152/ajplegacy.1945.143.3.385.

Small, J. A., E. Charmley, A. V Rodd, and A.H. Fredeen. 1997. Serum mineral concentrations in relation to estrus and conception in beef heifers and cows fed conserved forage. Can. J. Anim. Sci. 77: 55–62. doi: 10.4141/A96-042.

Smith, O. B., and O. O. Akinbamijo. 2000. Micronutrients and reproduction in farm animals. Anim. Reprod. Sci. 60: 549-560. doi:10.1016/S0378-4320(00)00114-7.

Smith, A. L., K. J. Stalder, T. V. Serenius, T. J. Baas, and J. W. Mabry. 2007. Effect of piglet birth weight on weights at weaning and 42 days post weaning. J.Swine Health Prod. 15(4): 213-218.

Sommer, A. L., and C. B. Lipman. 1926. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1(3), 231-249. doi: 10.1104/pp.1.3.231.

Sprietsma, J. E., and G. E. Schuitemaker. 1994. Diabetes can be prevented by reducing insulin production. Med. Hypotheses. 42: 15-23. doi: 10.1016/0306-9877(94)90029-9.

Stalder K. J., C. Lacy, T. L. Cross, and G. E. Conatser. 2003. Financial impact of average parity of culled females in a breed-to-wean swine operation using replacement gilt net present value analysis. J Swine Health Prod. 11(2): 69-74.

Stalder, K., M. Knauer, T. Baas, M. F Rothschild, and J. Mabry. 2004. Sow longevity. Pig News and Information. 25: 53-74.

Stalder, K. J., R. C. Lacy, T. L. Cross, G. E. Conatser, and C. S. Darroch. 2000. Net present value analysis of sow longevity and the economic sensitivity of net present value to changes in production, market price, feed cost, and replacement gilt costs in a farrow-to-finish operation. Prof. Anim. Sci. 16: 33-40. doi:10.15232/S1080-7446(15)31658-2.

Stickland, N. C. 1994. The influence of early events in utero on postnatal muscle growth: A review. In 40th International Congress of Meat Science and Technology (ICoMST). The Hague. Netherlands. p 17.

Surai, P. F. 2006. Selenium in nutrition and health. Nottingham University Press. Nottingham, UK.

Surai, P. F., and V. I. Fisinin. 2014. Selenium in poultry breeder nutrition: An update. Anim. Feed Sci. Technol. 191: 1-15. doi: 10.1016/j.anifeedsci.2014.02.005.

Surai, P. F., and V. I. Fisinin. 2016. Selenium in sow nutrition. Anim. Feed Sci. Technol. 211: 18-30. doi: 10.1016/j.anifeedsci.2015.11.006.

Sutherland, B., P., Strong, and J. C. King. 1998. Determining human dietary requirements for boron. Biol. Tr. Elem. Res. 66:193-204. doi: 10.1007/BF02783138.

Taranu, I., D. E. Marin, G. Manda, M. Motiu, I. Neagoe, C. Tabuc, M. Stancu, and M. Olteanu. 2011. Assessment of the potential of a boron-fructose additive in counteracting the toxic effect of Fusarium mycotoxins. Br. J. Nutr. 106(3):398-407. doi: 10.1017/S0007114511000341.

Theil, P. K., H. Jorgensen, A. Serena, J. Hendrickson, and K. E. B. Knudsen. 2011. Products deriving from microbial fermentation are linked to insulinaemic response in pigs fed breads prepared from whole-wheat grain and wheat and rye ingredients. Brit. J. Nutr. 105:373–383. doi:10.1017/s0007114510003715.

Thingnes, S. L., E. Hallenstvedt, E. Sandberg, and T. Framstad. 2015. The effect of different dietary energy levels during rearing and mid-gestation on gilt performance and culling rate. Livest. Sci. 172: 33-42. doi: 10.1016/j.livsci.2014.11.012.

Tirpan, M. B., and N. Tekin. 2015. Effects of boron (sodium pentaborate), added instead of Tris components, on freezing and post-thaw quality of Angora buck semen. Ankara Üniv Vet Fak Derg. 62: 295-302. doi: 10.1501/Vetfak_0000002695.

Tirpan, M. B., H. Gürler, K. T. Olğaç, and A. Daşkin. 2018. Effects of boron added bull semen extender on post-thaw spermatological parameters. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 65(2): 123-128. doi: 10.1501/Vetfak_0000002837.

Travis, N. J., and E. J. Cocks. 1984. The Tincal Trail. A history of borax. Harraps. London, UK. 115-124.

Tritton, S., R. King, R. Campbell, A. Edwards, and P. Hughes. 1996. The effects of dietary protein and energy levels of diets offered during lactation on the lactational and subsequent reproductive performance of first-litter sows. Anim. Sci. 62(3): 573-579. doi:10.1017/S1357729800015125.

Vallet, J. L., L. A. Rempel, J. R. Miles, and S. K. Webel. 2014. Effect of essential fatty acid and zinc supplementation during pregnancy on birth intervals, neonatal piglet brain myelination, stillbirth, and preweaning mortality. J. Anim. Sci. 92(6): 2422-2432. doi: 10.2527/jas.2013-7130.

Venn, J., R. McCance, and E. Widdowson. 1947. Iron metabolism in piglet anaemia. J. Comp. Pathol. Ther. 57:314-325. doi: 10.1016/s0368-1742(47)80037-2.

Warrington, K., 1923. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. 37: 629-672. doi: 10.1093/oxfordjournals.aob.a089871.

Weir Jr., R. J., and R.S. Fisher. 1972. Toxicologic studies on borax and boric acid. Toxicol. Appl. Pharm. 23:351-364. doi: 10.1016/0041-008x(72)90037-3.

Weldon, W.C., A.J. Lewis, G.F. Louis, J.L. Kovar, M.A. Giesemann, and P.S. Miller. 1994. Postpartum hypophagia in primiparous sows: I. Effects of gestation feeding level on feed intake, feeding behavior, and plasma metabolite concentrations during lactation. J. Anim. Sci. 72(2): 387-394. doi: 10.2527/1994.722387x.

Wiley, H. W. 1904. Influence of Food Preservation and Artificial Colors on Digestion and Health: I. Boric Acid and Borax. Washington, DC: Government Printing Office; US Department of Agriculture Bulletin No 84, Pt 1.

Wilson, J. H., and P. L. Ruszler. 1997. Effects of boron on growing pullets. Biol. Trace Elem. Res. 56:287-294. doi: 10.1007/BF02785300.

Wilson, J. H., and P. L. Ruszler. 1998. Long term effects of boron on layer bone strength and production parameters. Br. Poult. Sci. 39:11–15. doi: 10.1080/00071669889312.

Whittemore, C.T. 2006. The science and practice of pig production. Eds I. Kyriazakis and C. T. Whittemore, 3rd Edition. Blackwell Science Ltd., UK.

World Health Organization. 1996. International Atomic Energy Agency & Food and Agriculture Organization of the United Nations. Trace elements in human nutrition and health. World Health Organization. https://apps.who.int/iris/handle/10665/37931.

Ullrey, D. E. 1992. Basis for regulation of selenium supplements in animal diets. J. Anim. Sci. 70:3922-39270. doi: 10.2527/1992.70123922x.

United Nations Department of Economic and Social Affairs, Population Division. 2022. World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3.

Usuda, K., K. Kono, Y. Orita, T. Dote, K. Iguchi, H. Nishiura, M. Tominaga, T. Tagawa, E. Goto, and Y. Shirai. 1998. Serum and urinary boron levels in rats after a single administration of sodium tetraborate. Arch. Toxicol. 72(8):468-474. doi: 10.1007/s002040050530.

Vallet, J. L., and B. A. Freking. 2005. Research on uterine capacity and litter size in swine. USDA Agricultural Research Service. Clay Center, NE.

Yeni, D., F. Avdatek, and M. Gündoğan. 2018. The effect of Boron addition on spermatological parameters, oxidative stress and DNA damage after frozen-thawed process in ramlic ram semen. Sağlık Bilimleri Veteriner Dergisi, Fırat Üniversitesi, 32: 53-57.

Young, M. G., M. D. Tokach, F. X. Aherne, R. G. Main, S. S. Dritz, R.D. Goodband, J. L. Nelssen. 2004. Comparison of three methods of feeding sows in gestation and the subsequent effects on lactation performance. J. Anim. Sci. 82: 3058-3070. doi: 10.2527/2004.82103058x.

Zang, J. J., J. S. Chen, J. Tian, A. N. Wang, H. Liu, S. D. Hu, X. R. Che, Y. X. Ma, J. J. Wang, C. L. Wang, G. H. Du, and X. Ma. 2014. Effects of magnesium on the

performance of sows and their piglets. J. Anim. Sci. Biotechnol. 5: 39. doi: 10.1186/ 2049-1891-5-39.

VITA

Tyler B. Chevalier

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EDUCATION

Doctor of Philosophy in Animal Sciences, University of Kentucky 2020-2023 (Expected)

Concentration: Swine and Poultry Nutrition

Dissertation: Evaluation of boron supplementation in swine and poultry

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Master of Science in Animal Sciences, University of Kentucky 2018-2019

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Thesis: Improved iron status in weanling pigs leads to improved growth performance in the subsequent nursery period

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EXPERIENCE

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Graduate Research Assistant, January 2018 – December 2021

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Smithfield Foods, Inc. Science and Technology Intern, May 2017 - August 2017

Production Research for Hog Production Division

Undergraduate Research Assistant, October 2015 – December 2017

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Student Farm Assistant, August 2015 – December 2017

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PUBLICATIONS

First Author

Chevalier, T.B., and M.D. Lindemann. 2022. Injecting different amounts of iron – Effects on blood parameters during late nursery-early grower phase. Proceed. Midwest Swine Nutr. Conf. Indianapolis, IN.

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2021. Effects of iron dosage administered to newborn piglets on hematological measures, preweaning and postweaning growth performance, and postweaning tissue mineral content. Journal of Swine Health and Production. 29(4):189-199.

Chevalier, T.B. 2019. Improved iron status in weanling pigs leads to improved growth performance in the subsequent nursery period. Theses and Dissertations--Animal and Food Sciences. 111. University of Kentucky. doi: 10.13023/etd.2019.442.

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Elefson, S.K., N. Lu, **T. Chevalier**, S. Dierking, D. Wang, H.J. Monegue, J.C. Matthews, Y.D. Jang, J. Chen, G.K. Rentfrow, S.A. Adedokun, and M.D. Lindemann. 2021. Assessment of visceral organ growth in pigs from birth through 150 kg. Journal of Animal Science. 99(9): 1-11. skab249. doi: 10.1093/jas/skab249.

ABSTRACT/PRESENTATIONS

European Symposium of Porcine Health Management 2023 (Accepted)

Chevalier, T.B., Lyons J.W., D.B. Paczosa, G.K. Rentfrow, and M.D. Lindemann. 2023. Effects of an additional injection of iron administered to piglets on hemoglobin concentration, growth performance, and carcass characteristics through market weight. To be presented at ESPHM, Greece, May 31-June 2, 2023.

Pierce, J.L., J.W. Lyons, **T.B. Chevalier**, D.B. Paczosa, and M.D. Lindemann. 2023. Effects of an additional iron dextran injection administered to piglets on differential gene expression and metabolic pathway changes at weaning. To be presented at ESPHM, Greece, May 31-June 2, 2023.

AASV Annual Meeting 2023

Chevalier, T.B., J.W. Lyons, D.B. Paczosa, G.K. Rentfrow, and M.D. Lindemann. 2023. Effects of an additional iron dextran injection administered to piglets on hemoglobin, performance, and carcass responses. In: 54th Annual Meeting of the American Association of Swine Veterinarians. Aurora, CO.

Pierce, J.L., J.W. Lyons, **T.B. Chevalier**, D.B. Paczosa, and M.D. Lindemann. 2023. Effects of an additional iron dextran injection administered to piglets on

differential gene expression at weaning. In: 54th Annual Meeting of the American Association of Swine Veterinarians. Aurora, CO.

ASAS Midwest Section Meeting 2023

Paczosa, D.B., **T.B. Chevalier**, and M. D. Lindemann. 2023. Effects of feeding varying levels of mycotoxin-containing corn fines on diet choice and growth performance of nursery pigs.

Pearce, J.L, J.W. Lyons, **T.B. Chevalier**, D.B. Paczosa, and M.D. Lindemann. 2023. Effects of a second Uniferon injection at castration on weaning liver and intestinal gene expression.

Chevalier, T.B., D.B. Paczosa, J.W. Lyons, G.K. Rentfrow, and M.D. Lindemann. 2023. Effects of an additional injection of iron administered to piglets on hemoglobin concentration, growth performance, and carcass characteristics through market weight.

Paczosa, D.B., **T.B. Chevalier**, L. Zheng, F. Berry, and M.D. Lindemann. 2023. Evaluation of increasing levels of mycotoxin-containing corn fines fed to nursery pigs on growth performance.

The International Poultry Scientific Forum 2023

Chevalier, T.B., S.A. Adedokun, and M.D. Lindemann. 2023. Evaluation of boron supplementation to a semi-purified diet fed to broiler chicks on growth performance, tibia mineral composition, and apparent total tract digestibility of nutrients.

University of Kentucky 10th Annual Poster Symposium

Paczosa, D.B., **T.B. Chevalier**, and M. D. Lindemann, Effects of feeding varying levels of mycotoxins on diet choice and growth performance of nursery pigs. University of Kentucky, Lexington, KY.

ASAS Midwest Section Meeting 2022

Chevalier, T.B. and M.D. Lindemann. 2022. PSV-6 Effects of boron supplementation on apparent digestibility and retention of nutrients in growing pigs. Journal of Animal Science. 100(Suppl 2): 155. doi: 10.1093/jas/skac064.263.

ASAS-CSAS Annual Meeting 2021

Chevalier, T.B. and M.D. Lindemann. 2021. PSVI-9 Effects of dietary boron supplementation on growth performance, fasting and postprandial serum insulin, and glucose concentration of pigs. Journal of Animal Science. 99(Suppl 3): 398. doi: 10.1093/jas/skab235.724.

Lee, J.W., **T.B. Chevalier**, H.J. Monegue, and M.D. Lindemann. 2021. PSIV-B-18 Performance response of weanling and grower pigs fed graded levels of poultry byproduct meal (PBM) in the diet. Journal of Animal Science 99(Suppl 3): 392. doi: 10.1093/jas/skab235.714.

ASAS Midwest Section Meeting 2021

Lee, J.W., **T.B. Chevalier**, C. Sparks, T.D. Crenshaw, and M.D. Lindemann. 2021. Growth performance, bone mineralization, and nutrient digestibility of nursery-grower pigs fed phytase-supplemented calcium and phosphorus-deficient diets., Journal of Animal Science. 99(Suppl 1): 48–49. doi: 10.1093/jas/skab054.083.

Chevalier, T.B., O. Adeola, S.D. Carter, C.R. Dove, M.J. Estienne, C.L. Levesque, C.V. Maxwell, T.C. Tsai, and M.D. Lindemann. 2021. PSIV-9 A multistate evaluation of an additional iron injection administered to piglets before weaning. Journal of Animal Science. 99(Suppl 1): 184–185. doi: 10.1093/jas/skab054.308.

Chevalier, T.B., J. Ferrel, and M.D. Lindemann. 2021. PSIV-10 The effect of a dacitic (rhyolitic) tuff breccia, a hydrated sodium calcium aluminosilicate (HSCAS), inclusion in corn-soybean meal diets on growth performance of individually housed grower pigs, Journal of Animal Science. 99(Suppl 1):178. doi: 10.1093/jas/skab054.301.

ASAS Midwest Section Meeting 2020

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2020. Effects of increasing iron dosage at birth on the hematological profile and growth performance of piglets during the lactation period. Journal of Animal Science. 98(Suppl 3):41. doi: 10.1093/jas/skaa054.073.

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2020. PSVIII-12 Effects of increasing iron dosage at birth on hematological profile, growth performance, and tissue mineral concentrations of nursery pigs. Journal of Animal Science. 98(Suppl 3): 208–209. doi: 10.1093/jas/skaa054.361.

ASAS-CSAS Annual Meeting 2019

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2019. PSIV-12 Effects of an additional iron injection administered to piglets before weaning. Journal of Animal Science. 97(Suppl 3): 227. doi: 10.1093/jas/skz258.462.

University of Kentucky 9th Annual Poster Symposium

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2019. Effects of an additional iron injection administered to piglets before weaning. University of Kentucky, Lexington, KY.

University of Kentucky 8th Annual Poster Symposium

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2018. Assessment of the iron status of young pigs in a confinement herd. University of Kentucky, Lexington, KY.

ASAS Midwest Section Meeting 2018

Chevalier, T.B., H.J. Monegue, and M.D. Lindemann. 2018. Assessment of the iron status of young pigs in a confinement herd. Journal of Animal Science. 96(Suppl 2): 278. doi: 10.1093/jas/sky073.511.

AWARDS

2nd place MS student poster competition

ASAS-CSAS Annual Meeting 2019

1st place MS student poster competition

University of Kentucky 9th annual poster symposium 2019

2nd place MS student poster competition

University of Kentucky 8th annual poster symposium 2018