Nutritional and management practices for improved sow productivity and progeny performance

by

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B.S., Mississippi State University, 2020
M.S., North Carolina State University, 2022

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Abstract

A total of 7 experiments structured in 6 chapters were performed to investigate the effects of yeast probiotics in lactation and a pre/probiotic combination in nursery diets, evaluate the impact of increased histidine in lactation diets, assess increased standardized ileal digestible lysine levels through additional soybean meal in late gestation, examine allotment strategies in the nursery, assess calcium administration protocols around farrowing, and determine the optimal litter size in relation to functional teat count during lactation. Chapter 1 utilized 720 pigs to evaluate three allotment strategies aimed at assessing variation and determining replications required to detect statistical significance in nursery pig research. Overall, allotting pigs to pens using a bodyweight (BW) grouping strategy resulted in the least within-pen and pen-to-pen variation. The BW grouping method required the fewest pens to detect statistically significant differences. Chapter 2 utilized 28 mixed-parity sows and their offspring to evaluate live yeast supplementation during lactation with or without a pre/probiotic combination during the nursery period on lactation performance, lifetime growth performance, and immune response. The effects of combining a yeast probiotic in lactation diets and a pre/probiotic in nursery diets were not additive. However, feeding a live yeast probiotic during lactation resulted in increased progeny final BW and hot carcass weight. Chapter 3 utilized 87 mixed-parity sows and their offspring to evaluate the effects of increasing standardized ileal digestible (SID) lysine in late gestation diets on lactating sow and litter performance. Increased SID lysine through additional soybean resulted in increased late gestation sow weight gain and piglet average daily gain (ADG) during early lactation. However, litter ADG during late lactation and overall lactation was maximized in litters from sows fed 15.8 g/d of SID lysine. Chapter 4 utilized a total of 88 mixed-parity sows and their litters to evaluate the effects of increasing SID histidine:lysine ratio on sow and litter

performance. Overall, sows fed diets with an SID histidine:lysine ratio of 50% had heavier litters on day 7 of lactation and at weaning and greater calculated average daily milk yield. Chapter 5 utilized 1,005 mixed-parity sows and their litters to evaluate the effect of initial litter size relative to functional teat count on sow and litter performance. Overall, sows with 1 less pig than functional teats after cross-fostering had the lowest pig mortality and sow BW loss and greatest pig weaning weight. However, for the greatest number of pigs weaned per litter and pigs weaned per sow per year, 2 more pigs than functional teats after cross-fostering was ideal. Chapter 6 utilized 933 mixed-parity sows and their litters to evaluate calcium administration protocols around farrowing on sow performance. In the overall population, topdressing calcium chloride before farrowing or injecting calcium gluconate peripartum altered sow metabolites but did not influence farrowing performance. However, when comparing at-risk sows among the 3 treatments, administration of calcium gluconate decreased stillbirths and increased percentage of pigs born alive.

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Acknowledgements

The completion of this dissertation would not have been possible without the support, guidance, and mentorship of an incredible number of people over the past three years. First and foremost, I would like to thank my committee: Dr. Jason Woodworth, Dr. Jordan Gebhardt, Dr. Mike Tokach, Dr. Joel DeRouchey, and Dr. Bob Goodband. I am grateful for your endless wealth of knowledge and your unparalleled passion for helping swine producers find meaningful answers to important questions. Throughout my time at Kansas State, each of you challenged me to grow as a swine nutritionist, leader, mentor, and professional. It has truly been an honor and a privilege to learn from some of the best applied swine nutritionists in the world.

I have also been fortunate to collaborate with several outstanding industry partners. Thank you to Joe Loughmiller and Brian Kremer at Phileo by Lesaffre, Keith Haydon at CJ Bio America, and Jose Soto and Andrew Bents at Alltech. Your support made this dissertation possible, and I've learned so much from each of you, lessons that will continue to shape me as a swine nutritionist. A special thank you to Kyle Coble and PJ Corns at JBS Live Pork for your mentorship and trust in allowing me to conduct impactful research. I truly believe my experiences with JBS changed the trajectory of my career. I am also grateful to the Longview 100 farm crew, Erik Darrington, Dr. Rebecca Robbins, Sara Virdis, Olivia Cataldo, and Sierra Collier for your invaluable help collecting data for the two commercial sow trials that are part of this dissertation.

I have been lucky to work alongside an incredible group of graduate students across species and disciplines. To the Weber 217 team, thank you for the early mornings at the farm, 24-hour farrowing watches, and the collection of the 54,739 individual pig weights contained in this dissertation. More importantly, thank you for the friendships I will forever cherish. You

became my family away from home and brought me countless laughs along the way. To the Hillcrest girls: Mikayla, Ally, Ashley, Sara, and Sierra, thank you for the Sunday night ice cream runs, the vent sessions, the love, and the fun. I have been blessed with the most amazing roommates during my time at K-State.

To my family and friends: thank you isn't enough to sum up how much I have appreciated the love, support, and prayers each of you has given me the past three years. Mom and dad, thanks for encouraging me to always chase my big dreams and teaching me the value of hard work, respect, and compassion. The sacrifices that you both have made to ensure that I had the most amazing opportunities in life did not go unnoticed. Thank you mom for sharing your love of livestock with me, I have you to thank for introducing me to my passion in life. Emily, thank you for being my biggest cheerleader and the one that I can always count on to be in my corner. Christopher, thank you for always being there for much needed laugh or to distract me from the stress during this process. Travis and Alissa, thank you for being the best bonus siblings that I could ask for. Joey, Jordan, Jack, and Tanner: you have brought me endless joy along the way and taught me just how much love my heart can hold. Jessica, you are my very best friend. Even from across the country, you've supported me without hesitation, even as I subjected you to countless hours of pig talk.

Lastly and most importantly, I would like to thank my Lord and Savior, Jesus Christ for Your grace and mercy. Thank you for being faithful to complete the work that You began in me. Psalm 23:6.

Chapter 1 - Technical note: Utilization of various allotment strategies to evaluate variation and replications required to detect statistical significance in nursery pig research

ABSTRACT

A total of 720 barrows (Line 200 × 400, DNA Genetics) were used in two 42-d nursery trials (initially 6.20 ± 0.12 kg and 5.63 ± 0.16 kg, respectively) to evaluate strategies for allotting pigs to pens in randomized controlled trials. At placement, the population was split into 3 cohorts with similar average weight and standard deviation and randomly assigned to 1 of 3 allotment strategies. Strategy 1 (random) utilized a simple randomization strategy with each pig randomized to pens independent of all other pigs. Strategy 2 [body weight (BW) distribution] sorted each pig within the cohort into 1 of 5 BW groups. One pig from each weight group was then randomly assigned to a pen such that distribution of BW within pen was uniform across pens. Strategy 3 (BW grouping) sorted pigs within the cohort into 3 BW categories: light, medium, and heavy. Within each BW category, pigs were randomized to pen to create pens of pigs from each BW category. Within each experiment, there were 72 pens with 5 pigs per pen and 24 pens per allotment strategy. For all strategies, once pigs were allotted to pens, pens were allotted to 1 of 2 treatments for a concurrent trial. In Exp. 1, environmental enrichment using ropes tied near the pan of the feeder was compared to a control with no enrichment. In Exp. 2, treatment diets consisted of basal levels of Zn and Cu from the trace mineral premix for the duration of the study (110 and 17 mg/kg, respectively; control), or diets (supplemented control) with carbadox (50 g/ton; Mecadox, Phibro Animal Health, Teaneck, NJ) fed in phase 1 (d 0 to 22) and 2 (d 22 to 43), pharmacological levels of Zn and Cu (2,414 mg/kg Zn from ZnO; 168

mg/kg Cu from $CuSO_4$) fed in phase 1, and only pharmacological levels of Cu (168 mg/kg Cu from $CuSO_4$) fed in phase 2. These treatment designs were used to determine the impact on coefficient of variation (CV) and to estimate the number of replications required to find significant treatment differences based on allotment strategy. There were no meaningful allotment strategy × treatment interactions for either study. For between-pen CV, pigs allotted using BW distribution and BW grouping strategies had the lowest CV at allotment and final weight in both trials. For overall average daily gain in Exp. 1 and 2 in Exp. 2, the BW distribution strategy required the fewest replications to detect differences in performance. However, there is no meaningful difference between allotment strategies in replications required to detect significant differences for gain:feed ratio.

INTRODUCTION

Minimizing variation between experimental units is one of the foremost goals of conducting randomized controlled trials as reducing variability can lead to an increased likelihood of finding a statistically significant difference if one exists. There are numerous factors that can increase body weight (BW) variability during the nursery period, the largest of which are weaning weight and weaning stressors (Wellock et al., 2004). However, experimental design is perhaps the most influential method of reducing variability. Allotments are a crucial part of experimental design as it is the method in which animals are assigned to experimental units (most often this is the pen) and experimental units are then assigned to a treatment. Often, randomization is included as a component of allotments to reduce the effects of unknown factors or bias that may influence results (Aaron and Hays, 1991). During the process of randomizing experimental units or pens to treatments, a completely randomized design (CRD) is the simplest of the allotment strategies. In this allotment procedure, experimental units are allotted to

treatments independently of all factors, including body weight, gender, or age. Another common allotment strategy is the randomized complete block design (RCBD) which assigns experimental units to blocks based on an indicated factor, most commonly weight, and then randomly assigns experimental units to a treatment within block. Past research has characterized the effect of blocking on the power to detect differences, suggesting that the reduction of degrees of freedom in the error term leads to a reduced power to detect differences with an RCBD when compared to a CRD (Shelton et al., 2009). However, more research is needed to evaluate the variation that results from different approaches for allotment. A power analysis with an established power and sensitivity should be conducted when designing an experiment to determine the number of experimental units required to ensure that a significant effect will be found if one exists (Aaron and Hays, 2004). Research that investigates techniques for formation of experimental units (assignment of animals to pen) and their effects on statistical analysis are nonexistent in the literature. The most common method for construction of experimental units is a randomized approach that may include computer generated numbers to determine which animals are assigned to each experimental unit (Johnson and Besselsen, 2002), or by simply putting pigs in pens in a random manner as they enter the barn. Therefore, the objective of this study was to appraise multiple techniques to allot pigs to pens in swine nursery research to ascertain differences in variation within and between experimental units and how that may affect the number of replications required to detect treatment differences.

MATERIALS AND METHODS

Animal treatment and structure

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4506). A total of 720 barrows (Line

200 × 400, DNA Genetics; initially 6.20 ± 0.12 kg and 5.63 ± 0.16 kg, respectively) were used in two 42-d trials. Two barns were utilized for each trial with 72 pens per trial (36 pens per barn) and 5 pigs per pen. Within each experiment, there were 24 pens per allotment strategy. Pigs were weaned and transported to the research facility at approximately 21 d of age. Each pen (1.2 × 1.2 m) contained a 4-hole, dry self-feeder, and cup waterer for ad libitum access to feed and water.

Upon entry into the facility, pigs were individually weighed and tagged. Excel (Microsoft Corporation, Redmond, WA) was used to sort the pigs from lightest to heaviest after which cohorts of 3 were created such that the lightest 3 pigs were a cohort, the next 3 lightest pigs were a cohort, and this process continued until all animals were assigned a weight cohort. Then, pigs were randomized to allotment strategy within cohort so that each allotment strategy was assigned one pig from each cohort. This step ensured that underlying populations used in each allotment strategy were as similar as possible which was determined to be accurate based on summarizing the mean and standard deviation of starting weights of the populations assigned to each allotment strategy.

The first allotment strategy (random) utilized a completely randomized design to allot pigs to pens (Fig. 1). Strategy 2 (BW distribution) required that pigs be sorted by BW and assigned to 1 of 5 BW strata such that the lightest 20% of pigs were stratum 1, the next lightest 20% of pigs were stratum 2, and this process continued until all animals within this strategy were assigned to a BW stratum. Then, pigs were randomly assigned to pens such that each pen contained 1 pig from each BW stratum which created a relatively consistent distribution of BW across pen. In strategy 3 (BW grouping), pigs were sorted by BW and assigned to one of three BW groups: light, medium, and heavy. Then, within each BW group, pigs were randomized to

pens to create pens of a single BW group (pens of light pigs, pens of medium pigs, and pens of heavy pigs).

After pigs were allotted to pens, pens were allotted to 1 of 2 environmental enrichment treatments in Exp. 1. The first treatment consisted of no environmental enrichment. The second treatment, defined as environmental enrichment, utilized rope secured to the divider between feeding spaces in the feeder pan from d 0 to 10 as a way to try to stimulate pigs to find feed faster immediately after weaning. For Exp. 2, dietary treatments consisted of a control diet and the control supplemented with growth-promoting ingredients (Supplemental Table 1). Phase 1 diets were fed in pelleted form from d 0 to 22. Phase 2 treatments were fed in meal form from d 22 to 43. The control diets for both phases were formulated to meet the NRC (2012) requirement estimates for Zn (110 mg/kg) and Cu (17 mg/kg) with no feed-grade antibiotic. The phase 1 supplemented control diet was formulated to contain pharmacological levels of Zn (2,414 mg/kg from ZnO) and Cu (168 mg/kg from $CuSO_4$) with addition of carbadox (55 mg/kg; Mecadox, Phibro Animal Health, Teaneck, NJ). The phase 2 supplemented control diet contained pharmacological levels of copper (168 mg/kg from $CuSO_4$) and also contained carbadox (55 mg/kg; Mecadox, Phibro Animal Health, Teaneck, NJ). These dietary treatments in Exp. 2 were designed to increase the likelihood of finding a difference among the treatments.

During Exp. 1, pigs were individually weighed on d 0, 3, 10, 14, 21, and 42. Feeders were weighed daily from d 0 to 14 and again on d 21. During Exp. 2 feeders and pigs were individually weighed on d 0, 8, 15, 22, 29, 36, and 43 to ascertain average daily gain (ADG), coefficient of BW variation (CV), average daily feed intake (ADFI), and gain:feed ratio (G:F). Daily feed deliveries were recorded for both experiments. Records of injections administered were also kept and analyzed for dietary treatment differences (Exp. 2).

Statistical analysis

Growth performance data were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) with pen considered the experimental unit. To test the effect of strategy on growth performance outcomes, allotment strategy was included in the model as a fixed effect. Growth performance data were also analyzed to test the effect of dietary treatment (Exp. 2) or environmental enrichment (Exp. 1) using the emmeans function in R using a Tukey multiple comparison adjustment. The GLIMMIX procedure of SAS (version 9.4; Cary, NC) was used to analyze the effect of dietary treatment on number of injections per 1,000 d (Exp. 2). A Poisson distribution was utilized with an offset function of the log transformed number of days at risk for each experimental unit. Treatment was included in the model as a fixed effect and barn as a random effect. Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$. Using the SEM from the dietary treatment and environmental enrichment analyses, the standard deviation was calculated and used to calculate sample size required to detect a statistically significant difference of an indicated magnitude between two treatments using the equation described by Dohoo et al. (2014): $n = 2 \times \left[\frac{\left(Z_{\frac{\alpha}{2}} - Z_{\beta}\right)^2 \times \sigma^2}{(\mu_1 - \mu_2)^2}\right]$. $Z_{\frac{\alpha}{2}}$ (1.96) represented the Z score of α, when σ was 0.05. Z_{β} (-0.84) represented the Z score of β, when β was 0.20. The standard deviation (σ) of the statistical population for each parameter of interest was calculated by multiplying the SEM of that parameter of interest within each allotment strategy by the square root of the number of pens per allotment strategy. μ_1 was calculated by averaging the performance between the two dietary or environmental treatments. μ_2 was calculated by multiplying μ_1 by specified percentages of

improvement. Values reported within the BW grouping strategy are values for total pens within each treatment group.

RESULTS AND DISCUSSION

There were no meaningful allotment strategy × treatment interactions for either study. Within the BW grouping strategy, there were no BW group × treatment interactions for either experiment. In Exp. 1. there were no differences in average BW, ADG, ADFI, or G:F based on allotment strategy or environmental enrichment at any point in the study (data not shown). For Exp. 2, there were no diet × allotment strategy interactions. Pigs that were fed the diet supplemented with growth-promoting ingredients were heavier at each weighing event after allotment ($P < 0.05$; Supplemental Table 2) and exhibited increased ADG and ADFI throughout the duration of the study ($P < 0.001$). Phase 1 and overall G:F was improved in pigs fed the supplemented control diet ($P < 0.001$); however, there was only a marginally significant improvement in feed efficiency during phase 2 of the study ($P = 0.072$). Pigs fed the supplemented-control diet also required 7 less total injections per 1,000 pig days ($P = 0.020$). The lack of observed differences in growth performance due to environmental enrichment (Exp. 1) is consistent with previous research. The observed differences due to dietary treatment in Exp. 2 were expected and dietary treatment was used to create differences between treatment to further evaluate the effect of allotment strategy and estimate the sample size required to find statistical differences. Body weight and ADFI were not different between the three different allotment strategies throughout the entire study (Supplemental Table 3). Phase 1 G:F was improved in those pigs allotted with the BW grouping strategy compared to those pigs allotted using the random strategy ($P < 0.05$), with those allotted using the BW distribution strategy being intermediate. There was a marginally significant improvement overall G:F in pigs allotted

using the BW grouping strategy compared to the other allotment strategies ($P = 0.065$). However, pairwise comparisons revealed no means separation between allotment strategies for overall G:F. Additionally, there was a marginally significant ($P = 0.055$) increase in ADG from d 22 to 43 in pigs of the BW grouping strategy compared to those allotted using the random allotment strategy with the BW distribution being intermediate.

Coefficient of variation within pen is the variation between animals in each pen. This CV calculation is important in ascertaining the effect that different allotment strategies have on individual animals. By design, the within-pen CV was different for the different allotment strategies at the start of the trial. When considering CV of pig BW within pen, the BW grouping allotment strategy had the lowest CV at allotment and throughout the entirety of both trials (Fig. 2). In both experiments, the within-pen CV for the random and BW distribution strategies remained relatively similar throughout the trial. In Exp. 1, the within-pen CV for the BW distribution strategy was slightly lower than that of the random strategy but for Exp. 2, there was no discernable difference in within-pen CV between the two strategies.

Coefficient of variation between pens is the largest determinant in the sample size calculation. While within-pen CV characterizes the BW variation within a pen, between-pen CV depicts the variation of the average BW between pens. Minimizing the between-pen CV was the largest objective of this trial to decrease sample size required to detect statistically significant differences. Coefficient of variation of the mean body weight between pens was greatest for the random allotment strategy at the time of allotment for both trials (Fig. 3). While the difference between the random allotment strategy and the other two allotment strategies for between-pen CV decreased over time, the random allotment strategy still concluded both trials with a higher between-pen CV. In Exp. 1, the between-pen CV remained consistent over time, whereas in Exp.

2, there was an increase in between-pen CV at the completion of phase 1 and then a decline at the conclusion of the trial. Differences in between-pen CV based on allotment strategy were minimal at the conclusion of Exp. 2.

The population CV was the same for all 3 allotment strategies at the beginning of both trials (Supplemental Table 4). This was accomplished through the formation of cohorts which created extremely similar starting populations for all 3 strategies. The population CV remained fairly consistent for all strategies throughout Exp. 1. For Exp. 2, there was an increase in population CV until d 29 for all strategies and then a decline thereafter. This reduction in CV as BW increases is in agreement with other models that investigated change in CV over time (Tolosa et al., 2021). The population CVs for Exp. 2 were higher than those of Exp. 1 for the duration of the trial. This can be attributed to a slightly more variable population at allotment and the use of the control and supplemented dietary treatments which were formulated to cause greater differences in growth performance.

When considering the number of replications (pens) required to detect significant differences between treatments with different allotment strategies, there were differences in which strategy required the fewest replications based on parameter of interest (Table 1). For d 21 BW, the BW distribution and BW grouping strategies required less than half the replications than the random strategy for both experiments. For final BW, replicates required for the random allotment strategy were much higher than those of the BW distribution and BW grouping strategies for Exp. 1. However, for Exp. 2, the BW grouping strategy required the fewest replications followed by the random allotment strategy and then the BW distribution strategy. Differences in replications required to detect differences became minor as the expected response got larger. Replications required to detect significant differences were the highest for d 0 to 21

ADG and ADFI for both experiments. Replications required decreased dramatically for ADG and ADFI when the overall data was examined, which speaks to a decrease in variation between experimental units over time. For overall ADG in Exp. 1 and 2 and overall ADFI in Exp. 2, the BW grouping strategy required the fewest replications to detect differences in performance. The random strategy mandated the fewest replications to detect differences for overall G:F; however, differences between allotment strategies were minor at more than 6% expected improvement between treatments. Overall, fewer replications would be required to find similar percentage responses when allotting pigs using the BW grouping strategy for the largest number of growth performance measurements as compared to the other allotment strategies.

Previous research has shown no effect on growth performance based on uniformity of pens post-weaning (Cámara et al., 2016) but no research is available on how uniformity, or lack thereof may effect replications required in nursery research. While this experiment investigated the effects of accounting for variation prior to animal research beginning, others have looked at the use of a covariate to account for variation after the completion of the trial. Use of initial BW as a covariate and blocking based on previous diet consumed resulted in an increased ability to detect differences in digestibility (Jacobs et al., 2013).

When conducting nursery research with pen serving as the experimental unit, the data herein would support that allotting pigs to pens using a BW grouping strategy would result in the least within-pen and pen-to-pen variation. This is contrary to the more commonly utilized method of complete randomization when assigning animals to pens which often required the highest number of replications to find significant differences.

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Table 1.1 Number of replicates (pens) required to detect a significant difference between treatments with different allotment strategies[1,2]

	Expected response, %				
Item	2	4	6	8	10
d 21 BW					
Exp. 1					
Random[3]	723	181	81	46	29
BW distribution[4]	246	62	28	16	10
BW grouping[5]	238	60	27	15	10
Exp. 2					
Random[3]	240	60	27	15	10
BW distribution[4]	112	28	13	7	5
BW grouping[5]	114	29	13	8	5
d 42 BW					
Exp. 1					
Random[3]	236	59	27	15	10
BW distribution[4]	141	36	16	9	6
BW grouping[5]	110	28	13	7	5
Exp. 2					
Random[3]	77	20	9	5	4
BW distribution[4]	93	24	11	6	4
BW grouping[5]	52	13	6	4	3
ADG (d 0 to 21)					
Exp. 1 (d 0 to 21)					
Random[3]	1,977	495	220	124	80
BW distribution[4]	897	225	100	57	36
BW grouping[5]	906	227	101	57	37
Exp. 2 (d 0 to 22)					
Random[3]	3,618	905	402	227	145
BW distribution[4]	942	236	105	59	38
BW grouping[5]	619	155	69	39	25
ADFI (d 0 to 21)					
Exp. 1 (d 0 to 21)					
Random[3]	970	243	108	61	39
BW distribution[4]	668	167	75	42	27
BW grouping[5]	765	192	85	48	31
Exp. 2 (d 0 to 22)					
Random[3]	1,705	427	190	107	69
BW distribution[4]	377	95	42	24	16
BW grouping[5]	418	105	47	27	17
G:F (d 0 to 21)					
Exp. 1 (d 0 to 21)					
Random[3]	299	75	34	19	12
BW distribution[4]	138	35	16	9	6
BW grouping[5]	316	79	36	20	13
Exp. 2 (d 0 to 22)					

Random[3]	663	166	74	42	27
BW distribution[4]	311	78	35	20	13
BW grouping[5]	200	50	23	13	8
Nursery ADG					
Exp. 1 (d 0 to 42)					
Random[3]	236	59	27	15	10
BW distribution[4]	347	87	39	22	14
BW grouping[5]	215	54	24	14	9
Exp. 2 (d 0 to 43)					
Random[3]	295	74	33	19	12
BW distribution[4]	197	50	22	13	8
BW grouping[5]	141	36	16	9	6
Nursery ADFI					
Exp. 2 (d 0 to 43)					
Random[3]	261	66	29	17	11
BW distribution[4]	182	46	21	12	8
BW grouping[5]	129	33	15	9	6
Nursery G:F					
Exp. 2 (d 0 to 43)					
Random[3]	45	12	5	3	2
BW distribution[4]	72	18	8	5	3
BW grouping[5]	63	16	7	4	3

[1]A total of 720 barrows (DNA 200 × 400, initially 6.20 ± 0.12 kg and 5.63 ± 0.16 kg, respectively) were used in two 42-d nursery trials with 5 pigs per pen and 24 replications per allotment strategy. Sample size calculations assume an α of 0.05 with 80% power. Values reported as number of pens per treatment to detect a statistically significant difference between treatments of indicated magnitude. Values reported within body weight (BW) grouping strategy are values for numbers of pens total within each treatment group, not number of pens within each BW group.

[2]ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio.

[3]Pigs were allotted to pens in a completely randomized manner.

[4]Pigs were sorted by BW into five groups. Within each BW group, pigs were randomized to pen such that each pen consisted of 1 random pig from each BW group to ensure that distribution of BW within pen was relatively consistent across pens.

[5]Pigs were sorted into three groups by BW (light, medium, heavy). Within each BW group, pigs were randomized to pen such that each pen consisted of pigs from a single BW group (pens of light pigs, pens of medium pigs, pens of heavy pigs).

Figure 1.1 Allotment strategies.
Strategy 1 (random) utilized a simple randomization strategy with each pig randomized to pen independent of all other pigs. Strategy 2 [body weight (BW) distribution] sorted each pig within the cohort into 1 of 5 BW groups. One pig from each weight group was then randomly assigned to a pen such that distribution of BW within pen was uniform across pens. Strategy 3 (BW grouping) sorted pigs within the cohort into 3 BW categories: light, medium, and heavy. Within each BW category, pigs were randomized to pen to create pens of pigs from each BW category.

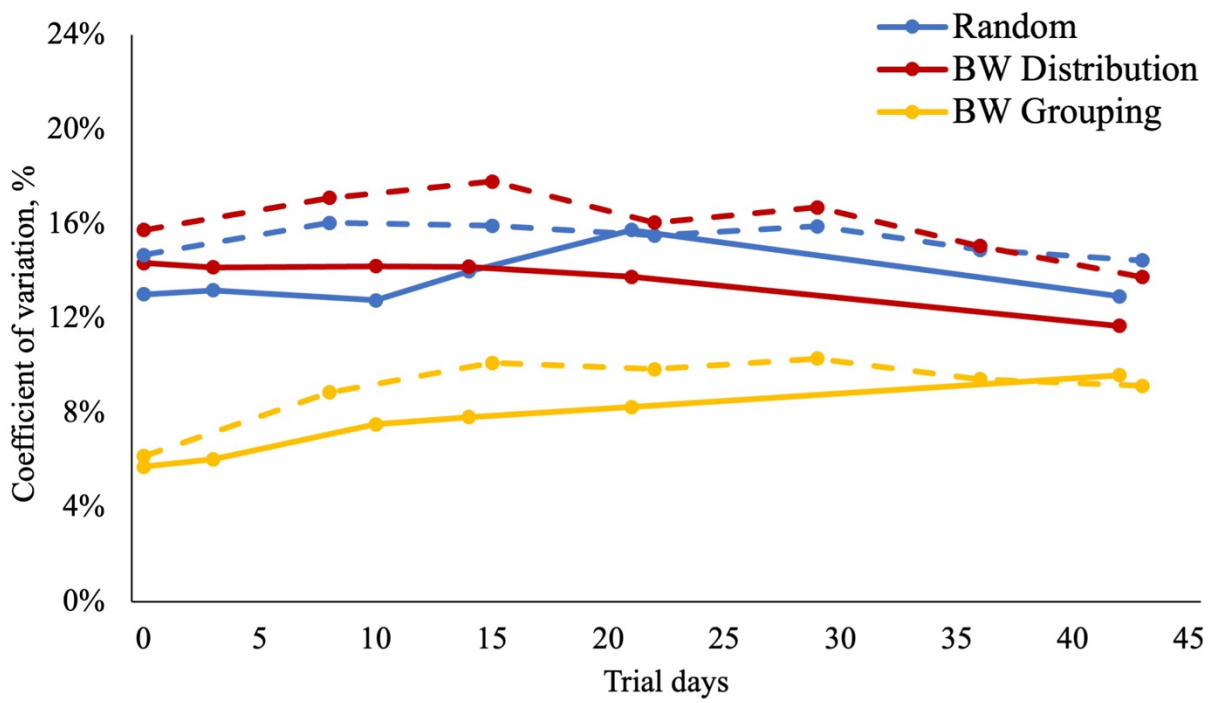

Figure 1.2 Coefficient of variation (CV) of body weight (BW) within pen.
Solid lines denote within-pen CV from Exp. 1 and dashed lines denote within-pen CV from Exp. 2. For the Random allotment strategy, pigs were allotted to pens in a completely randomized manner. For the BW Distribution strategy, pigs were sorted by BW into five groups. Within each BW group, pigs were randomized to pen such that each pen consisted of 1 random pig from each BW group to ensure that distribution of BW within pen was relatively consistent across pens. For the BW Grouping strategy, pigs were sorted into three groups by BW (light, medium, heavy). Within each body weight group, pigs were randomized to pen such that each pen consisted of pigs from a single BW group (pens of light pigs, pens of medium pigs, pens of heavy pigs).

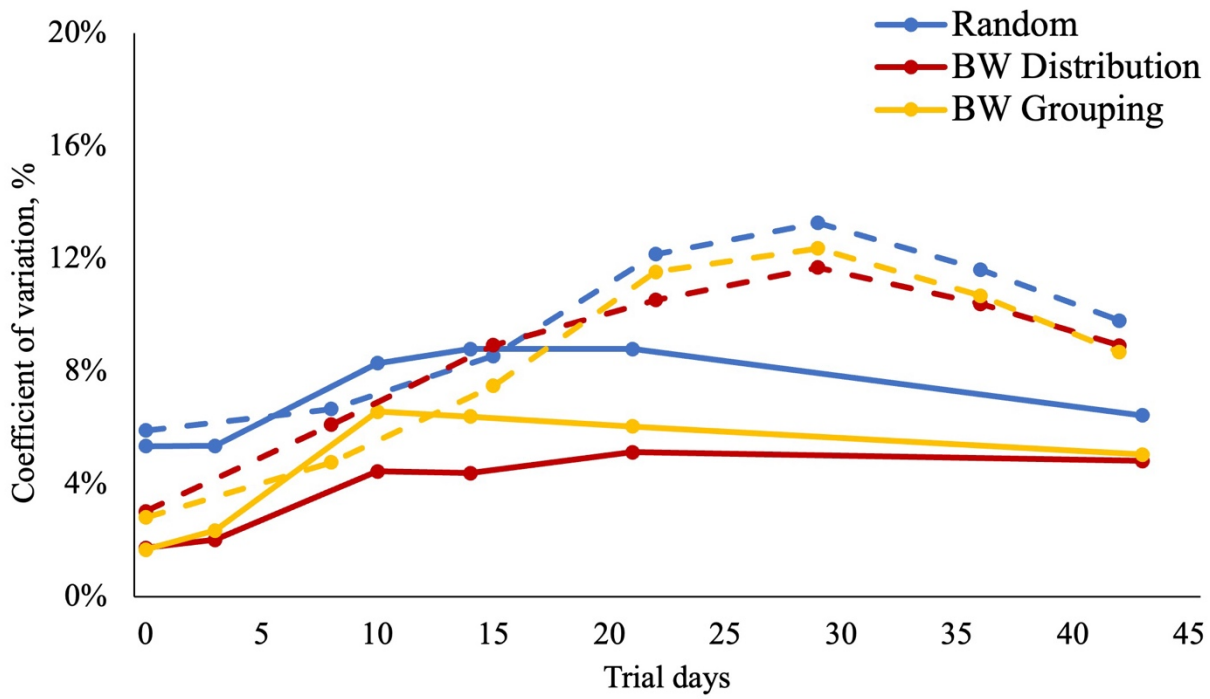

Figure 1.3 Coefficient of variation (CV) of body weight (BW) between pen.
Solid lines denote between-pen CV from Exp. 1 and dashed lines denote between-pen CV from Exp. 2. For the Random allotment strategy, pigs were allotted to pens in a completely randomized manner. For the BW Distribution strategy, pigs were sorted by BW into five groups. Within each BW group, pigs were randomized to pen such that each pen consisted of 1 random pig from each BW group to ensure that distribution of BW within pen was relatively consistent across pens. For the BW Grouping strategy, pigs were sorted into three groups by BW (light, medium, heavy). Within each body weight group, pigs were randomized to pen such that each pen consisted of pigs from a single BW group (pens of light pigs, pens of medium pigs, pens of heavy pigs).

Chapter 2 - Effect of yeast probiotics in lactation and yeast cell-wall prebiotic and *Bacillus subtilis* probiotic in nursery on lifetime growth performance, immune response, and carcass characteristics

ABSTRACT

Twenty-eight mixed parity sows (Line 241; DNA) and their offspring were used to evaluate live yeast supplementation during lactation with or without a pre/probiotic combination during the nursery period on lactation performance, lifetime growth performance, and immune response. On d 110 of gestation, sows were allotted to a lactation diet with or without a live yeast probiotic (0.10% Actisaf Sc 47 HR+; Phileo by Lesaffre, Milwaukee, WI). At weaning, their offspring (350 pigs; initially 6.1 ± 0.02 kg) were randomly assigned in a 2 × 2 factorial with main effects of sow treatment and nursery treatment consisting of a control diet or a diet with a yeast cell-wall prebiotic and *Bacillus subtilis* probiotic (0.10% YB; Phileo by Lesaffre, Milwaukee, WI) fed for 42 d followed by common diets fed until marketing. Two nursery pens were combined into 1 finishing pen, such that there were 5 and 10 pigs per pen with 17 or 18 and 8 or 9 replications per treatment during the nursery and finishing periods, respectively. There were no significant effects of yeast supplementation on lactation performance ($P \geq 0.079$). There was a sow × nursery diet interaction ($P = 0.024$) on nursery ADG. Pigs from yeast sows had increased ADG when fed control nursery diets compared to pigs from control sows fed the control nursery diet with pigs fed pre/probiotic nursery diets intermediate, regardless of sow diet. Pigs from yeast sows tended ($P = 0.067$) to have greater final BW (d 165). A subset of pigs was sampled throughout their lifetime to determine serum porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* antibody sample-to-positive (S/P) ratios and percentage inhibition of *Lawsonia intracellularis*. There was a

tendency for a PCV2 S/P ratio sow diet × day interaction ($P = 0.097$) where progeny from yeast sows had higher PCV2 S/P ratios at 101 d of age compared to control sow progeny ($P = 0.046$). There was a PCV2 S/P ratio nursery diet × day interaction ($P = 0.036$) where pigs fed a pre/probiotic combination had reduced S/P ratios at 66, 78, and 162 d of age ($P \leq 0.022$); however, at 22 d of age pigs fed a pre/probiotic combination tended to have an increased S/P ratio ($P = 0.051$). In conclusion, the effect of yeast in lactation diets on progeny nursery ADG was dependent on the post-weaning treatment. However, feeding a live yeast probiotic during lactation resulted in tendencies ($P \leq 0.10$) for increased progeny final BW and HCW.

INTRODUCTION

Yeast additives are currently being investigated for their potential improvements in swine growth performance, immune response, gut health, and nutrient digestibility (Liu et al., 2018). Yeast can be supplemented in the diet in many different forms including live yeast cells, yeast cell wall, yeast cell cultures, yeast extracts, and yeast cell wall components. Live yeast cells are kept intact and commonly dried to be fed as a pure, active yeast (Shurson, 2018). Yeast culture is a combination of the yeast biomass and fermentation metabolites that are produced during the fermentation process (Shurson, 2018). Yeast extracts consist only of the intercellular components of the yeast cell and are obtained through hydrolysis using either enzymes or acids (Sampath et al., 2023). Conversely, yeast cell wall products contain only the carbohydrates found in the cell wall (Shurson, 2018) and include mannanoligosaccharides and β-glucans. While there are numerous specie of yeast and a large diversity of yeast products within each specie, this research deals specifically with a live yeast product from the *Saccharomyces cerevisiae* strain in lactation diets and a pre/probiotic combination product consisting of a yeast cell-wall prebiotic and *Bacillus subtilis* probiotic fed during the nursery period. Peng et al. (2020) observed that

Saccharomyces cerevisiae live yeast supplementation during gestation increased immunoglobulin (Ig) G concentration in sow plasma on d 1 of lactation. Because sow IgG is transferred through colostrum (Jang et al., 2013), it has been shown to enhance piglet immunoglobulin concentrations post-weaning (Xia et al., 2022). In addition, progeny of sows fed *Saccharomyces cerevisiae* live yeast during lactation have exhibited improvements in growth during the nursery period (Chance et al., 2022a).

The addition of *Saccharomyces cerevisiae* live yeast and yeast cell walls in nursery diets has been observed to decrease the incidence of diarrhea and *Escherichia coli* present in feces, and improve nursery growth performance (Trckova et al., 2014). Yeast culture supplementation during the nursery phase may augment the gut immune response and improve intestinal morphology (Shen et al., 2009). However, other data has shown no improvement in pig growth when a yeast prebiotic was fed during the nursery period (Chance et al., 2022a).

Bacillus subtilis is a spore-forming bacteria that has been added to pig diets as a probiotic as it is resistant to high temperatures and can improve enzymes such as amylase, lipase, and maltase in the nursery pig (Deng et al., 2020). The inclusion of *Bacillus subtilis* in weanling pig diets may also enhance post-weaning growth performance (Wang et al., 2019). This improvement in growth may be attributed to an improvement in intestinal health as evidenced by an increase in villus height to crypt depth ratio, increased plasma levels of interleukin-2 and interleukin-10, and the up-regulation of genes involved in the metabolic pathways that influence intestinal microbiota maturation (Tian et al., 2021). Additionally, *Bacillus subtilis* in nursery diets increased serum immunoglobulin (Ig) A and immunoglobulin G concentrations which may indicate an improvement in immune response (Li et al., 2021).

Limited data is available evaluating the interactive effects yeast supplementation in lactation and nursery diets on lifetime growth performance and carcass characteristics when yeast is supplemented in both the lactation and nursery phases. Studies that have investigated this either show a benefit in finishing growth performance or no effect at all (Shen et al., 2017; Thayer et al., 2022).

While there has been evidence to suggest that *Saccharomyces cerevisiae* yeast supplementation in late gestation/lactation and nursery diets improves passive immunity by increasing immunoglobulin concentrations in piglets (Trckova et al., 2014; Jiang et al., 2015; Xia et al., 2022), little research has been conducted to determine if added yeast in the lactation or nursery periods has an effect on antibody production and active immunity following vaccination and again what data is available has yielded inconsistent results (Hiss and Sauerwein, 2003; Hildabrand et al., 2005; Pornanek and Phoemchalard, 2021). The effect of added yeast in lactation and nursery diets on immune response following vaccination for porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae*, and *Lawsonia intracellularis* will be investigated through this research. The three aforementioned diseases represent a substantial threat to global swine production but through vaccination their impact on both mortality and economics can been greatly reduced (Simionatto et al., 2013; Jacobs et al., 2020; Guo et al., 2022). For that reason, the authors of the current trial wanted to investigate the effect of yeast and yeast products in lactation and nursery diets on vaccine efficacy for these diseases. Our hypothesis was that the additions of the live yeast *Saccharomyces cerevisiae* strain NCYC Sc 47 during lactation and a yeast pre-probiotic combination during the nursery period would result in an improvement in nursery and finishing period growth performance and specific immunity. Therefore, the objective of this study was to determine if yeast supplementation during the lactation and nursery periods would

result in improvement in lifetime growth performance, serum antibody titers, and carcass characteristics.

MATERIALS AND METHODS

Animals and treatment structure

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4485.33). This study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Sows were housed individually in an environmentally controlled and mechanically ventilated barn during lactation. Farrowing stalls were equipped with an electronic feeding system (Gestal Quattro Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada) and an individual cup waterer. Farrowing stalls contained a heated mat for piglet comfort. Nursery pigs were housed in a single room environmentally controlled and mechanically ventilated nursery containing 86 pens. Each pen was equipped with a nipple waterer and 4-hole, dry self-feeder to allow ad libitum access to feed and water. Pens (1.3 × 1.3 m) had metal tri-bar floors and allowed approximately 0.34 m^2/pig. During the finishing period, pigs were housed in a mechanically ventilated barn containing 36 pens (2.44 × 3.05 m) with adjustable gates that allowed for 0.93 m^2/pig. Pigs were allowed ad libitum access to feed and water with a cup waterer and a single-sided, dry self-feeder. Pens were located over a completely slatted concrete floor with a manure storage pit underneath that measured 1.20 m deep.

A total of 28 mixed-parity sows (DNA 241, DNA Genetics) were used with 14 sows per treatment. Sows were weighed and moved into the farrowing house on approximately d 110 of gestation. Sows were blocked by parity and BW and allotted to one of two dietary treatments. The dietary treatments consisted of a standard corn-soybean meal lactation diet or the same basal

diet with the addition of yeast probiotics (0.10% Actisaf Sc 47 HR+; Phileo by Lesaffre, Milwaukee, WI) at the expense of corn (Table 1). The yeast-based probiotic contained the live yeast *S. cerevisiae* strain NCYC Sc 47. Sows were fed approximately 2.7 kg of their respective treatment diet from d 110 of gestation until farrowing. After farrowing, sows had ad libitum access to their experimental diet throughout lactation. Daily feed additions were recorded to determine daily feed intake.

Sow BW was measured at entry into the farrowing house and at weaning. Sow back fat depth (measured 8 cm from the midline of the 10th rib; Renco Lean Meter, S. E. C. Repro Inc., Golden Valley, MN) and caliper scores (Knauer and Baitinger, 2015) were measured upon entry to the farrowing house and at weaning. Piglets were cross fostered within 48 h of birth within sow treatment to equalize litter size. Piglets were individually weighed on d 2 and at weaning.

Pigs were weaned at approximately 17 d of age. While 367 pigs were weaned from the 28 sows, piglets that were more than 3 standard deviations above or below the average weaning weight (n = 7) or were deemed lame (n = 7) were removed from the study. Of the pigs that were 3 standard deviations above or below the average weaning weight, 4 were progeny of sows fed the live yeast probiotic and 3 were from sows fed the control diet. Of the pigs that were deemed lame at weaning, 4 were from sows fed the control diet during lactation and 3 were progeny of sows fed the live yeast probiotic. In addition, 3 average progeny from sows fed yeast were randomly selected to be excluded from the nursery portion of the trial due to the inability to make a complete nursery pen of 5 pigs. Thus, following weaning, a total of 350 weaned pigs (241 × 600, DNA; initially 6.1 ± 0.02 kg) were used in a 54-d nursery study. Within sow treatment, pigs were randomly assigned to nursery pens with pens then randomly allotted to treatment. Nursery pens had no more than two pigs from an individual sow, no more than two

gilt offspring, and no more than three pigs of the same gender. There were 5 pigs per pen and 17 or 18 replications per treatment. Nursery treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (previously described) and nursery treatment (control vs. 0.10% inclusion of a pre/probiotic combination of yeast cell-wall prebiotic and 1.0 $\log_5$ *Bacillus subtilis* probiotic (YB; Phileo by Lesaffre, Milwaukee, WI)) from d 17 to 71 of age. Pigs were fed treatment diets in three phases with phase 1 from placement until 27 d of age, phase 2 from d 27 to 40, and phase 3 from 40 to 59 d of age. Following phase 3, all pigs were fed a common phase 4 diet from d 59 to 71 of age. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). All diets were fed in meal form, except for the phase 1 nursery diets which were pelleted (Table 1). Pigs were individually weighed and feed disappearance was measured weekly during this portion of the study to determine pen ADG, ADFI, and G:F.

After the nursery period, a total of 336 pigs (initially 36.5 ± 0.41 kg) of the original 350 pigs included in the nursery portion of the trial were followed for an additional 94 days until marketing. Pigs that were not included in the finishing portion of the trial included mortalities (n=2) and pigs removed from the trial due to lameness (n=1) or rectal prolapse (n=1). Of the mortalities, one was a progeny of a control sow fed the pre/probiotic combination diet in the nursery while the other mortality was from a sow fed live yeast during lactation that was fed the pre/probiotic combination diet in the nursery. The pig removed from the trial due to lameness was the progeny of a control sow fed the control diet during the nursery period and the pig removed due to rectal prolapse was the progeny of a sow fed live yeast during lactation that was then fed the pre/probiotic combination in the nursery. In addition, one nursery pen of progeny from sows fed live yeast during lactation that were then fed the control nursery diet and one pen of progeny from

sows fed live yeast during lactation that were then fed the pre/probiotic nursery diet were randomly assigned to be excluded from the finishing portion of the trial due to an odd number of replications in the nursery portion of the trial for those treatment combinations and an inability to standardize pig space for those pens. Within sow × nursery treatment combination, two nursery pens of pigs were combined into one finishing pen to create 8 or 9 replications per treatment with 9 or 10 pigs per pen. All pigs were fed common diets throughout the grow-finish period in 3 phases that were formulated to meet or exceed NRC (2012) requirement estimates (Table 1). Pigs and feeders were weighed at the completion of each phase to determine ADG, ADFI, and G:F. At 165 d of age, final pen and individual pig weights were taken, and pigs were transported to a commercial packing plant (Triumph, St. Joseph, MO) for processing and carcass data collection which included hot carcass weight (HCW), backfat thickness, loin depth, and percent lean. Carcass yield was calculated as HCW divided by final live weight taken at the farm.

Immunoglobulin and antibody titer analyses

On d 5 after weaning (22 d of age), one average weight gilt was selected from each pen and bled. A sample of serum from each female was sent to the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* antibody analysis. All pigs were vaccinated for porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* with a single 2 mL dose (Circumvent PCV-M G2, Merck, Rahway, NJ) following the first blood draw. Blood was collected from the same average weight gilts and the antibody analysis for PCV2 and *Mycoplasma hyopneumoniae* was conducted 6 more times on d 38, 50, 66, 78, 101, and 162 of age. In addition, serum samples collected on d 22 and 50 were used for analysis of Immunoglobulin A and Immunoglobulin G using an enzyme-linked immunosorbent assay (Bethyl Laboratories, Inc, Montgomery, TX). At 50 d of age and all

successive bleeding events serum was analyzed for percent inhibition of *Lawsonia intracellularis*. All pigs were vaccinated for *Lawsonia intracellularis* with a single 2 mL dose (Porcilis Ileitis, Merck, Rahway, NJ) on d 50 following blood collection. All serum samples were analyzed in duplicate for immoglobulin and antibody titer analyses.

Statistical analysis

Performance data were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria). The sow portion of the trial was analyzed as a randomized complete block design. Blocking structure accounted for sow parity and BW. Sow served as the experimental unit. Treatment was included as a fixed effect and block was included as a random effect. Count data were analyzed using a Poisson distribution using a logit link function. Proportion data, including litter born alive, stillborn, born mummified, and pre-weaning mortality were analyzed using a binomial distribution using a logit link function. Performance data for the nursery and finishing portions of the trial were analyzed in a completely randomized design with pen serving as the experimental unit. Treatments were arranged as a 2 × 2 factorial of sow treatment and nursery treatment. The main effect of sow treatment and nursery treatment, as well as their interactions, were tested.

For analysis of carcass data, individual carcass served as the observation and pen was included in the model as a random effect. Hot carcass weight was used as a covariate for analysis of percentage lean, loin depth, and backfat to account for differences in carcass characteristics due to ending weight.

Antibody titer data and Immunoglobulin A and G concentrations were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Fixed effects of the model included sow diet, nursery diet, day, and their second- and third-order

interactions. A Kenward-Rogers adjustment was used in the statistical model, as well as a Tukey multiple comparison adjustment. For analysis of immunoglobulin and vaccine titer data, repeated measures over time was modeled using a first order antedependence covariance parameter matrix structure. For both immunoglobulin analyses, sow and ELISA plate also served as a random effect. Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.15$.

RESULTS

Growth performance

The addition of live yeast probiotics to the lactation diet resulted in no differences in sow BW, caliper score, back fat, ADFI, or wean to estrus interval ($P > 0.10$; Table 2). Litter birth characteristics, litter size, and preweaning mortality did not differ based on sow lactation diet. Litters from sows fed the control diet tended to be heavier at weaning ($P = 0.079$); however, mean piglet BW was not affected ($P = 0.858$).

During the nursery treatment period (d 17 to 59 of age), there was a tendency for an interaction between sow and nursery diet for ADG ($P < 0.052$) and ADFI ($P \leq 0.073$; Table 3). However, pairwise comparisons revealed no means separation by nursery treatment period. Numerically, progeny from sows fed live yeast that were then fed the control diet (YE-CON) or the pre/probiotic diets (YE-YE) in the nursery and progeny from control sows that were then fed the pre/probiotic in the nursery (CON-YE) had greater ADG and ADFI than progeny from control sows that were fed the control diet (CON-CON) during the nursery treatment period. This suggests that the effects of feeding the pre/probiotic in the nursery is dependent upon sow treatment during lactation, with no additive effects observed between lactation and nursery treatment.

On d 59 at the end of the nursery treatment feeding period, there was an interaction between sow treatment and nursery treatment for BW ($P = 0.047$). However, pairwise comparisons revealed no means separation for BW at the conclusion of the nursery treatment period. Numerically, CON-YE, YE-CON, and YE-YE pigs were heavier than CON-CON pigs. Similar to nursery treatment period for ADG and ADFI, the effects of the pre/probiotic treatment in the nursery may be dependent on the sow treatment during lactation with no additive effects between lactation and nursery treatment.

During the common nursery feeding period (d 59 to 71 of age), there was a tendency for an interaction for ADG ($P = 0.099$) for sow and nursery dietary treatments where there was a numeric increase in gain in YE-CON pigs. During the common nursery period, there was a main effect of sow treatment, as progeny of sows fed yeast during lactation had increased ADG and ADFI ($P \leq 0.032$) when compared to progeny of sows fed the control diet.

For the overall nursery period (d 17 to 71 d of age), there was an interaction between sow diet and nursery diet for ADG ($P = 0.024$) where YE-CON pigs had increased ADG when compared to CON-CON pigs ($P < 0.050$), with pigs fed the pre/probiotic combination in the nursery (CON-YE and YE-YE) being intermediate, regardless of sow diet. This lack of an additive effect suggests that the effect of the sow lactation treatment on overall nursery period ADG was dependent upon post-weaning supplementation. In addition, there was an interaction between sow and nursery treatments for ADFI ($P = 0.048$). This was the result of CON-CON pigs having numerically lower ADFI when compared to CON-YE pigs while YE-CON pigs had numerically higher ADFI compared YE-YE pigs. There was a tendency for a main effect of sow diet where progeny from sows fed live yeast during lactation had improved ADG ($P = 0.073$) compared to offspring from control sows.

At the completion of the finishing period (d 165 of age), progeny from sows fed yeast during lactation tended to have a heavier final BW ($P < 0.067$) and HCW ($P = 0.101$) when compared to pigs from control sows. There were no other main effects of sow or nursery treatment as well as no interactions between the two on overall finishing period growth performance ($P > 0.10$). There was an interaction between sow and nursery diets on backfat depth at harvest ($P = 0.034$). However, pairwise comparisons revealed no means separation between the treatments. Numerically, YE-CON pigs had decreased backfat depth compared to CON-CON or YE-YE pigs.

Antibody titers and immunoglobulin concentrations

There were no sow diet or nursery diet effects on $\log_{10}$ IgG concentrations on d 22 or 50 of age as well as no significant second or third order interactions ($P > 0.100$; Figure 1). For $\log_{10}$ IgA concentration, there was an interaction between sow and nursery diet ($P = 0.029$; Figure 2). Progeny from sows fed yeast during lactation tended to have decreased $\log_{10}$ IgA concentration when they were fed a pre/probiotic during the nursery treatment period when compared to those fed the control diet during the nursery period ($P = 0.066$). There was a significant day effect on both IgG and IgA concentrations, where IgG concentration was lower at 50 d of age and IgA concentration was higher at 50 d of age as compared to concentrations at 22 d of age ($P < 0.001$).

There was an interaction between nursery diet and day for porcine circovirus type 2 (PCV2) S/P ratio ($P = 0.036$; Figure 3). Pigs fed a pre/probiotic during the nursery had a higher PCV2 S/P ratio at 22 d of age ($P = 0.050$). However, at d 66, 78, and 162 of age pigs fed a pre/probiotic during the nursery period had lower PCV2 S/P ratios than pigs fed the control diet during the nursery period ($P \leq 0.022$) and tended to have lower PCV2 S/P ratios on d 50 and 101 of age ($P < 0.093$). There was a tendency for an interaction between sow diet and day for PCV2

S/P ratio ($P = 0.097$; Figure 4). Progeny from sows fed yeast had a higher PCV2 S/P ratio at 101 d of age when compared to progeny from sows fed the control diet ($P = 0.046$), with no evidence of a difference at all other sampling points. There was a tendency for an interaction between sow diet and nursery diet on *Lawsonia intracellularis* inhibition ($P = 0.071$; Figure 5). YE-CON pigs had numerically the highest percentage inhibition on d 66, 78, 101, and 162. Pairwise comparisons revealed no means separation within day of serum collection. For both antibody titers and *Lawsonia intracellularis* inhibition, there was a significant day effect ($P < 0.001$). For *Lawsonia intracellularis*, percentage inhibition rose drastically after vaccination on d 50 of age until d 78 of age, following which percent inhibition began to plateau. *Mycoplasma hyopneumoniae* S/P ratio peaked at d 22 of age, following vaccination S/P ratios dropped until d 38 and then began to rise until d 78 of age after which S/P ratios decreased once more until marketing (Figure 6). Porcine circovirus type 2 S/P ratios decreased following vaccination until d 38 of age, after which S/P ratios rose until d 66 of age and then began to decrease until marketing.

DISCUSSION

This study aimed to evaluate the effect of a live yeast probiotic fed to sows during lactation as well as a pre-probiotic combination consisting of a 3 strain *Bacillus* blend probiotic and yeast cell wall fed during the nursery on lifetime growth performance, specific immune response, and carcass characteristics.

The supplementation of yeast in late gestation and lactation has yielded variable lactation and subsequent reproductive performance. In the present study, we did not see any significant effects of lactation yeast supplementation on lactation performance. However, Thayer et al. (2023) observed that inclusion of whole-cell inactivated *Pichia guilliermondii* yeast starting at d

35 of gestation increased piglets born alive and born alive litter weights. In their study, pigs from control sows were heavier at weaning but the authors speculated that this may be due to smaller litter sizes and an increase in lactation length (Thayer et al., 2023). Therefore, this improvement in piglet weaning weight may not be a result of sow lactation diet as control pigs in this study had less competition at the underline potentially allowing for an increase in milk consumption and thus weight gain in addition to more days of lactation which helped to further improve piglet weaning weights. It should also be noted that while *Saccharomyces cerevisiae* and *Pichia guilliermondii* are both yeasts, *Pichia guilliermondii* has a smaller surface area and stronger hydrophobic bonds (Peisker et al., 2017), which may alter the effect observed with the inclusion of yeast in gestating and lactating sow diets. Zhao et al. (2022) observed that yeast culture (*Saccharomyces cerevisiae*) fed during late gestation and lactation improved average daily milk yield during early lactation leading to an increase in ADG in piglets. Also, Sun et al. (2022) provided *Saccharomyces cerevisiae boulardii* live yeast supplementation for 5 days pre-farrow until weaning and found a decrease in lactation backfat loss and increased feed intake during the first week of lactation. Jang et al. (2013) established that *Saccharomyces cerevisiae* live yeast supplementation in late gestation and lactation or supplementation only during lactation at 10^6 or 10^7 CFU/g of the diet resulted in a shortening of WEI. However, the authors of the aforementioned trial commented on the small sample size of the study and postulated that more research is needed to confirm the effect of yeast on WEI and define the mode of action. Results from our trial are similar to those seen by Chance et al. (2022b) that found little effect of lactation supplementation of *Saccharomyces cerevisiae* live yeast on lactation performance, including no differences in WEI when sows were supplemented during lactation. However, Chance et al. (2022b) did find an increase in ADFI with yeast inclusion in the lactation diet.

Lack of lactation effects in the present study may be due to differences in diet composition or in timing of supplementation, where most studies that saw impacts on lactation performance had higher levels of wheat products in the diet and began supplementation prior to d 90 of gestation whereas the present study did not begin supplementation until d 110 of gestation.

Saccharomyces cerevisiae yeast probiotics fed during late gestation and lactation have been shown to improve milk-derived immunity in suckling and early nursery pigs through increased immunoglobulin content (Jang et al., 2013; Peng et al., 2020). Swine possess an epitheliochorial placenta that is impermeable to immunoglobulins (Dividich et al., 2005). Thus, maternal immunity must be obtained through passive acquisition via ingestion of colostrum and milk or actively produced in response to disease challenge or vaccination. It has been shown that a higher serum immunoglobulin concentration is indicative of a more robust immune system (Ogawa et al., 2016).

Immunoglobulin G is the primary immunoglobulin found in piglet blood circulation and in sow colostrum while immunoglobulin A is typically found near the mucosal membranes in the body, most notably the gut. In swine diets, *Saccharomyces cerevisiae* live yeast supplementation during late gestation and lactation led to increased colostral IgG, milk IgG, and milk IgA (Zanello et al., 2013). It was further demonstrated that these effects are sustained post-weaning in the serum of piglets weaned from primiparous sows fed *Saccharomyces cerevisiae* live yeast during lactation (Xia et al., 2022). However, most literature that has investigated the effects of sow yeast supplementation has initiated treatment diets from d 80-90 of gestation whereas the present study did not begin yeast inclusion until d 110 of gestation. The lack of effect of sow diet on IgG and IgA piglet serum concentrations on d 22 and 50 of age may indicate that yeast needs to be present in sow diets earlier in gestation in order to have passive immunological effects. It

has been shown that IgG transfer from sow plasma to lacteal secretions begins at approximately d 104 of gestation, increases on d 110 of 111 of gestation, and is maximized on the day of parturition (Huang et al., 1992). However, the length of *Saccharomyces cerevisiae* supplementation required in order to induce a change in the IgA and IgG serum concentrations of the sow has not been researched. Researchers have also found an increase in serum IgG and IgA concentrations post weaning with the inclusion of *Saccharomyces cerevisiae* yeast in nursery diets (Trckova et al., 2014; Jiang et al., 2015; Long et al., 2021). Additionally, inclusion of *Bacillus subtilis* in post-weaning diets has been shown to increase piglet IgG and IgA levels (Li et al., 2021). While there was an interaction between sow and nursery diets for IgA concentration in our study, there was no main effect of nursery diet on $\log_{10}$ IgA concentration. To the best of our knowledge, this is the first trial to investigate the interactive effects of *Saccharomyces cerevisiae* inclusion in the lactation diet and a yeast cell wall and *Bacillus subtilis* product in the nursery diet on IgG and IgA levels. The numeric increase in IgA levels in pigs from control sows fed the pre/probiotic diet may be due to the stimulation of immunity in the intestinal mucosa which would cause an increase in the production of IgA (Xiong et al., 2015). However, it is unclear as to why the addition of the pre/probiotic combination in the nursery period would cause a decrease in IgA levels in pigs from sows fed a live yeast during lactation. More research is needed in order to determine the cause of this interaction.

Due to the improvement in Ig levels in the literature in response to dietary yeast supplementation, the authors of the current study hypothesized that differences may exist in specific immunity following vaccination with inclusion of yeast in lactation and nursery diets. Past research would indicate the potential for increased antibody transmission through the milk of sows fed yeast co-products. Decuypere et al. (1998) found increased K88ab and K99

Escherichia coli (*E. coli*) antibody titers in the milk of sows on d 14 of lactation when β-1,3/1,6 glucans were added to sow diets. Although there were differences in specific immune measurements in the milk, piglet blood samples exhibited no differences in *E.coli* antigen titers post-weaning, with the exception of a reduction in 987P titer on d 21 post weaning in progeny from sows fed β-glucans (Decuypere et al., 1998). Past research has found no improvement in porcine reproductive and respiratory syndrome virus titers with the inclusion of yeast cell wall components (β-glucans) in nursery diets (Hiss and Sauerwein, 2003). Conversely, Pornanek and Phoemchalard (2021) saw an improvement in classical swine fever virus antibody titers with β-glucan rich molasses yeast powder supplementation in nursery diets. The current study sought to further investigate the effect of yeast in lactation and nursery diets on specific immune response with other diseases effecting the swine industry. The current study measured sample to positive (S/P) ratios for PCV2, *Mycoplasma hyopneumoniae,* and *Lawsonia intracellularis* at the time of vaccination and multiple time points thereafter. A sample to positive ratio is a measure of the optical density or absorbance of a serum sample relative to a positive sample with a known antibody measurement. Therefore, a higher S/P ratio is indicative of greater antibody levels for the pathogen of interest for the given test. To the best of our knowledge, this is the first trial to elucidate the effects of live yeast supplementation in lactation and yeast cell wall and *Bacillus subtilis* in nursery diets on specific immune response to PCV2 and *Lawsonia intracellularis* vaccination. Past research found no improvement in *Mycoplasma hyopneumoniae* S/P ratios with the inclusion of a yeast product in nursery diets (Hildabrand et al., 2005) which is in agreeance with the results of the current study. Results from the present study show only a significant effect of sow diet on PCV2 immunity at one time point where pigs from sows fed yeast in lactation had higher S/P ratios. Interestingly, pigs fed the pre/probiotic combination during the nursery tended

to have a higher S/P ratio at the time of vaccination at 22 d of age but after d 50 of age had lower PCV2 S/P ratios when compared to pigs on control diets. The heightened S/P ratio at the time of vaccination may have led to a decrease in immune system response to the vaccination, causing a decrease in S/P ratios at successive bleeding events. In the present study, there was a numeric increase in percent inhibition of *Lawsonia intracellularis* at both d 78 and 101 when pigs were either from a sow fed live yeast in lactation or were fed the pre/probiotic combination during the nursery period. This may suggest that the inclusion of a probiotic or pre/probiotic combination may be beneficial for the specific immune response to *Lawsonia intracellularis*. Considering the mixed results we observed, more research is needed to fully determine what effects pre/post-weaning yeast product supplementation has on specific immunity.

Progeny from sows fed *Saccharomyces cerevisiae* live yeast during late gestation and lactation have been shown to have increased ADG during the nursery phase of growth (Lu et al., 2019; Chance et al., 2022a). Similarly, Loughmiller et al. (2021) found that *Saccharomyces cerevisiae* live yeast supplementation during gestation and lactation improved ADG and ADFI during the first 12 days post-weaning and over the entire nursery period. The results of the present study show that the effect of *Saccharomyces cerevisiae* supplementation during lactation on nursery growth performance is dependent on pre/probiotic supplementation in the nursery period with an improvement in nursery growth performance when pigs were from sows fed live yeast in lactation compared to those from control sows if they were fed the control diet in the nursery. This improvement in nursery ADG can be attributed to an increase in ADFI during the nursery period when pigs were from sows fed yeast during lactation. Similarly, Lu et al. (2019) observed a tendency for an increase in early nursery ADFI when pigs were from sows fed a *Saccharomyces cerevisiae* yeast during lactation. This improvement in nursery ADG may also be

due to an increase in apparent total tract digestibility of gross energy, which has previously been reported for piglets from sows supplemented with *Saccharomyces cerevisiae* yeast during lactation (Lu et al., 2019). They hypothesized that the improvement in apparent total tract digestibility of gross energy following maternal yeast supplementation may be the result of an improvement in piglet microbiome, allowing for an improvement in digestion of fiber. Indeed, differences in microbiome composition throughout the nursery period in progeny from sows fed a *Saccharomyces cerevisiae* live yeast product during gestation and lactation have been observed (Law et al., 2024). Similar to results from Chance et al. (2022a) and Loughmiller et al. (2021), the present trial did not see an additive effect of yeast based products fed in both lactation and nursery diets on nursery growth performance. The cause of the interaction between pre- and post-weaning supplementation on nursery growth observed in this study is unclear. This is the first study to investigate this combination of yeast and *Bacillus subtilis* products in lactation and nursery diets and thus, more research is needed to further elucidate the mechanism behind this interaction.

Research investigating the combined effects of nursery diet supplementation of yeast cell wall and *Bacillus subtilis* combinations are few in the literature. However, research is prevalent on supplementation of either *Bacillus subtilis* or yeast cell wall alone. Improvements in final BW, ADG, ADFI, and G:F ratio were observed when *Bacillus subtilis* was included in nursery pig diets (Hu and Kim, 2022). These improvements in growth performance may be due to the enhancement in digestibility of dry matter, crude protein, and energy observed in pigs fed *Bacillus subtilis* when compared to pigs fed a control diet (Hu and Kim, 2022). In addition to increased ADG, Deng et al. (2020) detected an enhancement in villus height:crypt depth ratio in the ileum and a modulation in the microbiota when pigs were fed *Bacillus subtilis* in the nursery

period. Conversely, Menegat et al. (2019) observed no improvement in nursery period growth performance or fecal consistency when *Bacillus subtilis* C-3102 was added in nursery diets. Past research has shown an improvement in ADG and apparent ileal digestibility of dry matter and energy and reduced frequency of diarrhea when yeast cell wall products were included in nursery diet (Lee et al., 2021). Xiong et al. (2015) found an increase in amino acid transporters in the ileum as well as increased amino acids in piglet serum when pigs were supplemented with yeast products. In the present study, numeric improvements in ADG during the nursery period can be attributed to an increase in nursery ADFI when pigs were fed a yeast cell wall and *Bacillus subtilis* product. Existing literature is inconclusive, with some observing an increase in ADFI (Deng et al., 2020; Hu and Kim, 2022) while others noted no differences in ADFI (Menegat et al., 2019; Lee et al., 2021) with the inclusion of either a yeast cell wall or *Bacillus subtilis* product. However, many of the aforementioned studies included either *Bacillus subtilis* or yeast cell wall in the nursery diet, thus direct comparisons to the present study that included both feed additives is challenging.

Little research exists on the carry-over effects of live yeast supplementation in lactation and yeast cell wall and *Bacillus subtilis* in nursery diets on finishing growth performance and carcass characteristics. Some studies have shown that yeast hydrolysate or β-glucan supplementation during the finishing period results in improvements in finishing ADG due to enhanced nutrient apparent total tract digestibility during the finishing period which may indicate an improvement in fiber digestibility (Luo et al., 2019; Sampath et al., 2022). Thayer et al. (2022) conducted a similar study to that of the current trial; however, that trial investigated the effects of whole-cell inactivated *Pichia guilliermondii* yeast in lactation and nursery/finishing diets whereas the current study investigated the *Saccharomyces cerevisiae* yeast and terminated

offspring yeast supplementation after d 42 post-weaning. Thayer et al. (2022) found that whole cell inactivated yeast (WCY) inclusion in gestation and lactation diets did not affect offspring nursery performance; however, it did improve grow-finish ADG and ADFI. Similar to the present study, final body weight and HCW were improved with maternal WCY supplementation (Thayer et al., 2022). The numeric improvement in grow-finish ADG in the present study can be partially attributed to an increase in grow-finish ADFI. It is unclear what the mechanism is that caused an increase in ADFI in the present study when pigs were from sows fed a live *Saccharomyces cerevisiae* yeast during lactation. Past research has shown that live yeast increases ADFI in finishing pigs due to an increase in feeding frequency (Serviento et al., 2023). However, it should be noted that their trial differs from the current study in that Serviento et al. (2023) fed yeast during the finishing period whereas the current trial ceased inclusion during the nursery period. More likely explanations for this carry-over effect found in the present study are continued impacts of improvements in hindgut microbiota and alleviation of oxidative stress in the nursery period in progeny from sows fed yeast during lactation (Kiros et al., 2018; Tan et al., 2021).

In conclusion, there were no significant effects of added live yeast in the lactation diet on lactation performance. While interactions between sow diet and day and nursery diet and day were observed in antibody titer analysis, the overall impact of yeast probiotics in sow lactation diets and a pro/prebiotic combination in the nursery diet on antibody titer, contrary to our original hypothesis, was minimal and requires additional research. However, progeny from sows fed live yeast, then fed the control nursery diet had improved ADG during the overall nursery period compared to offspring from control sows that were then fed the control nursery diet with pigs fed a pre-probiotic combination in the nursery being intermediate, regardless of sow

treatment. This may indicate that the effect of lactation live yeast supplementation on nursery growth is dependent on nursery pre/probiotic supplementation. Feeding a pre/probiotic combination in the nursery period did not result in differences in finishing growth performance or final BW. While there were no differences in overall finishing growth performance due to sow supplementation during lactation, progeny from sows fed live yeast tended to have a higher final BW and HCW.

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Table 2.1 Diet composition (as-fed basis)

		Nursery[1]				Finisher[2]		
Item	Lactation	Phase 1	Phase 2	Phase 3	Phase 4	Phase 1	Phase 2	Phase 3
Ingredient, %								
Corn	66.55	44.15	57.10	66.85	72.10	77.90	83.35	86.10
Soybean meal, 46.5% CP	27.70	18.10	25.75	29.10	24.40	19.05	14.00	11.70
Whey powder	---	25.00	10.00	---	---	---	---	---
Fish meal	---	4.50	2.00					
Enzymatically-treated soybean meal[3]	---	3.75	---	---	---	---	---	---
Soybean oil	2.00	1.50	1.00	---	---	---	---	---
Calcium carbonate	1.10	0.25	0.50	0.85	0.85	0.90	0.90	0.88
Monocalcium P, 21.5% P	1.20	0.55	1.15	1.00	0.70	0.68	0.38	0.25
Sodium chloride	0.50	0.30	0.55	0.60	0.50	0.35	0.35	0.35
L-Lysine-HCl	0.28	0.43	0.51	0.52	0.50	0.45	0.42	0.32
DL-Methionine	0.05	0.22	0.22	0.20	0.17	0.10	0.06	0.02
L-Threonine	0.10	0.19	0.23	0.22	0.20	0.16	0.14	0.10
L-Tryptophan	0.01	0.06	0.06	0.06	0.04	0.04	0.04	0.03
L-Valine	---	0.15	0.17	0.14	0.11	0.08	0.05	---
Trace mineral premix	0.25	0.15	0.15	0.15	0.15	0.15	0.15	0.13
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.15	0.13	0.10
Phytase[4]	---	0.08	0.08	0.08	0.04	0.04	0.04	0.04
Zinc oxide	---	0.40	0.27	---	---	---	---	---
Actisaf Sc 47 HR+[5]	+/-	---	---	---	---	---	---	---
YB[6]	---	+/-	+/-	+/-	---	---	---	---
Total	100	100	100	100	100	100	100	100

continued

Table 2.1 continued

Item	Lactation	Nursery[1]				Finisher[2]		
		Phase 1	Phase 2	Phase 3	Phase 4	Phase 1	Phase 2	Phase 3
Calculated analysis								
SID amino acids, %								
Lys	1.07	1.40	1.35	1.30	1.17	1.00	0.85	0.72
Ile:Lys	64	56	54	54	54	54	54	58
Leu:Lys	134	109	110	114	118	126	134	152
Met:Lys	29	38	37	36	36	33	31	30
Met and Cys:Lys	54	57	57	57	57	56	56	58
Thr:Lys	63	64	64	63	63	63	63	65
Trp:Lys	21	20	20	20	19	19	19	19
Val:Lys	70	70	70	70	68	68	68	69
His:Lys	42	32	34	36	36	37	39	43
Total Lys, %	1.20	1.54	1.49	1.44	1.30	1.11	0.95	0.81
ME, kcal/kg	3,363	3,418	3,341	3,281	3,299	3,310	3,323	3,330
NE, kcal/kg	2,523	2,575	2,500	2,434	2,472	2,507	2,545	2,560
SID Lys:NE, g/Mcal	4.24	5.44	5.40	5.34	4.73	3.99	3.34	2.81
CP, %	19.1	20.9	20.4	20.3	18.4	16.2	14.1	13.1
Ca, %	0.80	0.70	0.72	0.70	0.63	0.61	0.53	0.48
P, %	0.63	0.69	0.71	0.60	0.51	0.48	0.40	0.36
STTD P, %	0.50	0.61	0.59	0.47	0.39	0.38	0.31	0.28

[1]Nursery Phase 1 diets were fed from d 17 to 27 of age (approximately 6.1 to 7.5 kg BW), Phase 2 from d 27 to 40 of age (approximately 7.5 to 13.0 kg BW), Phase 3 from d 40 to 59 of age (approximately 13.0 to 25.4 kg BW), and Phase 4 from d 59 to 71 of age (approximately 25.4 to 36.5 kg BW).

[2]Finishing Phase 1 diets were fed from d 71 to 100 of age (approximately 36.5 to 67.2 kg BW), Phase 2 from d 100 to 128 of age (approximately 67.2 to 97.7 kg BW), Phase 3 from d 128 to 165 of age (approximately 97.7 to 132.2 kg BW).

[3]HP 300 (Hamlet Protein, Findlay, OH)

[4]Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Parsippany, NJ) provided a 0.14% release of STTD P with 2,027 FTU/kg in the nursery and 0.13% release of STTD P with 1,000 FTU/kg in the finisher.

[5]Actisaf 47 HR+ (Phileo by Lesaffre, Milwaukee, WI) was added at 0.1% at the expense of corn from the experimental diets.

[6]YB (Phileo by Lesaffre, Milwaukee, WI) was added at 0.1% at the expense of corn from the experimental diets.

Table 2.2 Evaluation of yeast inclusion on sow lactation performance[1]

	Sow treatment[2]			
Item	Control	Yeast	SEM	*P* =
Sow BW, kg				
Entry	268.0	268.3	7.72	0.943
Wean	228.9	234.0	8.65	0.228
Change (entry to wean)	-39.1	-34.3	4.07	0.363
Sow back fat, mm				
Entry	15.3	15.4	0.65	0.923
Wean	14.3	14.4	0.64	0.859
Change (entry to wean)	-1.0	-0.9	0.25	0.824
Sow caliper score[3]				
Entry	17.4	17.3	0.26	0.655
Wean	16.0	16.0	0.43	1.000
Change (entry to wean)	-1.4	-1.3	0.31	0.747
Sow ADFI, kg				
Pre-farrow	2.6	2.6	0.06	0.895
Farrow to wean	7.4	7.3	0.25	0.717
Wean to estrus interval, d	5.2	5.3	0.14	0.826
Litter size, n				
d 0	15.2	13.9	1.04	0.373
d 2	13.9	13.1	1.00	0.572
Wean	13.4	12.9	0.98	0.677
Litter weight, kg				
d 2	24.1	22.7	0.79	0.144
Wean	80.7	76.8	1.57	0.079
Mean piglet BW, kg				
d 2	1.76	1.75	0.075	0.937
Wean	6.02	6.06	0.162	0.858
Litter ADG d 2 to wean, kg	3.24	3.15	0.065	0.353
Piglet ADG d 2 to wean, kg	0.24	0.25	0.009	0.523
Preweaning mortality, %				
Birth to d 2	4.0	4.3	1.64	0.876
d 2 to wean	3.8	2.5	1.64	0.457

[1]A total of 28 mixed-parity sows (Line 241, DNA, Columbus NE) and litters were used from day 110 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size up to 48-h post farrowing.

[2]Sow diet consisted of providing a control diet or a diet with inclusion of a live yeast probiotic at 0.1% of diet (Actisaf Sc 47 HR+; Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

[3]A caliper score of 18 was used for sows that had a caliper score that was too large to be measured by the device (> 18). Caliper scores at entry > 18: Control: n = 7; Actisaf: n = 3. Caliper score at weaning > 18: Control: n = 2; Actisaf: n = 1.

Table 2.3 Interactive effects of yeast inclusion in lactation diets and yeast pro/prebiotic inclusion in nursery diets on growth performance of nursery and finishing pigs.[1,2]

Sow treatment[3]:	Control		Yeast			*P* =		
Nursery treatment[4]:	Control	Pre/ probiotic	Control	Pre/ probiotic	SEM	Sow × nursery	Sow	Nursery
BW, kg								
d 17 (Weaning)	6.1	6.1	6.1	6.1	0.02	0.876	0.512	0.988
d 59	24.8	25.7	25.8	25.5	0.32	0.047	0.268	0.381
d 71	35.7	36.6	37.1	36.4	0.41	0.047	0.123	0.783
d 165	129.9	131.4	133.2	132.9	1.29	0.470	0.067	0.628
d 17 to 59 (Nursery treatment period)								
ADG, kg	0.446	0.467	0.469	0.462	0.0071	0.052	0.214	0.334
ADFI, kg	0.669	0.696	0.693	0.683	0.0103	0.073	0.565	0.404
G/F	0.666	0.672	0.677	0.676	0.0062	0.549	0.177	0.652
d 59 to 71 (Common nursery period)								
ADG, kg	0.896	0.904	0.944	0.910	0.0132	0.099	0.032	0.313
ADFI, kg	1.523	1.534	1.606	1.555	0.0211	0.140	0.013	0.328
G/F	0.589	0.590	0.589	0.586	0.0053	0.736	0.601	0.928
d 17 to 71 (Overall nursery period)								
ADG, kg	0.545[b]	0.564[ab]	0.575[a]	0.561[ab]	0.0073	0.024	0.073	0.729
ADFI, kg	0.857	0.881	0.896	0.875	0.0124	0.048	0.157	0.882
G/F	0.636	0.640	0.642	0.641	0.0042	0.474	0.348	0.618
d 71 to 165 (Overall finishing period)								
ADG, kg	0.995	1.006	1.020	1.018	0.0134	0.570	0.149	0.720
ADFI, kg	2.618	2.641	2.679	2.691	0.0362	0.870	0.127	0.627
G/F	0.380	0.381	0.381	0.378	0.0021	0.386	0.592	0.677
Carcass Data								
HCW, kg	97.0	98.4	98.9	100.0	1.09	0.852	0.101	0.249
Carcass yield, %	75.0	74.9	74.5	74.9	0.21	0.269	0.317	0.534
Lean, %[5]	55.1	55.2	55.3	55.0	0.14	0.146	0.662	0.417
Loin depth, cm[5]	67.03	67.30	66.67	66.35	0.569	0.594	0.246	0.961
Back fat depth, cm[5]	15.14	14.93	14.45	15.25	0.235	0.034	0.425	0.202

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 350 weaned pigs (initially 6.1 ± 0.02 kg) were used in a 54-day nursery trial with 17 to 18 replications per treatment and 5 pigs per pen.

[2]A total of 336 pigs (initially 36.5 ± 4.67 kg) were used in a 94-day finishing trial with 8 to 9 replications per treatment and 10 pigs per pen.

[3]Sow diet consisted of providing a control diet or a diet with inclusion of a live yeast probiotic at 0.1% of diet (Actisaf Sc 47 HR+; Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

[4]Nursery treatment consisted of providing a control diet or a diet with the inclusion of a pre/probiotic combination consisting of a yeast cell wall prebiotic and Bacillus subtilis probiotic at 0.1% of diet (YB; Phileo by Lesaffre, Milwaukee, WI) in phases 1, 2, and 3 of the nursery.

[5]Adjusted using HCW as a covariate.

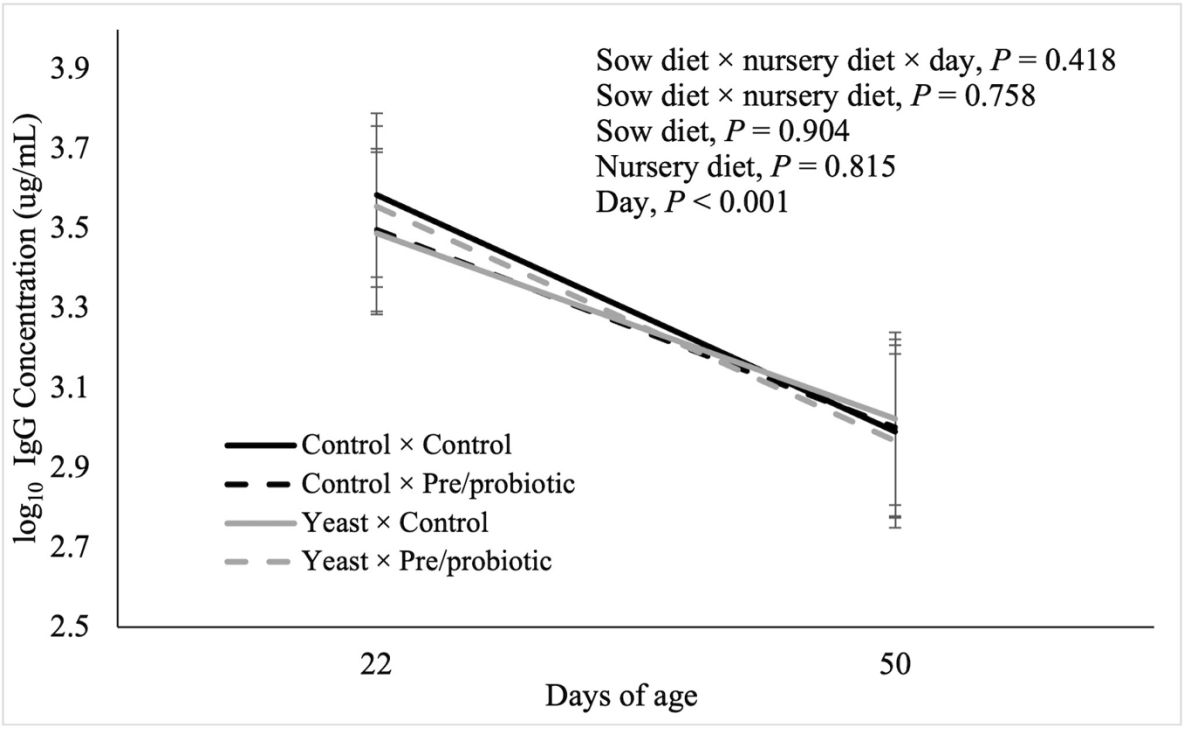

Figure 2.1 Effect of sow and nursery diet on $\log_{10}$ immunoglobulin G concentration at 22 and 50 d of age (n = 17 to 18). Data are expressed as means ± SE.

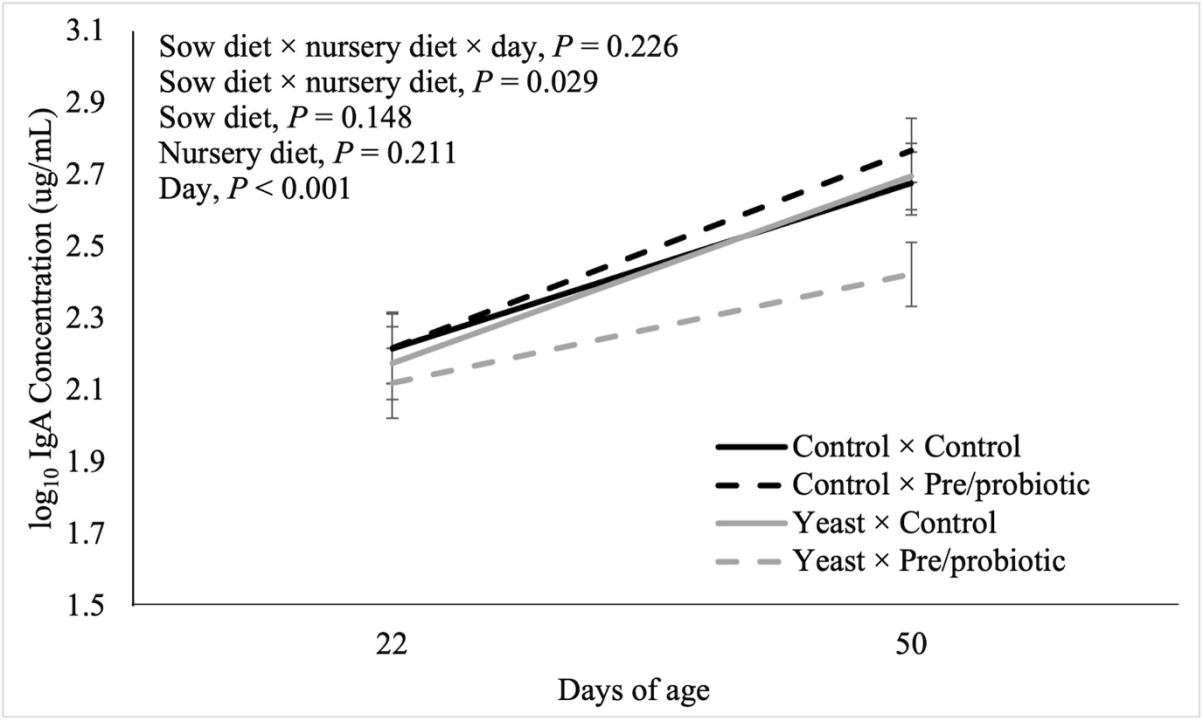

Figure 2.2 Effect of sow and nursery diet on $\log_{10}$ immunoglobulin A concentration at 22 and 50 d of age (n = 17 to 18). Data are expressed as means ± SE.

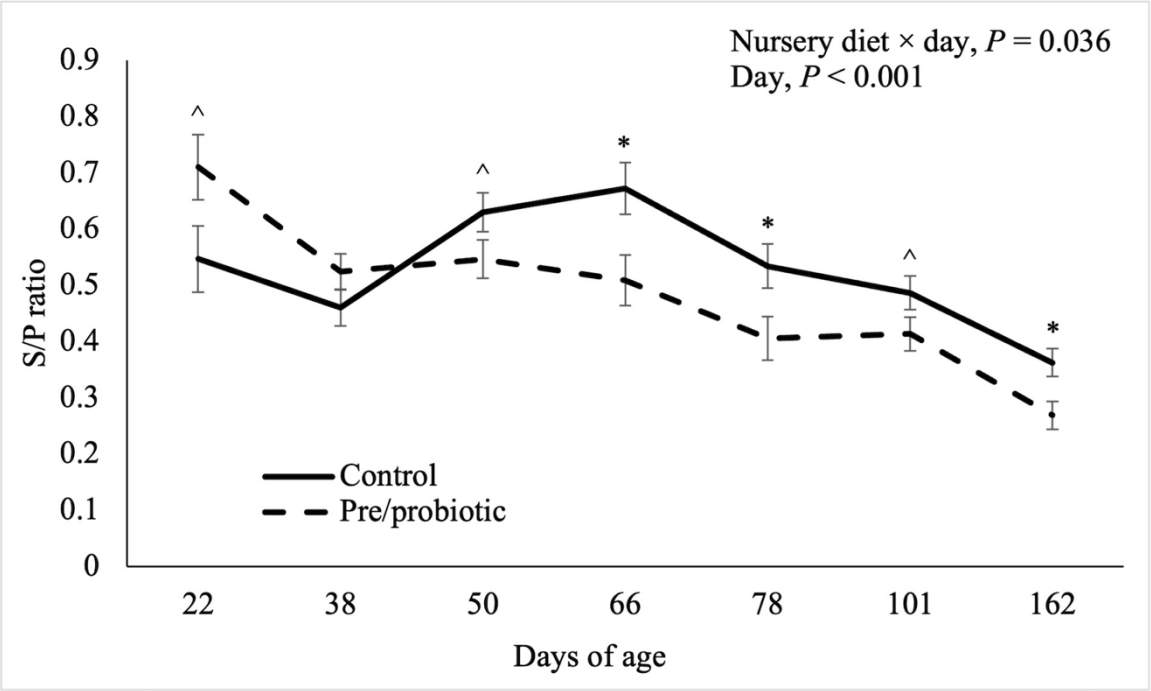

Figure 2.3 Effect of nursery diet on porcine circovirus type 2 sample-to-positive (S/P) ratio at 22, 38, 50, 66, 78, 101, and 162 d of age (n = 35). Data are expressed as means ± SE.

* Indicates significant differences ($P < 0.05$) and ^ shows marginally significant differences ($0.05 < P \leq 0.10$).

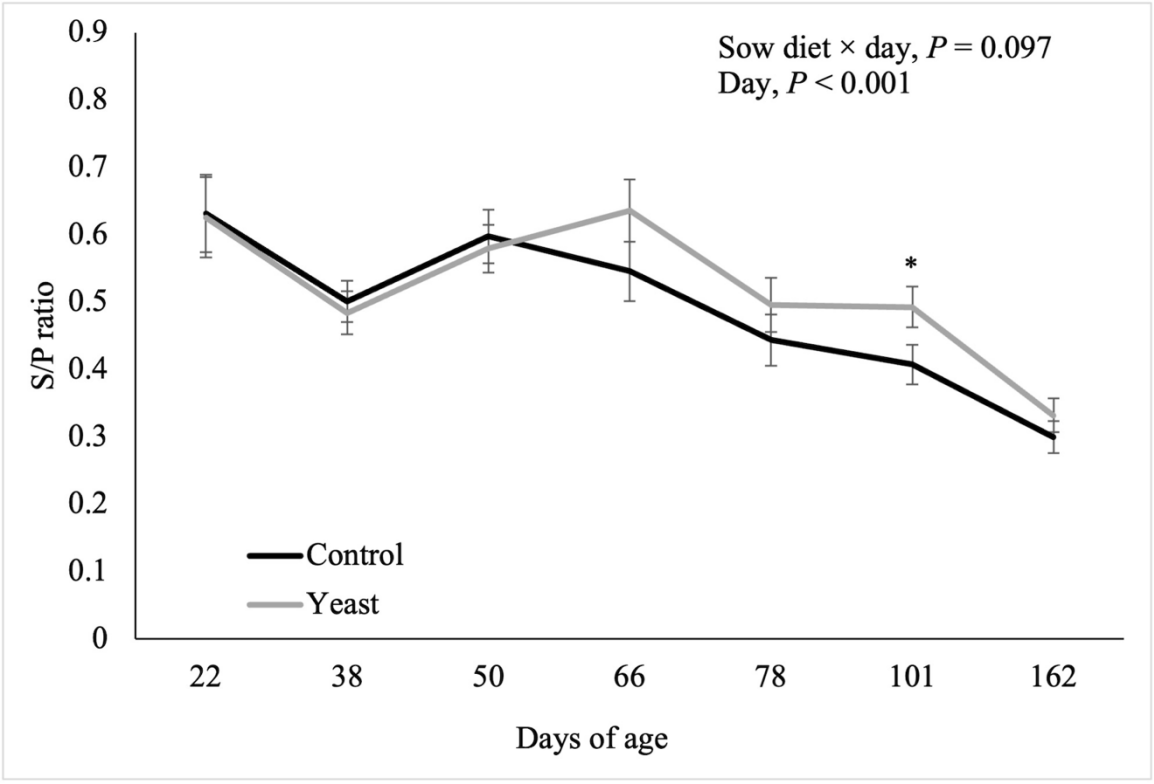

Figure 2.4 Effect of sow diet on porcine circovirus type 2 sample-to-positive (S/P) ratio at 22, 38, 50, 66, 78, 101, and 162 d of age (n = 34 or 36). Data are expressed as means ± SE.

* Indicates significant differences ($P < 0.05$).

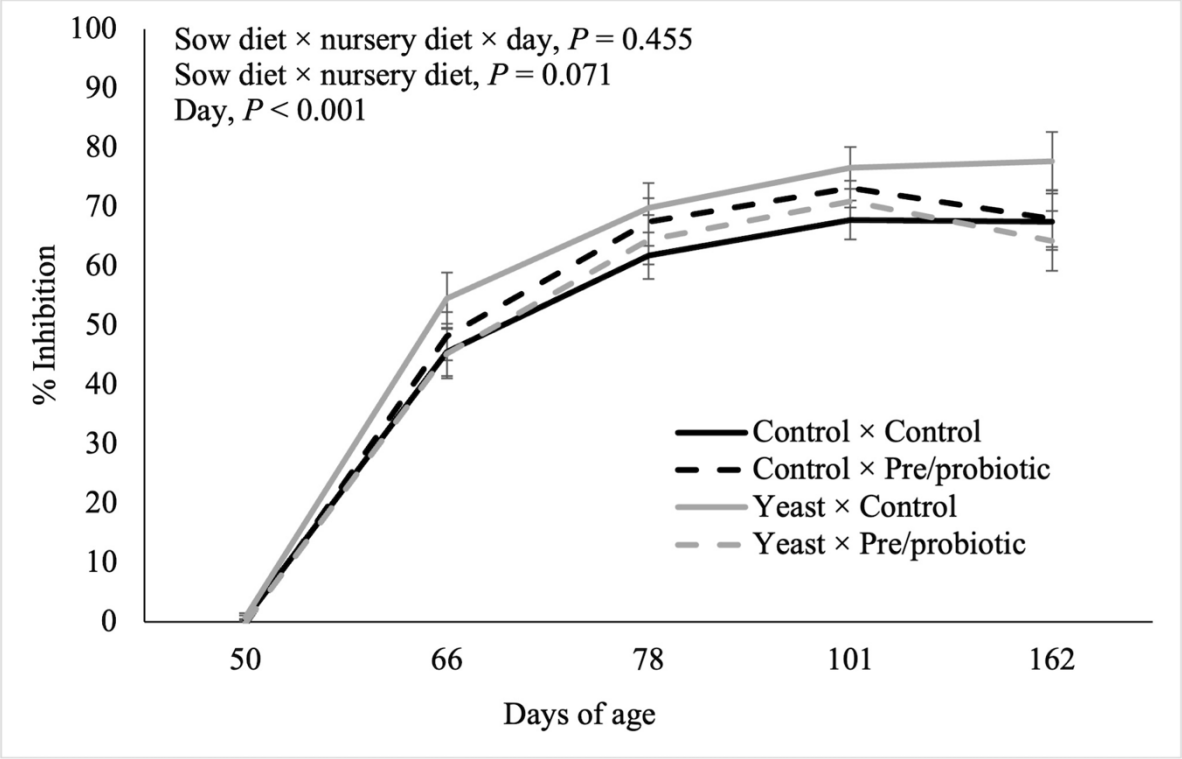

Figure 2.5 Effect of sow and nursery diet on *Lawsonia intracellularis* inhibition at 22, 38, 50, 66, 78, 101, and 162 d of age (n = 17 to 18). Data are expressed as means ± SE.

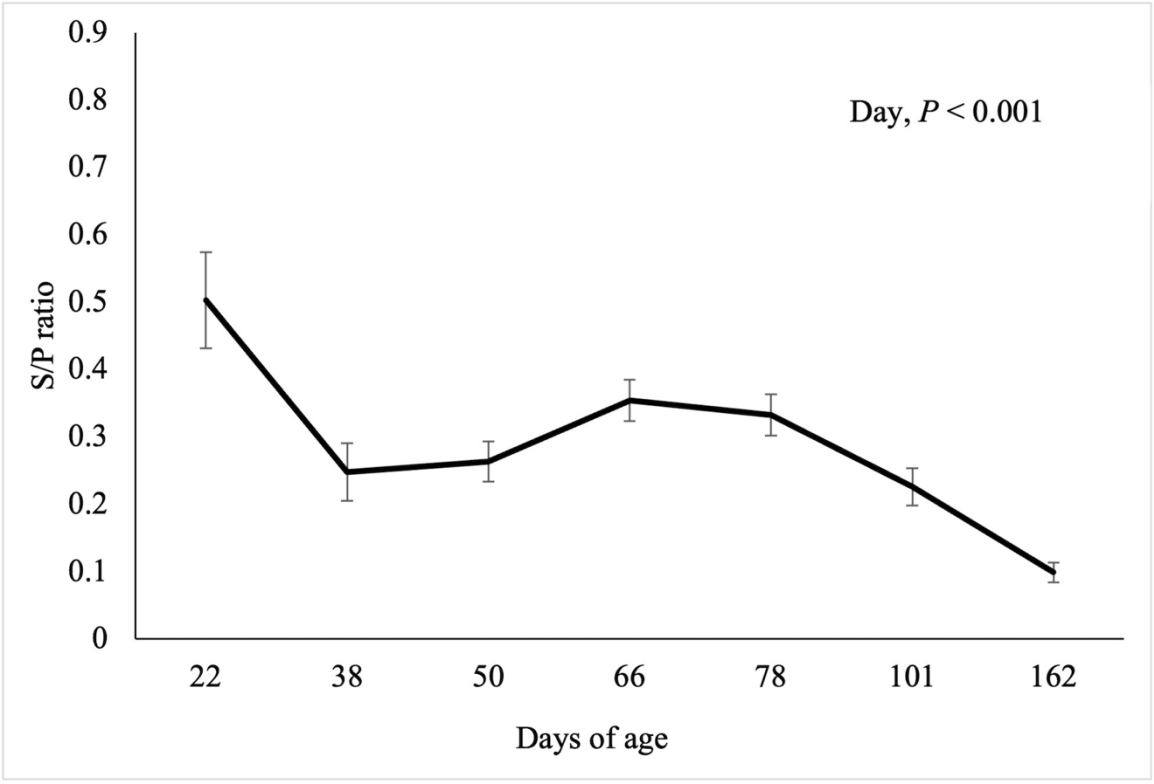

Figure 2.6 Effect of day on *Mycoplasma hyopneumoniae* sample-to-positive (S/P) ratio at 22, 38, 50, 66, 78, 101, and 162 d of age (n = 70). Data are expressed as means ± SE.

Chapter 3 - The effect of increased standardized ileal digestible lysine through increased soybean meal during late gestation on sow lactation performance

ABSTRACT

A total of 87 sows (Line 241, DNA) and their offspring were used to evaluate the effects of increasing standardized ileal digestible (SID) Lys in late gestation diets on lactating sow and litter performance. Sows were blocked by parity and body weight (BW) on d 90 of gestation and allotted to one of three treatments with 29 replications per treatment. Diets included increasing SID Lys (0.60, 0.80, or 1.00%) accomplished by increasing soybean meal (14, 21, or 29% of the diet). Sows were fed 2.04 kg/d of their treatment diet from d 90 of gestation until farrowing for average SID Lys intakes of 11.9, 15.8, or 19.9 g/d. After farrowing, sows had *ad libitum* access to a common lactation diet containing 1.10% SID Lys. Urine samples were collected on d 90 and 110 of gestation to determine urinary creatinine levels. Litters were cross-fostered within dietary treatment by 48 h after farrowing to equalize litter size. Parity was included in the statistical model as a fixed effect with classifications of primiparous (n = 35) or multiparous (n = 52) sows. Weight gain from d 90 to 110 of gestation increased (linear, $P < 0.001$) as SID Lys increased. Change in urinary creatinine level from d 90 to 110 of gestation tended to decrease (linear, $P = 0.063$) as SID Lys increased suggesting that muscle catabolism decreased with increasing SID Lys. There were no differences in starting litter size or piglet birth weight with increasing SID Lys in late gestation. Piglet average daily gain (ADG) from d 2 to 10 of lactation increased (linear, $P = 0.017$) as SID Lys increased. From d 2 until weaning, litters from sows fed 15.8 g/d of SID Lys in gestation had the greatest (quadratic, $P = 0.044$) litter weight gain. Pre-weaning

mortality from birth until d 2 of lactation was greatest for sows fed 15.8 g/d of SID Lys (quadratic, $P = 0.025$). There was a parity × gestation diet interaction ($P = 0.049$) for pre-weaning mortality from d 2 to weaning where mortality increased as SID Lys increased in primiparous sows but decreased in multiparous sows. However, the differences in mortality did not influence the number of pigs weaned per treatment. In conclusion, increased SID Lys through increased soybean meal linearly increased late gestation sow weight gain and piglet ADG during early lactation. Litters from sows fed 15.8 g/d of SID Lys had the greatest litter ADG during late lactation and overall.

INTRODUCTION

Total pigs born per litter has steadily increased as genetic selection continues to produce more prolific sows. During late gestation, there is an increase in both fetal and mammary growth with Lys being the first limiting amino acid for both. Due to the increase in litter size it has been hypothesized that today's prolific sow may have an increased requirement for Lys in late gestation (Feyera and Theil, 2017). For this reason, interest in defining the Lys requirements of late gestating sows has increased. The NRC (2012) estimates a standardized ileal digestible (SID) Lys intake of 16.7 g/d after d 90 of gestation for primiparous sows, decreasing as parity increases with a requirement estimate of 11.5 g/d for fourth parity or older sows. Various indicators have been used to estimate the SID Lys requirement of late gestating sows including whole body protein deposition, AA oxidation, and total piglets born alive with SID Lys requirement estimates ranging from 11.0 to 18.7 g/d depending on parity and response criteria used (Samuel et al., 2012; Thomas et al., 2021; Jo and Kim, 2023).

Because of the substantial increase in fetal and mammary growth during late gestation, Farmer et al. (2022) observed that an increase in SID Lys intake above NRC (2012) estimates

was needed to optimize late gestation mammary development in primiparous sows. Increasing SID Lys through increased soybean meal (SBM) intake in late gestation led to enhanced fetal and mammary growth at day 110 of gestation, as indicated by increased total parenchymal fat, protein, DNA, and RNA (Farmer et al., 2022). However, when a second study was conducted utilizing the same treatment structure with multiparous sows, there was no improvement in fetal or mammary growth as SID Lys increased (Farmer et al., 2023). These two studies ended on d 110 of gestation and the effects of the increased SID Lys in late gestation on lactation performance were not investigated. Therefore, the objective of this trial was to determine if increased SID Lys through increased SBM fed in late gestation diets improves lactating sow and litter performance. We hypothesized that increased SID Lys through increased SBM would result in improved milk production due to changes in mammary gland development as evidenced improved litter growth during lactation.

MATERIALS AND METHODS

Animals and treatment structure

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4485.46). This study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. During gestation, sows were housed in group pens by farrowing group with pens containing approximately 30 sows. Gestation pens had completely slatted concrete floors and allowed approximately 2.14 m^2/sow. Gestation pens were equipped with two nipple waterers and an electronic feeding system (Gestal 3G Feeding System, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada) to allow individual feed intake. During lactation, sows were housed individually in an environmentally controlled and mechanically ventilated barn. Farrowing stalls

were equipped with an electronic feeding system (Gestal Quattro Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada) and an individual cup waterer. Farrowing stalls also contained a heated mat for piglet comfort.

A total of 87 sows (Line 241, DNA Genetics, Columbus, NE) were used across three batch farrowing groups from May to September 2023. On approximately d 90 of gestation, sows were weighed, then blocked by body weight (BW) and parity and assigned to one of three dietary treatments. Two corn-soybean meal-based diets were formulated to contain 0.60 or 1.00% SID Lys with increasing levels of SBM (14 or 29% of the diet) and feed grade L-Lys (0.10 or 0.15% of diet; Table 1). The electronic feeding system was used to blend the two diets (50:50 blend) to provide the intermediate 0.80% SID Lys diet. Sows were allowed approximately 2.04 kg/day of their treatment diet from d 90 of gestation until farrowing for average SID Lys intakes of 11.9, 15.8, or 19.9 g/d. Sows were moved to the farrowing house on d 110 of gestation but remained on their treatment diet until the day they farrowed at which time they were switched to *ad libitum* access to a common lactation diet containing 1.10% SID Lys. All diets for this trial were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). All diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates (Table 1) and fed in meal form. Samples of gestation diets were collected upon movement of each group of sows from gestation to the farrowing barn (d 110 of gestation). Lactation diets were collected on the day before weaning each group of sows. Nitrogen content of complete feed samples was determined by combustion using a Leco TruMac N with TruMac operating software (Leco Corporation, St. Joseph, MI). A protein factor of 6.25 was used to convert N content to crude protein (Table 1).

Sow BW was measured on d 90 and 110 of gestation, after farrowing, and at weaning. Sow backfat depth and loin depth were measured on d 90 and 110 of gestation and at weaning at the last rib, 8 cm from the midline using a real-time ultrasound machine (IBEX Pro, E.I. Medical Imaging, Loveland, CO). Sow caliper score (Knauer and Baitinger, 2015) was measured at the last rib on d 90 and 110 of gestation and at weaning. Pigs were cross fostered by 48 h of birth within sow treatment to equalize litter size. Litters were weighed within 24 h after birth, on d 2 and 10 of lactation, and at weaning (approximately d 18 of lactation).

Coefficient of variation (CV) of pig weight within a litter was calculated on d 2, d 10, and at weaning by dividing the standard deviation of the average pig weight of the litter by the average pig weight of the litter. Milk yield was estimated using the model developed by Hansen et al. (2012). This model used litter size at weaning and litter average daily gain (ADG) to estimate daily milk yield from d 2 until weaning. Total milk yield was calculated as the summation of daily milk yield from d 2 of lactation until weaning. Total milk yield was then divided by the difference of weaning age from d 2 to determine average milk yield per day.

Colostrum and urine analysis

Colostrum was collected from each sow from the 3rd, 4th, and 5th teat on either side of the underline within 1 h of the start of farrowing to determine crude protein content. The birth of the first piglet in the litter was considered the start of farrowing. Nitrogen content of colostrum samples was determined by combustion using a Leco TruMac N with TruMac operating software (Leco Corporation, St. Joseph, MI). The N content was multiplied by 6.38 to determine crude protein content (AOAC, 2023). Sow urine samples were collected on d 90 and 110 of gestation to determine urinary creatinine levels. Sow urine samples were collected via the method described by Nickel et al. (2017). Urine was extracted into a conical tube containing 1 mL of HCl to prevent

volatilization of N. All samples were frozen and stored at -20°C until analysis. Urinary creatinine content was measured via a urinary creatinine colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI).

Statistical analysis

Performance data for the trial were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a randomized complete block design. Blocking structure accounted for parity and BW. Sow served as the experimental unit. Treatment and parity (multiparous or primiparous sows) and their interaction were included as fixed effects while farrowing group was included as a random intercept. Count data were analyzed using a Poisson distribution using a logit link function. Proportion data, including litter born alive, stillborn, born mummified, and pre-weaning mortality were analyzed using a binomial distribution using a logit link function. Urinary creatinine was analyzed as a repeated measure using the lmer function of R software with treatment, sample time point, parity, and their second- and third-order interactions included as a fixed effect. Plate and group were included as a random effect. Change in urinary creatinine from d 90 to 110 of gestation was analyzed using the lme4 package of R with treatment, parity, and their interaction included as fixed effects with plate and group included as a random effect. Preplanned linear and quadratic contrast statements were used to evaluate increasing SID Lys. Differences were considered significant at $P \leq 0.05$ and marginally significant or a tendency at $0.05 < P \leq 0.10$.

RESULTS

Sow gestation performance

There was an SID Lys × parity interaction ($P = 0.039$) observed for sow loin depth at entry into the farrowing house where primiparous sows fed 15.8 g/d of SID Lys had numerically

the least loin depth when compared to primiparous sows fed 11.9 or 19.9 g/d of SID Lys whereas multiparous sows fed 11.9 g/d of SID Lys had numerically the least loin depth relative to the multiparous sows fed greater levels of SID Lys (Table 2). Increasing SID Lys increased (linear, $P < 0.001$) late gestation BW gain (Table 3). Late gestation SID Lys tended (quadratic, $P = 0.080$) to influence backfat thickness at farrowing house entry where sows fed 15.8 g/d of SID Lys had numerically the greatest backfat depth compared to sows fed either 11.9 or 19.9 g/d of SID Lys in late gestation. Sow loin depth changes over late gestation behaved in an opposite manner as there was a tendency (quadratic, $P = 0.081$) for sows fed 15.8 g/d of SID Lys to have a smaller increase in loin depth during late gestation when compared to sows fed 11.9 or 19.9 g/d of SID Lys. As expected, increasing SID Lys increased (linear, $P < 0.001$) Lys intake/d during late gestation. Multiparous sows were heavier and had greater backfat depth ($P \leq 0.033$) than primiparous sows at d 90 and entry into the farrowing house. Multiparous sows had greater ($P = 0.001$) average daily feed intake (ADFI) during gestation when compared to primiparous sows which resulted in a slight increase (0.3 g/d) in SID Lys intake/d in multiparous sows as compared to primiparous sows ($P = 0.001$).

Sow lactation performance

There was a tendency for a treatment × parity interaction ($P \leq 0.070$) observed for sow BW change from entry into the farrowing house until weaning and sow BW change from d 90 of gestation until weaning. Primiparous sows fed 19.9 g/d of SID Lys had a numeric decrease in lactation BW loss (gaining weight from d 90 to weaning) when compared to primiparous sows fed lower levels of SID Lys in late gestation. Multiparous sows fed 19.9 g/d of SID Lys in late gestation had a numeric increase in lactation BW loss when compared to multiparous sows fed lower levels of SID Lys in late gestation. There was a treatment × parity interaction ($P = 0.018$)

observed for caliper score at weaning. Primiparous sows fed 15.8 g/d of SID Lys had lower caliper scores at weaning when compared to multiparous sows fed 15.8 g/d of SID Lys with multiparous and primiparous sows fed 11.9 or 19.9 g/d of SID Lys in late gestation intermediate. There was a tendency for a treatment × parity interaction ($P = 0.090$) for backfat depth at weaning in which responses were similar to caliper score at weaning but means did not separate based on the Tukey analysis. During lactation, sows fed 15.8 g/d of SID Lys during late gestation had an increase (quadratic, $P = 0.043$) in backfat loss compared to those fed 11.9 or 19.9 g/d of SID Lys during late gestation.

Multiparous sows tended ($P \leq 0.096$) to lose more weight during lactation and backfat from d 90 of gestation until weaning compared to primiparous sows. Multiparous sows lost ($P = 0.025$) more weight from d 90 of gestation until weaning compared to primiparous sows. However, multiparous sows were still heavier ($P < 0.001$) than primiparous sows at weaning. Multiparous sows had greater ADFI during lactation when compared to primiparous sows ($P < 0.001$).

Litter performance

There was a treatment × parity interaction ($P = 0.045$) observed for litter gain and litter ADG from d 10 to weaning. Primiparous sows previously fed 19.9 g/d of SID Lys in late gestation had the lowest litter gain and litter ADG, while multiparous sows fed either 15.8 or 19.9 g/d of SID Lys had the greatest litter gain. Litter ADG in primiparous sows fed either 11.9 or 15.8 g/d of SID Lys and multiparous sows fed 11.9 g/d of SID Lys in late gestation were intermediate. There was a treatment × parity interaction ($P = 0.049$) on d 2 to weaning mortality. In primiparous sows, d 2 to weaning mortality increased as SID Lys fed in late gestation increased but it decreased in multiparous sows.

The percentage of pigs born alive tended (linear, $P = 0.093$) to decrease and stillborns tended (linear, $P = 0.068$) to increase as SID Lys intake in late gestation increased (Table 4). Increasing SID Lys tended (linear, $P = 0.080$) to decrease litter birth weights. However, when litter birth weight was standardized by litter size, there were no effects of SID Lys level in late gestation on pig birth weight. Litter ADG from d 2 to 10 of lactation tended (linear, $P = 0.060$) to increase as SID Lys fed in late gestation increased. This corresponded with an increase (linear, $P = 0.014$) in pig ADG from d 2 to 10 as SID Lys increased. However, there were no differences in pig ADG from d 10 until weaning or during the overall lactation period based on SID Lys level fed in late gestation. Overall, sows fed 15.8 g/d of SID Lys in late gestation had the greatest (quadratic, $P = 0.044$) litter gain and tended (quadratic, $P = 0.075$) to have the heaviest litter weaning weights when compared to litters from sows fed 11.9 or 19.9 g/d of SID Lys.

Litter CV was not different on d 0 of lactation. However, on d 2 of lactation there was a tendency for an increase (linear, $P = 0.089$) in litter CV with increasing SID Lys fed in late gestation. On d 10 of lactation, litter CV was greater (quadratic, $P = 0.044$) in litters from sows fed 15.8 g/d of SID Lys in late gestation when compared to litters from sows fed 11.9 or 19.9 g/d of SID Lys in late gestation. However, at weaning there were no late gestation SID Lys level effects on litter CV. Litters from sows fed 15.8 g/d of SID Lys had the greatest (quadratic, $P = 0.018$) birth to d 2 piglet mortality compared to sows fed either 11.9 or 19.9 g/d of SID Lys in late gestation. Calculated total milk yield and average daily milk yield did not differ based on SID Lys level fed in late gestation or by parity.

The total number of pigs born was greater, percentage of pigs born alive was lower, and incidence of mummies was greater ($P \leq 0.025$) in multiparous sows compared to primiparous sows. Litter weight was greater ($P \leq 0.008$) at all time points and mean piglet BW was greater (P

≤ 0.046) on d 0, d 2, and at weaning and tended (P = 0.089) to be greater on d 10 in pigs born from multiparous sows compared to primiparous sows. Multiparous sows tended ($P \leq 0.100$) to have greater litter ADG from d 10 to weaning and d 2 to weaning compared to primiparous sows. On d 2 and d 10 of lactation, there was a tendency ($P \leq 0.089$) for a parity effect on litter CV where litters from primiparous sows had a lower CV when compared to litters from multiparous sows.

Colostrum and urine analyses

Colostrum protein content did not differ based on late gestation diet. There was a tendency for a treatment × day of gestation interaction (P = 0.076) observed for urinary creatinine concentration (Table 5). Primiparous sows fed 19.9 g/d of SID Lys in late gestation had the greatest urinary creatinine concentration on d 90 of gestation but the lowest urinary creatinine concentration on d 110 of gestation when compared to primiparous sows fed 11.9 or 15.8 g/d of SID Lys. Conversely, multiparous sows fed 15.8 g/d of SID Lys during late gestation had the lowest urinary creatinine concentration at both time points when compared to multiparous sows fed either 11.9 or 19.9 g/d of SID Lys. There was a tendency for a treatment × parity interaction (P = 0.087) observed for change in urinary creatinine from d 90 to d 110 of gestation (Figure 1). The change in urinary creatinine concentration progressed from positive to negative as SID Lys in late gestation increased in primiparous sows, whereas in multiparous sows, the SID Lys level in late gestation diets did not influence the change in urinary creatinine. Overall, increasing late gestation SID Lys intake decreased (linear, P = 0.034) the directional change in urinary creatinine concentration.

DISCUSSION

This trial increased SID Lys in late gestation sow diets through increased SBM. When SBM is increased in the diet it also contributes high levels of crude protein and other amino acids. However, based on the ratio of other amino acids to Lys, it was still considered the first limiting amino acid for the sows in this trial.

Farmer et al. (2022) evaluated the effects of a 40% increase in SID Lys above NRC (2012) requirement estimates through increasing SBM in diets for primiparous sows. They observed that fetal weight, total mammary parenchymal mass, and total parenchymal fat, protein, DNA, and RNA increased as SID Lys intake increased from 18.6 to 26.0 g/d (Farmer et al., 2022). However, in a second study, Farmer et al. (2023) observed no differences in fetal weight or mammary development in multiparous sows with increased SID Lys. Both studies euthanized sows on d 110 of gestation to collect mammary tissues, thus, the effects on litter performance could not be measured. Therefore, our study was conducted to see if there were differences in milk production as evidenced by differences in litter weight gain due to increased SID Lys through increased SBM in late gestation.

It is unclear what the SID Lys requirement of the late gestating sow is as past research in this area has produced varying estimates depending on which indicators are used. Using the number of total pigs born alive per litter as a response criteria, Jo and Kim (2023) estimated the late gestation (d 77 to 103) SID Lys requirement of multiparous sows (parity = 5.1 ± 2.0) to be 16.1 g/d. Conversely, Thomas et al. (2021) modeled SID Lys requirements based on predicted whole body protein deposition and concluded that 11.0 g/d of SID Lys was sufficient throughout most of gestation with the exception of the last 5- to 10-d of gestation when both multiparous and primiparous sows enter a negative SID Lys-balance and need to be provided with at least

13.5 g/d of SID Lys. Samuel et al. (2012) used the indicator AA oxidation method with simultaneous determination of heat production and set the SID Lys requirement of late gestating (d 86 to 110 of gestation) second-parity sows at 18.7 g/d and third-parity sows at 13.0 g/d. Samuel et al. (2012) suggested segregation of parities in gestation to more precisely feed sows to their SID Lys requirement.

While the aforementioned studies sought to determine the SID Lys requirement of late gestating sows, others, including the current study, aimed to determine the effects of increased SID Lys in late gestation on lactation performance. Many studies investigating the effects of increased SID Lys in late gestation have observed an increase in late gestation BW gain (Heo et al., 2008; Yang et al., 2009; Gonçalves et al., 2016; Che et al., 2019) which is in agreement with the results of the present study. Similar to this trial, Farmer et al. (2022), increased SID Lys through increased SBM and also observed an increase in late gestation BW gain in primiparous sows. However, when Farmer et al. (2023) employed a similar treatment design in multiparous sows, they observed no difference in late gestation BW gain.

Studies investigating the effects of increased SID Lys in late gestation on backfat change are more variable. Some studies observed an increase in backfat change when SID Lys increased (Heo et al., 2008; Yang et al., 2009); while others, similar to the current trial, saw no difference based on late gestation diet (Farmer et al., 2022; 2023). The variability in this response criteria may be due to differences in methods utilized to accomplish the increase in SID Lys through L-Lys-HCl or SBM wherein trials that utilized L-Lys-HCl did see an increase in backfat with increased SID Lys in the late gestation diet while trials that utilized SBM did not. This difference in backfat change during late gestation may be due to differences in digestion of Lys when it is supplemented as a free amino acid compared with protein-bound Lys. Free amino acids are

absorbed more rapidly in the small intestine when compared to protein-bound amino acids (Batterham, 1985). This could result in differences in Lys utilization, especially in gestating sows that are only fed once daily, as protein synthesis requires that all necessary amino acids are available at the same time. Thus, the additional Lys supplied by SBM in the diet may have been utilized for protein synthesis in fetal or maternal tissues while the additional Lys supplied by L-Lys-HCl may not have been utilized for protein synthesis and was converted to glucose through gluconeogenesis.

Due to differences in parenchymal tissue composition observed by Farmer et al. (2022), we hypothesized that there would be an increase in protein content of colostrum when SBM (and thus crude protein) were increased in the late gestation diet. Both Yang et al. (2008) and Heo et al. (2008) observed increased colostrum protein content when sows were fed high levels of SID Lys in late gestation. However, similar to the results of Yang et al. (2009) and Che et al. (2019), the present study did not observe a difference in colostrum protein content when SID Lys was increased via increased SBM in the late gestation diet.

Due to the substantial increase in both fetal and mammary growth during late gestation, the SID Lys requirement of sows should increase during the last month of gestation (Ji et al., 2005; Kim et al., 2009). For this reason, late gestating sows may have to mobilize and catabolize their own muscle protein to meet these demands. Creatinine is generated as a metabolic waste product during the process of muscle catabolism and therefore serves as a marker of protein mobilization. Due to the increase in SID Lys requirements in late gestating sows, we speculated that the change in urinary creatinine would decrease from d 90 to d 110 of gestation with increasing SID Lys. The decrease in urinary creatinine in the present study as SID Lys increased may point to a decrease in muscle catabolism. Heo et al. (2008) measured blood creatinine levels

post-farrowing and observed a decrease in creatinine levels with increased SID Lys. This is contrary to results by Yang et al. (2008, 2009) who observed no differences in blood creatinine levels post-farrowing with increased SID Lys in late gestation diets.

Due to the increase in fetal weight in primiparous sows observed by Farmer et al. (2022), we hypothesized that there may be differences in pig or litter birth weight as SID Lys intake (through added SBM) increased. Yang et al. (2009) observed an increase in pig birth weight and Che et al. (2019) saw a tendency for an increase in litter birth weight when SID Lys was increased in late gestation diets. However, the present trial observed a tendency for a linear decrease in litter birth weight when SID Lys was increased in late gestation. When litter birth weight was standardized by litter size, there were no differences in mean pig birth weight due to dietary treatment. While there were no differences in litter size at any time point in the study, there were numeric differences in total born and d 0 litter size. The number of total born piglets per litter would have been determined earlier in pregnancy than the start of dietary treatments and thus numeric differences would likely be a function of chance. However, this difference in total born was further heightened by the tendency for an increase in stillbirths with increasing SID Lys in late gestation diets. The biggest increase in stillbirths was observed when comparing sows fed 19.9 g/d of SID Lys to those fed 15.8 g/d of SID Lys in late gestation. The observation of increased stillbirths in the present study is in contrast with previously published data that either found no differences in stillbirths (Che et al. 2019) or a decrease in stillbirths (Gonçalves et al., 2016; Thomas et al., 2021) when SID Lys intake in late gestation was increased through added SBM. However, past research has shown increased stillbirths and farrowing duration when crude protein intake was increased in late gestating sows, potentially due to an increase in heat increment (Tydlitát et al., 2008).

Similar to results of Gonçalves et al. (2016), there were no differences in birth weight CV in the current study as SID Lys intake increased. Che et al. (2019) observed no differences in the percentage of intrauterine growth restricted piglets when SBM was increased in the late gestation diet. It is unclear as to why differences in CV were observed at only d 2 and 10 but no effect was observed at d 0 or weaning.

Due to the improvement in mammary development observed when feeding increased SID Lys in late gestation in primiparous sows (Farmer et al., 2022) but not in multiparous sows (Farmer et al., 2023), we hypothesized that an interaction between SID Lys level and parity classification may be observed for pig ADG, litter ADG and milk yield. Although an interaction between SID Lys intake and parity classification was observed in litter gain and litter ADG from day 10 to weaning, the reason for the reduced litter ADG in primiparous sows fed 19.9 g/d of SID Lys compared to those fed lower levels remains unclear, particularly given that increasing SID Lys intake in multiparous sows led to improved litter ADG over the same period. Che et al. (2019) observed an improvement in overall pig ADG when sows were fed 20.6 g/d of SID Lys compared to those fed 14.7 g/d of SID Lys in late gestation while in the present study litter ADG was optimized in sows fed 15.8 g/d of SID Lys in late gestation. Interestingly, although there were differences in litter and pig ADG at various time points due to gestation dietary treatment, there were no differences in calculated total milk yield or average milk yield. This may be due, in part, to numerical differences in litter size between treatments.

The quadratic increase in PWM observed in the current study in the first 48 h may be due to the numeric increase in number born alive also observed in sows fed 15.8 g/d of SID Lys. In larger litters, the amount of colostrum consumed per pig decreases, leading to more lethargic pigs that are more likely to die due to crushing or starve out (Edwards and Baxter, 2015). It is

unclear why there was an interaction between late gestation diet SID Lys level and parity classification for d 2 to weaning mortality. Past research in this area has shown either no difference (Che et al., 2019) or a decrease in pre-weaning mortality (Gonçalves et al., 2016) when SID Lys was increased through increased SBM.

Results from the current study indicated no differences in lactation backfat BF change or lactation ADFI with increasing SID Lys intake in late gestation diets which is in agreement with past literature (Che et al., 2019). In the current trial, there was an interaction between SID Lys intake in late gestation and parity classification for sow BW change from d 90 of gestation until weaning. This difference in response to increasing late gestation SID Lys between multiparous and primiparous sows is due, in part, to differences in average litter size at weaning. In addition, primiparous sows in this trial were still growing, and thus, additional SID Lys was more beneficial for these sows as opposed to multiparous sows. Previous research has reported no effect of increasing SID Lys intake during late gestation on WEI (Che et al., 2019) which aligns with the findings of the present study.

In conclusion, increasing SID Lys intake from 11.9 to 19.9 g/d during from d 90 of gestation to farrowing increased late gestation BW gain and early lactation pig ADG. Overall litter gain and litter weaning weight were optimized when sows were fed 15.8 g/d of SID Lys in late gestation. Results from this study suggest that the NRC (2012) estimates for primiparous sows (16.7 g/d) appears to be adequate to optimize lactation performance.

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Table 3.1 Diet composition (as-fed basis)

Item	Gestation[1]		Lactation
	Low Lys	High Lys	
Ingredient, %			
Corn	82.30	67.55	65.95
Soybean meal, 47.7% CP	14.00	28.75	28.15
Soybean oil	---	---	2.00
Calcium carbonate	1.15	1.10	1.25
Monocalcium P, 21.5% P	1.35	1.15	1.15
Sodium chloride	0.50	0.50	0.50
L-Lys-HCl	0.10	0.15	0.30
DL-Met	0.05	0.15	0.08
L-Thr	0.05	0.16	0.11
L-Trp	---	---	0.01
Trace mineral premix	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Standardized ileal digestible (SID) amino acids			
Lys, %	0.60	1.00	1.10
Ile:Lys	76	70	63
Leu:Lys	189	148	132
Met:Lys	42	42	31
Met and Cys:Lys	76	69	55
Thr:Lys	75	75	63
Trp:Lys	20.6	20.5	19
Val:Lys	88	77	68
His:Lys	54	47	41
Total Lys, %	0.70	1.14	1.23
ME, kcal/kg	3,265	3,261	3,358
NE, kcal/kg[2]	2,549	2,522	2,615
SID Lys:NE, g/Mcal	2.36	3.96	4.21
CP, %	13.6	19.6	19.3
Ca, %	0.80	0.79	0.89
P, %	0.60	0.63	0.62
STTD P, %	0.50	0.50	0.50
Chemical analysis			
CP, %	11.4	18.4	18.3

[1]Diets were fed from d 90 of gestation until farrowing. Low and high Lys diets were blended at a 50:50 ratio to form the intermediate treatment.

[2]NE value for soybean meal (91.4% of corn) was derived based on Sotak-Peper et al. (2015), who used the equation from Noblet et al. (1994) to determine NE from ME. Other ingredient NE values were based on NRC (2012).

Table 3.2 Interactive effects of increased standardized ileal digestible (SID) Lys through increased soybean meal in late gestation and parity on sow lactation and litter performance[1]

Parity:	Primiparous[2]			Multiparous				*P* =		
SID Lys, g/d[3]:	11.9	15.8	19.9	11.9	15.8	19.9	SEM	Treatment × parity	Treatment	Parity
Sow BW change (d 90 to weaning), kg	-7.2	-5.5	4.0	-9.0	-6.6	-10.5	4.67	0.059	0.308	0.025
Sow BW change (entry to weaning), kg	-18.8	-22.2	-13.8	-20.3	-21.1	-27.0	5.32	0.070	0.832	0.096
Sow backfat (weaning), mm	11.7	11.3	11.9	11.7	13.2	12.3	0.75	0.090	0.502	0.047
Sow caliper score (weaning)	12.6^{ab}	11.4^{b}	12.7^{ab}	12.7^{ab}	14.1^{a}	12.8^{ab}	1.11	0.018	0.967	0.022
Sow loin depth (entry), mm	51.9	49.3	51.0	49.4	51.2	51.5	1.98	0.039	0.547	0.956
Litter gain (d 10 to weaning), kg	29.3^{ab}	28.9^{ab}	24.3^{b}	27.9^{ab}	30.6^{a}	29.8^{a}	2.38	0.045	0.145	0.080
Litter ADG (d 10 to weaning), kg	3.66^{ab}	3.61^{ab}	3.03^{b}	3.49^{ab}	3.82^{a}	3.73^{a}	0.297	0.045	0.145	0.080
d 2 to weaning mortality, %	1.3	2.7	4.3	7.7	6.4	4.3	1.90	0.049	0.146	0.015

[a-b]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 87 mixed-parity sows (Line 241, DNA, Columbus NE) and litters were used from day 90 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size within 48-h after farrowing.

[2]Parity was included as a fixed effect in the data analysis model. Sows were either classified as primiparous (n=35) or multiparous (n=52).

[3]Sow treatment consisted of providing a low Lys control diet (SID Lys = 0.60%), a high Lys diet, accomplished by adding additional SBM and L-Lys-HCl (SID Lys = 1.00%), or a 50:50 blend of the two diets (SID Lys = 0.80%) from d 90 of gestation until farrowing. After farrowing, sows were fed a common lactation diet until weaning.

Table 3.3 Effects of increased standardized ileal digestible (SID) Lys through increased soybean meal in late gestation and parity on sow lactation performance[1]

	SID Lys, g/d[2]				SID Lys, *P* =		Parity[3]			
	11.9	15.8	19.9	SEM	Linear	Quadratic	Primiparous	Multiparous	SEM	Parity, *P* =
No. of sows	29	29	29	---	---	---	35	52		---
Parity	1.7	1.7	1.7	0.28	0.970	0.983	1.0	2.9	0.23	0.001
Gestation length, d	117.0	116.9	117.4	0.27	0.196	0.314	117.2	117.0	0.26	0.336
Lactation length, d	18.0	17.9	17.5	0.26	0.106	0.533	17.7	17.8	0.24	0.784
Sow BW, kg										
d 90	223.5	221.2	221.8	5.18	0.740	0.735	191.8	252.6	5.18	< 0.001
d 110	235.0	236.6	239.0	4.86	0.428	0.937	207.1	266.6	4.61	< 0.001
Weaning	215.1	215.6	218.5	4.09	0.528	0.794	188.4	244.3	3.69	< 0.001
Gestation change (d 90 to 110)	11.6	15.4	17.1	1.88	0.001	0.419	15.4	14.0	1.82	0.299
Lactation change (entry to weaning)[4]	-19.6	-21.6	-20.4	4.55	0.798	0.568	-18.3	-22.8	4.42	0.096
Overall change (d 90 to weaning)[4]	-8.1	-6.0	-3.3	3.86	0.128	0.901	-2.9	-8.7	3.72	0.025
Sow backfat, mm										
d 90	12.7	13.7	13.5	0.42	0.172	0.222	12.6	14.0	0.37	0.005
Entry	12.8	13.8	13.2	0.38	0.433	0.080	12.8	13.8	0.34	0.033
Weaning[4]	11.7	12.3	12.1	0.64	0.389	0.419	11.6	12.4	0.62	0.047
Gestation change (d 90 to entry)	0.2	0.1	-0.2	0.36	0.352	0.624	0.3	-0.2	0.34	0.154
Lactation change (entry to weaning)	-1.2	-1.7	-1.1	0.75	0.667	0.043	-1.3	-1.4	0.74	0.458
Overall change (d 90 to weaning)	-1.0	-1.5	-1.3	0.70	0.487	0.429	-0.9	-1.6	0.68	0.068
Sow caliper score, units										
d 90	16.2	16.5	16.2	0.57	0.877	0.428	16.1	16.5	0.55	0.139
Entry	14.9	15.4	15.2	1.16	0.461	0.331	14.9	15.5	1.15	0.118
Weaning[5]	12.6	12.7	12.8	1.02	0.808	0.932	12.2	13.2	1.01	0.022
Gestation change (d 90 to entry)	-1.2	-1.0	-1.0	0.64	0.367	0.661	-1.1	-1.0	0.63	0.654
Lactation change (entry to weaning)	-2.4	-2.8	-2.5	1.56	0.849	0.254	-2.8	-2.4	1.56	0.145
Overall change (d 90 to weaning)	-3.6	-3.7	-3.5	1.10	0.770	0.600	-3.9	-3.3	1.09	0.151
Sow loin depth, mm										

d 90	49.5	50.1	49.6	1.66	0.878	0.543	49.5	50.0	1.63	0.607
Entry[5]	50.7	50.3	51.2	1.83	0.505	0.380	50.7	50.7	1.81	0.956
Wean	50.4	50.8	50.9	1.29	0.554	0.815	50.8	50.6	1.26	0.753
Gestation change (d 90 to entry)	1.2	0.2	1.6	0.62	0.570	0.081	1.2	0.7	0.57	0.465
Lactation change (entry to weaning)	-0.4	0.5	-0.4	0.68	0.974	0.112	-0.1	-0.1	0.65	0.986
Overall change (d 90 to weaning)	0.8	0.7	1.2	0.80	0.683	0.669	1.1	0.7	0.73	0.561
Sow ADFI, kg										
Gestation (d 90 to farrowing)	1.82	1.84	1.88	0.041	0.150	0.927	1.79	1.90	0.039	0.001
Farrow to weaning	6.40	6.59	6.12	0.253	0.294	0.148	5.69	7.04	0.233	< 0.001
Late gestation average Lys intake, g/d	11.94	15.76	19.88	0.098	< 0.001	0.104	15.71	16.00	0.092	0.001
Wean to estrus interval, d	5.4	5.4	5.2	0.09	0.205	0.412	5.3	5.3	0.08	0.551

[1]A total of 87 mixed-parity sows (Line 241, DNA, Columbus, NE) and litters were used from day 90 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size within 48-h after farrowing. No treatment × parity interactions ($P > 0.10$) were observed unless otherwise noted.

[2]Sow treatment consisted of providing a low Lys control diet (SID Lys = 0.60%), a high Lys diet, accomplished by adding additional SBM and L-Lys-HCl (SID Lys = 1.00%), or a 50:50 blend of the two diets (SID Lys = 0.80%) from d 90 of gestation until farrowing. After farrowing, sows were fed a common lactation diet until weaning.

[3]Parity was included as a fixed effect in the data analysis model. Sows were either classified as primiparous (n=35) or multiparous (n=52).

[4]Treatment × parity $P < 0.10$.

[5]Treatment × parity $P < 0.05$.

Table 3.4 Effects of increased standardized ileal digestible (SID) Lys through increased soybean meal in late gestation and parity on litter performance[1]

	SID Lys, g/d[2]				SID Lys, *P* =		Parity[3]			
	11.9	15.8	19.9	SEM	Linear	Quadratic	Primiparous	Multiparous	SEM	Parity, *P* =
Litter characteristics										
Total born, n	16.0	16.3	15.7	0.78	0.754	0.630	14.0	18.3	0.64	0.010
Born alive, %	91.3	92.3	87.3	1.79	0.093	0.155	94.1	85.2	1.12	0.005
Stillborn, %	5.0	4.9	8.4	1.40	0.068	0.301	4.4	8.0	0.96	0.115
Mummy, %	3.5	2.6	3.5	1.25	0.995	0.512	1.4	6.9	0.82	0.025
Litter size, n										
d 0	14.6	15.0	14.0	0.75	0.548	0.465	14.0	15.1	0.64	0.511
d 2	14.2	14.1	13.8	0.72	0.651	0.894	13.6	14.5	0.63	0.568
Weaning	13.6	13.6	13.2	0.72	0.706	0.804	13.3	13.7	0.63	0.759
Litter weight, kg										
d 0	23.0	21.9	20.9	0.87	0.080	0.974	19.3	24.6	0.79	< 0.001
d 2	23.6	23.2	22.3	0.80	0.117	0.718	21.3	24.8	0.76	< 0.001
d 10	43.1	45.1	42.9	1.47	0.909	0.183	41.7	45.7	1.35	0.008
Weaning	72.0	75.0	69.8	1.96	0.419	0.075	69.0	75.5	1.77	0.003
Mean pig BW, kg										
d 0	1.57	1.47	1.50	0.068	0.438	0.430	1.38	1.65	0.061	0.001
d 2	1.66	1.65	1.63	0.044	0.632	0.945	1.58	1.71	0.039	0.007
d 10	3.16	3.30	3.25	0.141	0.443	0.356	3.15	3.32	0.136	0.089
Weaning	5.29	5.53	5.34	0.154	0.786	0.193	5.22	5.55	0.141	0.046
Litter gain, kg										
d 2 to d 10	19.5	21.9	20.6	1.37	0.468	0.152	20.4	21.0	1.28	0.656
d 10 to weaning[4]	28.6	29.7	27.0	2.76	0.254	0.112	27.5	29.4	2.04	0.080
d 2 to weaning	48.2	51.9	47.5	1.62	0.768	0.044	47.7	50.7	1.44	0.110
Litter ADG, kg										
d 2 to d 10	2.45	2.75	2.79	0.130	0.060	0.398	2.64	2.68	0.117	0.765
d 10 to weaning[4]	3.58	3.71	3.38	0.261	0.254	0.112	3.43	3.68	0.255	0.080
d 2 to weaning	2.69	2.90	2.73	0.109	0.748	0.075	2.69	2.86	0.101	0.100
Pig ADG, g										
d 2 to d 10	180	202	213	13.4	0.014	0.617	202	194	12.7	0.481
d 10 to weaning	262	273	259	16.6	0.824	0.263	260	270	16.0	0.335

d 2 to weaning	197	214	210	8.0	0.219	0.201	204	210	7.4	0.512
Litter CV, %										
d 0	18.2	17.6	18.6	1.47	0.805	0.557	16.5	19.8	1.38	0.011
d 2	18.4	19.2	20.9	1.42	0.089	0.734	18.4	20.6	1.34	0.089
d 10	17.7	19.5	16.8	0.93	0.498	0.044	17.0	19.0	0.83	0.060
Weaning	17.5	18.2	16.2	1.03	0.380	0.278	16.6	18.0	0.91	0.260
Milk yield[5]										
Total yield (d 2-weaning), kg	198.3	203.2	188.3	6.70	0.246	0.188	192.0	201.2	6.08	0.195
Average yield, kg/d	12.4	12.8	12.2	0.48	0.571	0.219	12.2	12.8	0.48	0.104
Colostrum protein content, %	18.9	19.4	19.3	0.43	0.545	0.590	19.2	19.1	0.39	0.828
Preweaning mortality, %										
Birth to d 2	1.9	4.6	2.0	1.35	0.959	0.018	1.8	3.7	0.97	0.150
d 2 to weaning[4]	3.2	4.2	4.8	1.17	0.339	0.879	2.7	6.0	0.88	0.015

[1]A total of 87 mixed-parity sows (Line 241, DNA, Columbus NE) and litters were used from day 90 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size within 48-h post farrowing. No treatment × parity interactions ($P > 0.10$) were observed unless otherwise noted.

[2]Sow treatment consisted of providing a low Lys control diet (SID Lys = 0.60%), a high Lys diet, accomplished by adding additional SBM and L-Lys-HCl (SID Lys = 1.00%), or a 50:50 blend of the two diets (SID Lys = 0.80%) from d 90 of gestation until farrowing. After farrowing, sows were fed a common lactation diet until weaning.

[3]Parity was included as a fixed effect in the data analysis model. Sows were either classified as primiparous (n=35) or multiparous (n=52).

[4]Treatment × parity $P < 0.05$.

[5]Total milk yield was estimated utilizing the model developed by Hansen et al. (2012). Average milk yield = Total milk yield / (Wean age – 2).

Table 3.5 Evaluation of increased standardized ileal digestible (SID) Lys level through increased soybean meal in late gestation on sow urinary creatinine excretion[1]

Parity:	Primiparous:			Multiparous:				*P*=		
SID Lys, g/d[2]:	11.9	15.8	19.9	11.9	15.8	19.9	SEM	Treatment × day	Treatment	Day
Urinary creatinine, mg/dL[3]										
d 90	378.6	381.4	446.6	465.7	248.8	430.6	90.19	0.076	0.816	0.268
d 110	477.7	337.0	249.3	402.2	303.9	350.0				

[1]A total of 85 mixed-parity sows (Line 241, DNA, Columbus NE) and litters were used from day 90 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size up to 48-h after farrowing.

[2]Sow treatment consisted of providing a low Lys control diet (SID Lys = 0.60%), a high Lys diet, accomplished by adding additional SBM and L-Lys-HCl (SID Lys = 1.00%), or a 50:50 blend of the two diets (SID Lys = 0.80%) from d 90 of gestation until farrowing. After farrowing, sows were fed a common lactation diet until weaning.

[3]Parity included in the model as a main effect. No third or second order interactions or main effects ($P > 0.10$) were observed unless otherwise noted.

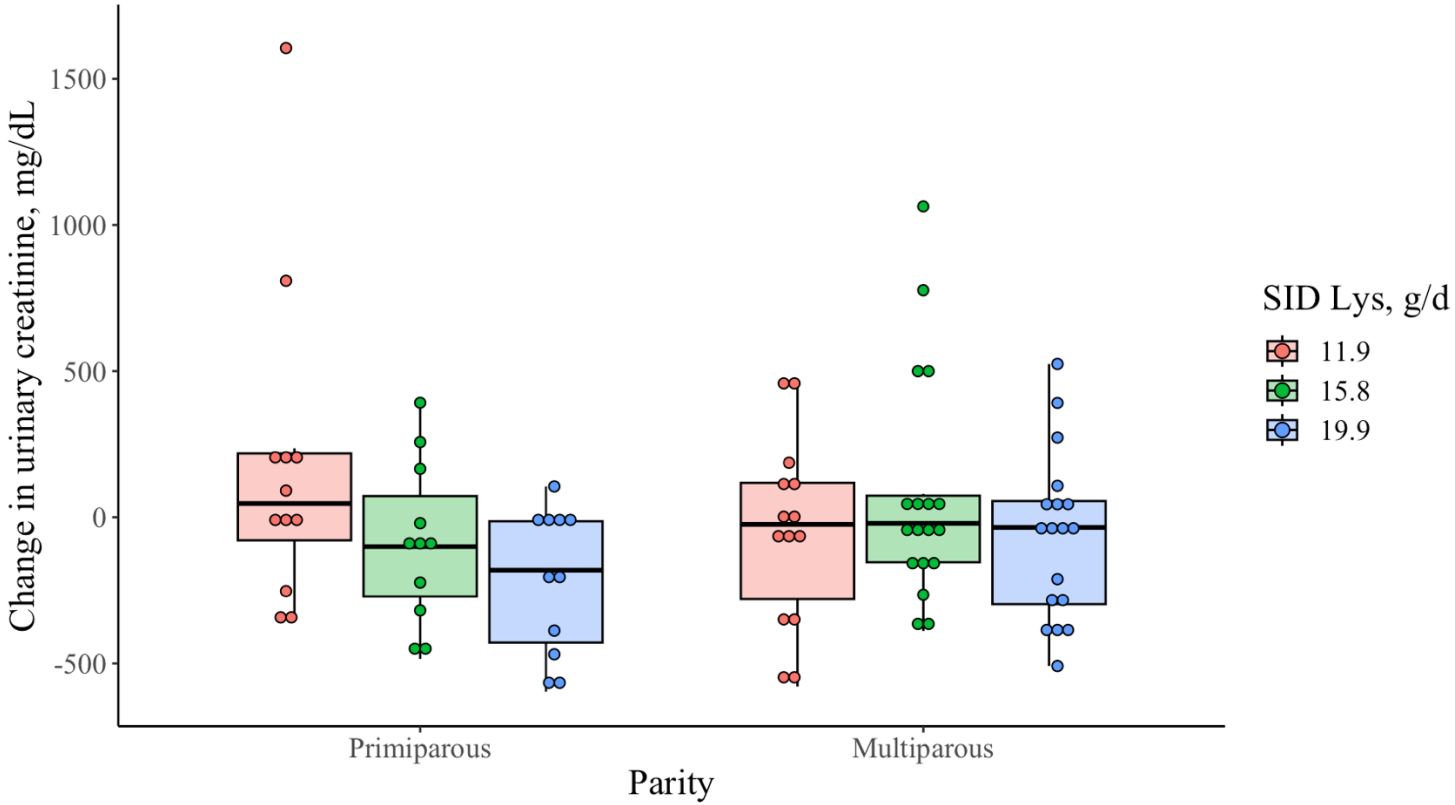

Figure 3.1 Evaluation of increased standardized ileal digestible (SID) Lys intake through increased soybean meal in late gestation on change in urinary creatinine from d 90 to d 110 of gestation.
Treatment × Parity, $P = 0.087$; Linear, $P = 0.034$; Quadratic, $P = 0.627$

Chapter 4 - The effect of standardized ileal digestible histidine:lysine ratio during lactation on sow and litter performance

ABSTRACT

The NRC (2012) requirement estimate for His is approximately 20 g/d for lactating sows. This estimate is based on factorial models and there is limited empirical data available. A total of 88 lactating sows (Line 241, DNA) and their litters were used to evaluate the effects of increasing standardized ileal digestible (SID) His:Lys ratio on sow and litter performance. Sows were blocked by parity and body weight (BW) on approximately d 110 of gestation and allotted to one of three dietary treatments containing increasing SID His:Lys ratios (40, 50, or 60% of Lys). The 40% His:Lys ratio diet was corn and soybean meal-based and formulated to 1.05% SID Lys with no added L-His. Sows were allowed 2.72 kg/d of their treatment diet from d 110 of gestation until farrowing after which they were provided *ad libitum* access to treatment diets. Pigs were cross-fostered by d 2 of lactation within treatment to equalize litter size (approximately 14.2 pigs). Sow BW, backfat, caliper score, and loin depth on d 110 of gestation and at weaning was not affected by increasing SID His:Lys ratio. Increasing SID His:Lys ratio tended to decrease (linear, $P = 0.085$) percentage of pigs born alive due to an increase (linear, $P = 0.049$) in the percentage of stillborn pigs. Litter weight on d 7 and at weaning tended to increase and then decrease (quadratic, $P \leq 0.062$) as SID His:Lys ratio increased. Pig average daily gain (ADG) from d 7 to weaning tended to increase (linear, P = 0.10) with increasing SID His:Lys ratio, though litter ADG and pre-weaning survival remained unaffected. Increasing SID His:Lys ratio increased and then decreased (quadratic, $P = 0.027$) calculated total milk yield from d 2 of lactation until weaning and tended to increase and then decrease calculated average

daily milk yield (quadratic, $P = 0.078$), but did not affect milk composition. Daily feed intake averaged 7.1 kg/d across the three treatments and SID Lys intake averaged 74.6 g/d. Average SID His intake was 29.0, 38.0, and 44.9 g/d with increasing SID His:Lys ratio, exceeding NRC (2012) requirement estimates. These results suggest that a SID His:Lys ratio of 50% tended to increase calculated average daily milk yield and litter weight on d 7 and at weaning. Additional research is warranted to determine the optimal SID His:Lys ratio in lactating sow diets, as typical corn-soybean meal-based diets contain a SID His:Lys ratio of approximately 40%.

INTRODUCTION

Feed-grade amino acids (AA) have been increasingly used in swine nutrition for the past 25 years to more precisely meet the AA requirements of pigs which has environmental and economic benefits (Wang et al., 2020; Cappelaere et al., 2021). Commercially available feed-grade AAs include Lys, Met, Thr, Trp, Val, and, most recently, Ile. With the commercial availability of feed-grade Ile, His becomes the next limiting AA in corn-soybean meal-based diets. Therefore, a His requirement estimate will determine the amount of Ile to be added to diets.

Currently, there is no published literature that evaluates the standardized ileal digestible (SID) His requirement for lactating sows. The NRC (2012) SID His requirement estimates range from 16.9 to 20.8 g/d depending on parity and projected litter weaning weight. However, these requirement estimates are based on factorial estimates and not empirical data. When the NRC (2012) requirement estimates are calculated as a ratio to the SID Lys requirement estimates for sows with similar parity and production level, it equates to an SID His:Lys ratio of approximately 40%.

Räisänen et al. (2023) summarized the effects of increasing His on lactating dairy cow performance. In this meta-analysis, His was added using rumen-protected His or infusion of His into the abomasum or jugular vein. This review indicated that added His (30 to 60 g/d) increased both milk- and milk protein-yield (Räisänen et al., 2023). Given the results observed in lactating dairy cows, increasing SID His in lactating sow diets might have similar benefits. Therefore, the objective of this experiment was to determine the effects of increasing the SID His:Lys ratio in lactating sow diets on milk production and litter performance. Our hypothesis was that, similar to the lactating dairy cow, increasing SID His:Lys ratio in lactating sow diets would result in an improvement in milk yield and consequently litter performance.

MATERIALS AND METHODS

Animals and treatment structure

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4485.51). The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Sows and litters were housed individually in an environmentally controlled and mechanically ventilated barn. Farrowing stalls were equipped with an electronic feeding system (Gestal Quattro Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada) and an individual cup waterer. Farrowing stalls contained a heated mat for pig comfort.

A total of 88 sows (Line 241, DNA, Columbus, NE) were used across three farrowing groups. On approximately d 110 of gestation, sows were moved into the farrowing house, weighed, blocked by body weight (BW) and parity, and randomly assigned to one of three dietary treatments (SID His:Lys ratios of 40, 50, and 60% of Lys). Two corn-soybean meal-based experimental diets were formulated to contain SID His:Lys ratios of 40% or 60% of Lys

with the 60% ratio diet created by the addition of L-Histidine (CJ Bio America, Downers Grove, IL; Table 1). The electronic feeding system was used to blend the two diets at a 50:50 ratio to form the intermediate treatment (SID His:Lys ratio of 50%). Sows were allowed 2.72 kg/day of their treatment diet from d 110 of gestation until farrowing. After farrowing, sows had *ad libitum* access to their treatment diet. Lactation diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates and fed in meal form. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS).

Sow BW was measured on d 110 of gestation, after farrowing, and at weaning. Sow backfat depth and loin depth were measured on d 110 of gestation and at weaning at the last rib approximately 7 cm from the midline using real-time ultrasound machine (IBEX Pro, E.I. Medical Imaging, Loveland, CO). Sow caliper score (Knauer and Baitinger, 2015) was measured on d 110 of gestation and at weaning at the last rib. Pigs were cross fostered within 48 h of birth within sow treatment to equalize litter size. Litters were weighed at birth, on d 2 and 7, and the day before weaning (d 17). All instances of pig mortality were recorded to determine pre-weaning mortality (PWM).

Milk composition and piglet plasma AA analyses

Milk was collected from 10 sows per treatment on approximately d 7 after farrowing and the day before weaning (d 17). To obtain milk samples, 10 IU of oxytocin was administered in an ear vein of the sow to induce milk letdown, then milk was manually expressed. Milk samples (50 mL total) were collected from the third, fourth, and fifth mammary glands on either side of the underline. To determine which sows were sampled for milk collection, blocks of sows (containing one sow from each treatment with similar entry BW and parity) were selected with the intent of creating a representative distribution of the overall trial population. The same sows

used for milk collection on d 7 after farrowing were also collected the day before weaning. From each litter milk was collected, the medium-weight barrow was selected using the individual piglet weights collected on d 7 after farrowing, and blood was drawn to determine plasma free AA concentration. The same barrow sampled on d 7 after farrowing was also sampled on the day before weaning. At both sampling time points, a 9-mL blood sample was collected from each pig in a 10-mL lithium heparin vacutainer tube (LH Lithium Heparin; Greiner Bio One International, Kremsmünster, Austria) by puncturing the jugular vein of the pig. The vacutainers were inverted 10 times to prevent coagulation of the blood samples and were then placed on ice until centrifuged. The blood samples were centrifuged at 1,800 × *g* for 30 min at 4°C and the resulting aliquots were then harvested into 1.5-mL tubes and stored at -20°C until analysis.

The frozen milk samples were later thawed and analyzed for percentages of fat, protein, lactose, total solids, and somatic cell count (MQT Lab Services, Kansas City, MO). The samples were analyzed with Bentley FTS/FCM equipment (Bentley Instruments, Chaska, MN) using a Fourier Transform Spectrometer based on infrared absorption. Somatic cell count was determined through flow cytometry methodology. All analyses were performed using AOAC methodology (AOAC, 2023).

Pig plasma samples (0.75 mL) were deproteinized with an equal volume of 10% sulfosalicylic acid (SSA). This SSA mixture also contained an internal standard of Norvaline. This 1:1 mixture of plasma and SSA was cooled in an ice bath for one h with recurring agitation every 15 minutes. Then, samples were centrifuged at 17,000 × g for 20 min at 4°C after which the supernatant was filtered through a 0.22μ filter and frozen until processed for AA analysis. The samples were analyzed by UPLC methods with a Waters ACQUITY UPLC TUV system (Waters, Milford, MA) and an AccQ Tag Ultra column to determine free AAs.

Milk yield and milk protein yield estimations

Milk yield was calculated utilizing the model developed by Hansen et al. (2012). This model utilized the number of pigs at weaning and litter ADG to estimate daily milk yield from d 2 until weaning. Total milk yield and average daily milk yield were calculated from these values. Total protein yield was calculated by multiplying the calculated total milk yield for each sow by the average protein content of all the milk samples collected from that treatment. Total protein yield was then divided by the difference of weaning age from d 2 to determine average daily protein yield.

Nitrogen balance

Nitrogen balance was determined to measure differences in N utilization. Nitrogen intake was determined by multiplying the N content in feed by the feed intake for each sow. Nitrogen content in milk was calculated by dividing the sow's calculated average daily protein yield (g/d) by the milk N conversion factor of 6.38 (AOAC, 2023). Nitrogen utilized from body reserves was calculated from changes in empty sow body weight and backfat from farrowing to weaning according to NRC (2012). Then, N excretion was determined by adding the N intake from feed and the N utilized from body reserves and then subtracting the N excreted in milk. This value was then divided by the N intake of the sow to determine the proportion of N excretion.

Statistical analysis

Performance data were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a randomized complete block design. Blocking structure accounted for parity and BW. Sow or litter served as the experimental unit. Treatment was included as a fixed effect and block within farrowing group was included as a random effect. Count data were analyzed using a Poisson distribution using a logit link function.

Proportion data, including litter born alive, stillborn, born mummified, and pre-weaning mortality were analyzed using a binomial distribution using a logit link function. Milk composition and pig AA concentrations were analyzed as a repeated measure using the lme4 function of R software with treatment, sampling time point, and their interaction included as fixed effects. For all data, preplanned linear and quadratic contrast statements were used to evaluate increasing SID His:Lys ratio. Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS

Sow BW at entry and weaning, as well as sow lactation BW loss did not differ based on lactation diet SID His:Lys ratio (Table 2). There were no linear or quadratic effects on sow backfat, caliper score, or loin depth upon entry into the farrowing house or at weaning, nor entry-to-wean change with increasing SID His:Lys ratio.

Increasing SID His:Lys did not affect sow average daily feed intake (ADFI) before farrowing and from farrowing until weaning. As expected, average daily SID His intake increased (linear, $P < 0.001$) with increasing SID His:Lys ratio. SID His intake per kg of litter gain increased (linear, $P < 0.001$) as SID His:Lys ratio increased. There were no differences in N intake or excretion with increasing SID His:Lys ratio. However, N secreted in milk increased then decreased (quadratic, $P = 0.032$) with increasing SID His:Lys ratio where sows fed the diet with a 50% SID His:Lys ratio had the numerically greatest amount of N secreted in milk. Nitrogen utilization from body reserves tended to decrease (linear, $P = 0.068$) as SID His:Lys ratio increased. Wean-to-estrus interval was unaffected by dietary treatment.

There were no differences observed in the number of total pigs born per litter (Table 3). Increasing SID His:Lys ratio tended to decrease (linear, $P = 0.085$) percentage born alive. This

decrease in percentage born alive was due to an increased percentage of stillbirths (linear, P = 0.049) observed with increasing SID His:Lys ratio. The percentage of mummified fetuses did not differ based on sow lactation diet.

Litter size and preweaning mortality were unaffected by increasing SID His:Lys ratio on d 0, 2, 7, or at weaning. There were no effects of increasing SID His:Lys ratio on d 2 litter weight. However, on d 7 and weaning, litter weight tended to increase then decrease (quadratic, $P \leq 0.062$) with increasing SID His:Lys ratio. Sows fed the diet with the 50% SID His:Lys ratio had numerically the greatest litter weight on d 7 and at weaning. There was no effect of increasing SID His:Lys ratio on mean pig BW on d 0, 2, 7, or at weaning. Pig ADG from d 7 to weaning tended to increase (linear, P = 0.10) with increasing SID His:Lys ratio. However, there were no effects of increased SID His:Lys ratio on litter ADG from d 2 to 7, d 7 to weaning, or from d 2 to weaning. Increasing SID His:Lys ratio increased then decreased (quadratic, P = 0.027) calculated total milk yield where sows fed 50% SID His:Lys ratio had numerically the greatest total calculated milk yield. When lactation length was accounted for, calculated average daily milk yield tended to increase then decrease (quadratic, P = 0.078), with sows fed the 50% SID His:Lys ratio diet having the greatest calculated average daily milk yield.

Milk composition was not affected by increasing SID His:Lys ratio, day of lactation, or their interaction (Table 4). When pig blood samples were analyzed for free AA concentrations, there were no significant interactions between increasing SID His:Lys ratio and day of lactation (Table 5). Plasma Trp tended to decrease (linear, P = 0.059) and Tyr decreased (linear, P = 0.031) as SID His:Lys ratio increased. Pig plasma Trp levels were greater (P = 0.014) at weaning than on d 7.

DISCUSSION

To our knowledge there is no existing data in lactating sows that has defined the effects of increasing SID His:Lys ratio nor any studies that have established the SID His requirement of lactating gilts and sows. The NRC (2012) SID His requirement estimates range from 16.9 to 19.5 g/d and 18.1 to 20.8 g/d for lactating gilts and sows, respectively. This requirement estimate is based on a set litter size of 11 and 11.5 pigs/litter for gilts and sows, respectively. Within this range of SID His intake, the requirement is determined based on a pig ADG of 190, 230, or 270 g/d. While the pig ADG of the current study most closely aligns with the 230 g/d category, the litter size in the current study (14.2 pigs weaned/litter) exceeded the set litter size used to model these requirement estimates. Thus, when requirements were determined based on litter ADG instead of pig ADG, the sows in the study herein most closely align with the upper range of the requirement estimates of 19.5 and 20.8 g/d for lactating gilts and sows, respectively. When this requirement estimate is calculated as a ratio of the SID Lys requirement estimate of lactating gilts and sows with the same production level (49.3 and 52.6 g/d Lys, respectively), it equates to an SID His:Lys ratio of 39.6% (NRC, 2012). This SID His requirement estimate is based on factorial estimates and empirical studies are not available to validate the estimate. Additionally, today's sow often exceeds the litter size for which these estimates were developed, similar to the sows included in this study. Lactation ADFI in this trial averaged 7.1 kg/d which led to an average SID Lys intake of 74.6 g/d. The SID His intake of the sows in this study was 144%, 189%, and 223% of NRC (2012) requirement estimates as SID His increased from 40 to 60% of Lys. A typical corn-soybean meal-based lactation diet with moderate feed-grade L-Lysine HCl levels will contain a SID His:Lys ratio of 40%. Therefore, this study was not intended to determine a SID His requirement of lactating sows, but rather to determine the effects of

increased His intake above that which would be observed with a typical corn-soybean meal-based diet.

Although no empirical studies have evaluated the effects of increased His intake in lactating sows, research has been conducted in other species, particularly dairy cattle. A meta-analysis conducted in dairy cattle included 22 studies in which His was supplemented by rumen-protected His or through infusion of His into the abomasum or jugular vein (Räisänen et al., 2023). The authors determined that total lactation milk yield and milk protein yield were increased in lactating dairy cows with increased His supplementation. Räisänen et al. (2023) concluded that His may be a limiting AA in the lactating cow diets. Indeed, His has been documented as the first limiting AA in cows when consuming grain and grass silage-based diets (Kim and Lee, 2021). However, His is the fifth limiting AA in corn-soybean meal-based lactating sow diets when tissue mobilization is 40% or less and falls out of the top 5 limiting AA when tissue mobilization during lactation is greater (Kim et al., 2001). Variation in the order of the limiting AA in lactating sow diets due to differences in tissue mobilization can be attributed to differences in the SID His:Lys ratio of mobilized body tissue (47%) compared to the SID His:Lys ratio in milk (43%) and mammary gland tissue (35%; NRC, 2012; Hurley, 2015). On average, the sows in the current study gained weight from the time of farrowing until weaning, suggesting that tissue mobilization was unlikely.

Similar to the data published in dairy cattle, calculated milk yield increased when the SID His:Lys ratio was increased to 50%, which corresponded with greater litter weight at weaning. However, this was not observed as His:Lys ratio increased to 60%, likely due to smaller litter size at weaning in addition to a slightly shorter lactation length. This is evidenced by the lack of differences in lactation pig ADG in sows fed the 50 and 60% SID His:Lys ratio diets.

In addition to increased calculated milk yield, milk protein yield was also increased in dairy cattle as His intake increased (Räisänen et al., 2023). This may be due to the up-regulation of the mTOR pathway in the mammary gland (Gao et al., 2015). The mTOR pathway controls milk protein production in the mammary gland by regulating translation and gene expression and increasing the production of important milk proteins including casein and whey (Li et al., 2025). The increase in milk protein yield of sows fed the 50% SID His:Lys ratio resulted from the increase in total milk production in the 50% SID His:Lys ratio sows as there were no differences in percentage milk protein content. In fact, no differences were evident in any of the milk composition measurements. Likewise, the meta-analysis by Räisänen et al. (2023) found no difference in lactose levels when dairy cattle were fed increased His. However, Räisänen et al. (2023) observed a decrease in percentage of milk fat in dairy cattle fed increased His which the authors speculated may also be due to the increase in milk yield.

Results in the current study showed an increase in the percentage of stillbirths and tendency for a decrease in the percentage of pigs born alive as SID His:Lys ratio increased. However, this was likely due in part to numeric differences in the total number of pigs born which increased by 0.8 and 0.6 pigs when SID His:Lys ratio increased to 50 and 60%, respectively. An increase in total born has been linked to increases in the percentage of stillbirths (van den Bosch et al., 2022). These numeric differences in total born were likely not caused by differences in SID His:Lys ratio as total born is established much earlier in the gestational period than when the treatments were first administered (d 110) in this trial.

A concern documented in other species with feeding diets containing excess His is a potential decrease in feed intake (Moro et al., 2020). This is because His can cross the blood-brain barrier, where excess His is then converted to histamine (Brosnan and Brosnan, 2020).

Neurons located throughout the paraventricular and ventromedial hypothalamus possess histamine receptors that regulate energy homeostasis and satiety signals which have a large impact on feed intake (Khouma et al., 2023). This decrease in feed intake due to increased histamine in the brain has been documented in both rats (Sheiner et al., 1985; Yoshimatsu et al., 2002) and humans (Henkin, 1977). In humans, increased His supplementation of 8 g/d was enough to induce anorexia (Henkin, 1977), while in rats an injection of 0.35 mmol/kg BW caused a significant decrease in feed intake (Yoshimatsu et al., 2002). Conversely, a meta-analysis in lactating dairy cows reported increased dry matter intake with higher dietary His, though it was unclear whether this was due to signaling or increased nutrient demand from higher milk yield (Räisänen et al., 2023). In the present study, ADFI numerically increased with SID His:Lys ratio, and prior work in weanling pigs has observed reduced intake only when His was deficient (Cemin et al., 2018). While elevated histamine levels have been reported in pigs fed high His diets, feed intake did not decline (Li et al., 2002), possibly due to species-specific regulation or adaptation to dietary protein level (Schirmer and Harper, 1970).

In conclusion, increasing SID His:Lys ratio increased litter weight on d 7 and at weaning where sows fed the diet with 50% SID His:Lys ratio had numerically the heaviest litters and greatest calculated milk yield. These results suggest that further research evaluating increasing SID His:Lys ratios above current NRC (2012) requirement estimates is warranted.

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Table 4.1 Diet composition (as-fed basis)

	Standardized ileal His:Lys ratio	
Item	40%	60%
Ingredient, %		
Corn	69.60	69.35
Soybean meal, 47.7% CP	24.40	24.45
Soybean oil	2.00	2.00
Calcium carbonate	1.13	1.13
Monocalcium P, 21.5% P	1.20	1.20
Sodium chloride	0.50	0.50
L-Lys-HCl	0.35	0.35
L-Met	0.08	0.08
L-Thr	0.18	0.18
L-Trp	0.03	0.03
L-Val	0.05	0.05
L-His	---	0.22
Trace mineral premix	0.25	0.25
Vitamin premix	0.25	0.25
Total	100.00	100.00
Calculated analysis		
SID AA, %[2]		
Lys	1.05	1.05
Arg:Lys	97	97
His:Lys	40	60
Ile:Lys	60	60
Leu:Lys	129	129
Met:Lys	31	31
Met and Cys:Lys	55	55
Phe and Tyr:Lys	117	117
Thr:Lys	65	65
Trp:Lys	20	20
Val:Lys	70	70
Total Lys, %	1.18	1.18
ME, kcal/kg	3,364	3,364
NE, kcal/kg	2,540	2,340
SID Lys:NE, g/Mcal	4.13	4.14
CP, %	17.9	17.9
Ca, %	0.80	0.80
P, %	0.61	0.61
STTD P, %[3]	0.50	0.50

[1]Diets were fed from approximately d 110 of gestation until weaning. The 40 and 60% His:Lys ratio diets were blended at a 50:50 ratio to form the intermediate treatment.

[2]SID, standardized ileal digestible.

[3]STTD, standardized total tract digestible.

Table 4.2 Effects of increasing standardized ileal digestible (SID) His:Lys ratio in lactation diets on sow lactation performance[1]

	SID His:Lys ratio, %[2]				*P*=	
	40	50	60	SEM	Linear	Quadratic
Count, n	30	30	28	---	---	---
Parity	2.2	2.2	2.2	0.32	0.989	0.896
Lactation length, d	17.8	18.1	17.7	0.22	0.973	0.136
Sow BW, kg						
Entry	244.5	244.4	245.2	5.73	0.725	0.794
Post-Farrow	231.6	227.7	228.0	5.71	0.215	0.381
Weaning	226.2	226.4	226.4	6.63	0.957	0.977
Lactation change (post-farrow to wean)	-4.3	-1.5	-1.8	2.04	0.252	0.426
Overall change (entry to wean)	-16.6	-18.5	-18.8	2.57	0.489	0.775
Sow caliper score						
Entry	16.4	16.0	15.9	0.30	0.243	0.553
Weaning	15.0	14.7	14.4	0.43	0.278	0.960
Lactation change (entry to wean)	-1.4	-1.3	-1.5	0.31	0.821	0.641
Sow backfat, mm						
Entry	13.5	13.3	13.5	0.39	0.956	0.604
Weaning	12.4	11.9	11.8	0.41	0.163	0.561
Lactation change (entry to wean)	-1.0	-1.4	-1.7	0.33	0.109	0.960
Sow loin depth, mm						
Entry	57.1	57.6	56.3	0.78	0.337	0.233
Weaning	54.4	54.9	54.2	0.72	0.813	0.504
Lactation change (entry to wean)	-2.6	-2.8	-2.1	0.80	0.551	0.580
Sow ADFI, kg						
Pre-farrow	2.6	2.5	2.5	0.03	0.139	0.786
Farrow to weaning	6.9	7.2	7.1	0.20	0.334	0.241
Average SID His Intake, g/d	29.0	38.0	44.9	1.07	< 0.001	0.295
SID His intake/kg litter gain	9.4	11.3	13.7	0.42	< 0.001	0.551
N balance, g/d						
Intake[3]	201.0	207.2	204.7	5.66	0.526	0.380
In milk[4]	85.0	89.9	86.9	1.54	0.387	0.032
From body reserves[5]	3.3	-2.0	-2.2	2.70	0.068	0.336
Excreted[6]	119.3	115.0	115.4	5.45	0.572	0.688
Excreted, %[7]	58.5	55.2	56.1	1.52	0.266	0.258
Wean-to-estrus interval, d	5.0	5.0	5.2	0.17	0.336	0.932

[1]A total of 88 mixed-parity sows (Line 241, DNA, Columbus NE) and litters were used from d 110 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size up to 48-h post farrowing.
[2]Sow treatment consisted of providing a control diet (SID His:Lys 40%), a high histidine diet, accomplished by adding synthetic histidine (SID His:Lys 60%), or a 50:50 blend of the two diets (SID His:Lys 50%) from d 110 of gestation until weaning.
[3]Calculated by N content in feed × feed intake.
[4]Calculated by dividing each sow's average protein milk yield (g/d) by the nitrogen in milk conversion factor of 6.38 (AOAC, 2023).
[5]Calculated from empty sow body weight and backfat according to equations in NRC (2012).

[6]Calculated from: N intake + N from body reserves – N in milk.
[7]Calculated from: (N intake + N from body reserves – N in milk) ÷ N intake.

Table 4.3 Effects of increasing standardized ileal digestible (SID) His:Lys ratio in lactation diets on litter performance[1]

	SID His:Lys ratio, %[2]				*P* =	
	40	50	60	SEM	Linear	Quadratic
Litter characteristics						
Total born, n	15.9	16.7	16.5	0.81	0.569	0.542
Born alive, %	92.9	90.7	89.7	1.58	0.085	0.629
Stillborn, %	3.9	6.3	6.8	1.29	0.049	0.344
Mummy, %	2.5	2.4	2.8	0.88	0.764	0.732
Litter size, n						
d 0	14.8	15.1	14.8	0.73	0.989	0.823
d 2	14.2	14.3	14.1	0.71	0.925	0.863
d 7	13.9	14.2	13.8	0.70	0.909	0.731
Weaning	13.9	14.0	13.6	0.70	0.768	0.767
Litter weight, kg						
d 2	23.5	24.2	24.2	0.69	0.449	0.640
d 7	39.7	41.9	39.5	1.08	0.926	0.062
Weaning	74.5	79.0	76.2	1.74	0.441	0.053
Mean piglet BW, kg						
d 0	1.57	1.59	1.57	0.054	0.921	0.794
d 2	1.66	1.69	1.71	0.047	0.432	0.882
d 7	2.86	2.95	2.86	0.071	0.995	0.250
Weaning	5.39	5.64	5.61	0.135	0.151	0.274
Litter ADG, kg/d						
d 2 to d 7	2.98	3.06	2.88	0.081	0.378	0.171
d 7 to weaning	3.36	3.58	3.53	0.109	0.204	0.211
d 2 to weaning	3.22	3.40	3.31	0.086	0.443	0.135
Piglet ADG, g/d						
d 2 to d 7	215	216	208	6.0	0.411	0.484
d 7 to weaning	244	256	260	8.6	0.100	0.676
d 2 to weaning	234	243	243	6.8	0.218	0.497
Milk yield[3]						
Total yield (d 2-weaning), kg	203.8	215.2	201.3	4.68	0.702	0.027
Average yield, kg/d	12.9	13.3	12.8	0.23	0.719	0.078
Total protein yield (d 2-weaning), kg	8.6	9.3	8.7	0.20	0.580	0.012
Average protein yield, kg/d	0.54	0.57	0.55	0.010	0.387	0.032
Preweaning mortality, %						
Birth to d 2	1.4	1.0	2.0	0.92	0.369	0.167
d 2 to weaning	2.6	2.0	3.0	0.96	0.767	0.341

[1]A total of 88 mixed-parity sows (Line 241, DNA, Columbus NE) and litters were used from d 110 of gestation until weaning. Litters were cross-fostered within treatment group to equalize litter size up to 48-h post farrowing.

[2]Sow treatment consisted of providing a control diet (40% SID His:Lys ratio), a high His diet, accomplished by adding feed-grade His (60% SID His:Lys ratio), or a 50:50 blend of the two diets (50% SID His:Lys ratio) from d 110 of gestation until weaning.

[3]Total milk yield was estimated utilizing the model developed by Hansen et al. (2012). Average milk yield = Total milk yield / (Wean age – 2).

Table 4.4 Effects of increasing standardized ileal digestible (SID) His:Lys ratio in lactation diets on sow milk composition[1]

	SID His:Lys ratio, %[2]				*P*=[3]		
	40	50	60	SEM	Linear	Quadratic	Day
Fat, %							
d 7	6.9	7.5	6.7	0.39	0.883	0.445	0.480
d 17	6.6	6.4	6.7				
Protein, %							
d 7	4.5	4.5	4.4	0.10	0.255	0.438	0.624
d 17	4.4	4.4	4.3				
Lactose, %							
d 7	5.5	5.4	5.6	0.08	0.812	0.258	0.180
d 17	5.6	5.6	5.5				
Total solids, %							
d 7	11.2	11.1	11.2	0.12	0.327	0.936	0.561
d 17	11.3	11.3	11.0				
Somatic cell count, cells/mL							
d 7	720	1104	493	267.1	0.974	0.148	0.916
d 17	754	997	996				

[1]Milk was collected from 10 sows per treatment on approximately d 7 after farrowing and the same sows the day before weaning (d 17). To obtain milk samples, 10 IU of oxytocin was administered via the ear vein of sows to induce milk letdown and milk was manually expressed. Milk (50 mL total) was collected from the third, fourth, and fifth teats on either side of the underline.

[2]Sow treatment consisted of providing a control diet (40% SID His:Lys ratio), a high-His diet, accomplished by adding feed-grade His (60% SID His:Lys ratio), or a 50:50 blend of the two diets (50% SID His:Lys ratio) from d 110 of gestation until weaning.

[3]Linear × day and Quadratic × day, $P > 0.10$ for all sow milk composition measurements.

Table 4.5 Effects of increasing standardized ileal digestible (SID) His:Lys ratio in lactation diets on pig plasma AA level (μmol/L)[1]

	d 7			d 17					
	SID His:Lys ratio, %[2]			SID His:Lys ratio, %[2]				*P*=[3]	
	40	50	60	40	50	60	SEM	Linear	Day
Ala	564	580	560	517	497	562	35.6	0.528	0.312
Arg	201	183	178	171	159	153	20.9	0.263	0.146
Asn	98	91	86	55	56	54	6.60	0.279	< 0.001
Glu	301	293	288	257	262	272	18.5	0.936	0.005
Gln	619	640	587	502	519	517	45.4	0.850	0.060
Gly	852	859	822	917	988	996	65.9	0.692	0.458
His	106	106	104	83	80	77	7.1	0.548	0.021
Ile	118	120	109	96	86	105	9.4	0.968	0.068
Leu	182	171	170	181	175	165	12.1	0.449	0.947
Lys	196	189	162	178	155	178	20.7	0.420	0.393
Met	94	87	83	61	60	59	6.0	0.235	< 0.001
Orn	121	120	114	107	92	105	9.9	0.703	0.212
Phe	82	81	78	87	94	84	6.8	0.549	0.465
Pro	662	720	665	517	522	534	45.0	0.808	0.006
Ser	279	284	253	213	220	227	22.4	0.725	0.004
Tau	179	161	162	139	127	132	12.7	0.274	0.001
Thr	314	293	273	213	244	205	30.0	0.367	0.002
Trp	51	45	44	63	62	56	3.9	0.059	0.014
Tyr	256	228	211	175	171	161	20.5	0.031	< 0.001
Val	254	273	261	236	253	236	18.6	0.820	0.375

[1]From each litter that milk was collected (n = 10/treatment), the medium-weight barrow was selected using the individual piglet weights collected on d 7 after farrowing, and blood was drawn to determine plasma free amino acid concentration. The same barrow bled on d 7 after farrowing was also bled on the day before weaning.

[2]Sow treatment consisted of providing a control diet (40% SID His:Lys ratio), a high His diet, accomplished by adding feed-grade His (60% SID His:Lys ratio), or a 50:50 blend of the two diets (50% SID His:Lys ratio) from d 110 of gestation until weaning.

[3]Linear SID His:Lys × day, Quadratic SID His:Lys × day, and Quadratic SID His:Lys, $P > 0.10$ for all piglet plasma AA.

Chapter 5 - The effect of litter size relative to functional teat count on lactating sow and litter performance

ABSTRACT

A total of 1,005 sows and their litters were used to evaluate the effect of initial litter size relative to functional teat count on sow and litter performance. Sows were blocked by parity (1, 2 to 4, or 5+) and functional teat count (≤ 13, 14 to 15, or ≥ 16) categories and allotted to one of four treatments with 251 or 252 replications. Treatments consisted of 1 less pig than functional teats (-1), same number of pigs as functional teats (0), 1 more pig than functional teats (+1), or 2 more pigs than functional teats (+2). Pigs were individually weighed after cross-fostering and before weaning. Pigs born < 0.9 kg were fostered onto sows not included in this study. Replacement pigs were not added to litters after a removal or mortality. Sow body weight (BW), caliper score, and backfat depth were collected at farrowing house entry and weaning (d 22). Parity category, treatment, and their interaction were fixed effects in the model along with teat category. As litter size relative to functional teat count increased, sows lost more BW and caliper units, but culling rate due to non-conception decreased (linear, $P \leq 0.038$). Litter size and weight increased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased at d 2 and weaning. Litter average daily gain (ADG) exhibited a quadratic relationship ($P = 0.045$) where -1 and +2 sows had numerically greater litter ADG compared to 0 and +1 sows. Mean pig weaning BW (linear, $P < 0.001$) and pig ADG decreased (quadratic, $P = 0.042$) and removals and mortality (d 2 to weaning) increased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased. Pigs weaned per sow per year (PSY) increased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased. Wean-to-estrus interval (WEI) exhibited a quadratic relationship ($P = 0.049$) where 0 sows had longer WEI compared to

+2 sows with -1 and +1 sows intermediate. Subsequent farrowing rate did not differ; however, subsequent liveborn increased (linear, $P = 0.017$) and total born tended to increase (linear, $P = 0.061$) as previous litter size relative to functional teat count increased. In conclusion, sows with 1 less pig than functional teats after cross-fostering had the lowest piglet mortality and sow BW loss and greatest piglet weaning BW. However, for the greatest number of pigs weaned per litter, litter WW, and PSY, 2 more pigs than functional teats after cross-fostering was ideal.

INTRODUCTION

The modern sow often gives birth to more pigs than the number of functional teats that she possesses. Recent benchmarking reports for United States swine production systems have reported that the top 10% of farms are achieving an average liveborn per litter of 15.7 pigs (PigChamp, 2024). However, recent research has shown that sows in some commercial swine herds have average functional teat counts of 13.9 (Obermier et al., 2023). This imbalance in liveborn pigs and functional teat count requires alternative rearing strategies such as artificial rearing, fostering extra pigs onto nurse sows, or allowing sows to nurse more pigs than they have functional teats. Artificial-rearing systems involve housing piglets in specialized enclosures that provide warmth, milk replacer, and solid feed, allowing them to be removed from the sow between 2 and 14 d of age (Baxter et al., 2013). While these systems can help manage surplus piglets, they have been associated with increased piglet tail lesion scores (Schmitt et al., 2019), impaired immune function (Han et al., 2024), and inconsistent effects on growth performance (Vergauwen et al., 2017). Alternatively, a nurse sow is a sow whose own litter has been weaned or removed and is then replaced with a new litter of pigs (Alexopoulos et al., 2018). This practice can reduce overall farm productivity due to lower milk yield (Kobek-Kjeldager et al., 2020), greater culling rates (Osotsi et al., 2024), and increased biosecurity risks by disrupting the all-

in/all-out system and facilitating disease spread (Garrido-Mantilla et al., 2020). In addition, nurse sow reduce the number of sows that can farrow because they require keeping empty farrowing stalls available for their transfer (Osotsi et al., 2024).

Given the limitations and challenges associated with artificial rearing or using nurse sows, some producers opt to allow sows to nurse more piglets than they have functional teats. However, existing literature on this practice is equivocal and thus, the optimal pig-to-functional-teat ratio to enhance litter performance and reduce pre-weaning mortality (PWM) and effects on subsequent reproduction remains unclear. Zanin et al. (2024) observed no differences in PWM when litter size relative to teat count was increased from the same number of pigs as functional teat count to 1 more pig than functional teat count. Whereas Vande Pol et al. (2021) observed that mortality increased from 7.7 to 17.9% when litter size was increased from 2 less pigs than functional teat count to 2 more pigs than functional teat count. To date, there are no studies using a large sample size that have examined a broad range of incremental increases in pig-to-functional-teat count ratios, while simultaneously assessing subsequent reproductive outcomes. Therefore, the objective of this study was to determine the effects of initial litter size relative to functional teat count on sow BW and body condition, litter performance, and subsequent reproductive performance under commercial conditions. We hypothesized that increasing initial litter size relative to functional teat count would increase litter size and weight at weaning but adversely affect sow body condition and subsequent reproduction.

MATERIALS AND METHODS

General

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4915.01). This study was conducted at

a commercial sow farm in northwest Texas. Sows were individually housed in an environmentally controlled and mechanically ventilated barn. Farrowing stalls measured 1.52 × 2.06 m. Each stall was equipped with a dry *ad-libitum* sow feed dispenser (SowMax, HogSlat, Newton Grove, NC) and an adjacent nipple waterer placed at sow shoulder height. An additional nipple waterer was located at the base of the stall, along with a heat lamp for piglets. Sows were provided *ad-libitum* access to a common sorghum-soybean meal-based lactation diet formulated to 1.05% standardized ileal digestible Lys throughout the duration of the study. Piglet needle teeth were not clipped during this study. Creep feed and supplemental milk were not offered to litters throughout this study.

Animals and treatment structure

A total of 1,005 sows (average parity 3.5, Line 1050, PIC, Hendersonville, TN) and their litters were used from June to August 2024. On approximately d 112 of gestation, sows were moved from the gestation facility into farrowing rooms which contained 148 farrowing stalls. Sows were blocked by parity category (1, 2 to 4, and 5+) and functional teat count category (≤ 13, 14 to 15, and ≥ 16 teats). Within block, sows were randomly assigned to treatment after farrowing was complete to equalize parity and functional teat count. Treatments were also balanced based on sow entry body weight (BW) and backfat depth. Treatments were based on initial litter size relative to functional teat count with treatment establishment (piglet movement) performed within 24 h of the completion of farrowing. Treatments consisted of: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2). The determination and counting of functional teats and cross-fostering were performed by the same individual for all litters. In the context of this study, a functional teat was one that produces

enough milk to sustain the life of a pig (Alexopoulos et al., 2018), is elongated and pointed with no visual defects, and can be suckled by the pig (Arend et al., 2023). Therefore, any blunt teats, blind teats, or teats connected to mammary glands with severe edema were considered non-functional. Additionally, functional teat count was determined after farrowing occurred which gives more certainty in the number of functional teats when compared to counting at the time of entry into the farrowing house (Alexopoulos et al., 2018). The average functional teat count in this study was 14.7. Pigs that were born less than 0.9 kg were not included in this study and were fostered onto sows where litters were provided specialized care per the standard operating procedure of this farm. To attain the correct number of pigs relative to functional teat count, average birthweight pigs were utilized for cross-fostering to maintain the normal bodyweight distribution within the litter.

Data collection

A walk-on platform scale was used to collect sow BW at entry into the farrowing house and at weaning. Body weight loss after farrowing was modeled utilizing equations developed by Estrada et al. (2024). First, pre-farrowing adjusted body weight was calculated to account for conceptus growth between the time of weighing and farrowing. This was done using the equation: pre-farrow adjusted weight (kg) = (total pigs born × 0.039 × days from weighing to farrowing) + pre-farrow weight (kg). Next, conceptus weight was estimated as: conceptus weight (kg) = 0.137 + (1.329 × total pigs born × average pig birth weight (kg)). Finally, sow body weight after farrowing was calculated by subtracting conceptus weight from the pre-farrow adjusted weight.

Sow backfat depth was measured at entry into the farrowing house and weaning at the last rib, 8 cm from the midline (Renco Lean Meter, S.E.C. Repro Inc., Golden Valley, MN). Sow

caliper score (Knauer and Baitinger, 2015) was measured at entry into the farrowing house and weaning at the last rib. Pigs were individually weighed after cross-fostering and the day before weaning. The average weaning age was 22 d. Beginning on d 3 of lactation, pigs that appeared gaunt, were not competitive at the udder, and were losing weight were deemed fall-behind pigs and removed from the litter. These pigs were only removed if two independent individuals of the research team agreed upon their removal. At the time of removal from the farrowing stall, all removals and mortalities were weighed and recorded. A replacement pig was not added to the litter in the incidence of a removal or mortality.

At weaning, pigs were classified into the following pre-determined BW categories: < 3.6 kg, 3.6 to 4.5 kg, 4.5 to 5.4 kg, 5.4 to 6.4 kg, 6.4 to 7.3 kg, and > 7.3 kg. The proportion of the litter that fell into each BW category was determined. In addition, the litter weight coefficient of variation (CV) was determined on d 2 and at weaning by dividing the standard deviation of pig weights within the litter by the average weight of pigs within the litter.

After weaning the insemination date was recorded using the CloudFarms System (BASF, Florham Park, NJ) and used to calculate wean-to-estrus interval (WEI) and percentage of sows bred by d 7 post-weaning. Sows culled for any reason or re-bred due to failure to conceive were not included in the subsequent farrowing data analysis. Subsequent farrowing data was obtained from the CloudFarms system. Estimated pigs weaned/sow/year (PSY) was calculated assuming a litters/sow/year of 2.43 which was the average of the farm at the time this study was conducted.

Statistical analysis

Data were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a generalized randomized complete block design. Blocking structure accounted for parity category and functional teat count category. Parity

categories were: first parity (n = 206), parity 2 to 4 (n = 487), and parity 5 and greater (n = 312). Functional teat count categories were: 13 or less (n = 163), 14 to 15 (n = 549), and 16 or more (n = 293). Sow (litter) served as the experimental unit. Treatment, parity category, and their interaction were included as fixed effects. Functional teat count category was also included in the model as a fixed effect. Preplanned linear and quadratic contrast statements were used to evaluate increasing initial litter size relative to functional teat count. Backfat depth at entry was included in the model as a covariate in the analysis of backfat depth at weaning and backfat change over lactation. The glm function was used to analyze count data using a negative binomial distribution with a log link function. The glm function (binomial distribution) using a logit link function was used to analyze proportion data, including pre-weaning mortality and removals. Percentage of sows bred by d 7 post weaning, percentage culled, and subsequent farrowing rate were analyzed using the glm function (binary distribution). Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$. All treatment × parity interactions were $P > 0.05$ unless otherwise indicated.

RESULTS

Interactive effects

Backfat depth at entry was the only treatment × parity interaction ($P = 0.022$) observed. Parity 1 sow backfat depth was numerically greater in +2 sows, whereas in parity 2 to 4 sows, backfat depth at entry was numerically greater in -1 sows, and numerically greater in +1 sows in parity 5+ sows (Table 1). There were no other interactions between initial litter size and sow parity category.

Effect of initial litter size relative to functional teat count

Sow BW did not differ based on initial litter size relative to functional teat count at entry or after-farrowing. However, there was a tendency (linear, $P = 0.080$) for decreased sow BW at weaning as initial litter size relative to functional teat count increased. Sow BW change (loss) during lactation increased (linear, $P \leq 0.006$) as initial litter size relative to functional teat count increased. Sow backfat depth at weaning decreased (linear, $P = 0.046$) as initial litter size relative to functional teat count increased. Sow caliper score was decreased at weaning (linear, $P = 0.055$) reflecting more caliper unit loss over lactation (linear, $P = 0.001$) as initial litter size relative to functional teat count increased, similar to the change in backfat depth.

Litter size on d 2 and weaning increased (linear, $P < 0.001$) as litter size relative to functional teat count increased (Table 2). The reduction of litter size due to removals and mortality based on day of lactation followed a consistent trend, regardless of the initial litter size relative to the number of functional teats (Figure 1). The number of pigs weaned per functional teat increased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased. Notably, 47% of the -1 sows weaned three or more pigs below their teat count (Figure 2). However, as initial litter size increased relative to teat count, the proportion of sows that weaned three or more pigs below teat count decreased, dropping to just 15% of +2 sows. In contrast, 48% of +2 sows weaned at or above their teat count. This percentage declined as initial litter size decreased, with no -1 sows that weaned at or above teat count because none in this group began with a litter size matching their teat count.

Litter weight on d 2 and at weaning increased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased. While there were no differences in total litter weight gain as initial litter size relative to functional teat count increased, there was a quadratic

relationship ($P = 0.045$) for litter average daily gain (ADG) where pigs from -1 and +2 sows had a numerically greater increase in litter ADG compared to 0 and +1 sows. There was no difference in mean pig BW on d 2; however, pig ADG and BW at weaning decreased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased.

Litter weight coefficient of variation did not differ on d 2 or at weaning as initial litter size increased relative to functional teat count. When the litter was divided into the proportion of pigs in the litter weaned in pre-determined BW categories, -1 sows, had a numerically lower percentage of small pigs (< 4.5 kg) at weaning and a greater proportion of pigs weaned > 7.3 kg compared to other treatments (Figure 3). However, between the other three treatments, the proportion of pigs in the litter weaned within each BW category was relatively similar. The percentage of pig removals increased (quadratic, $P < 0.001$) as initial litter size relative to functional teat count increased. The percentage of pig mortality and combined percentage of pig removals and mortalities increased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased. The percentage of litters with no pig removals or mortalities decreased (linear, $P < 0.001$) as initial litter size relative to functional teat count increased.

Initial litter size relative to functional teat count affected WEI (quadratic, $P = 0.049$) where +2 sows had a numerically shorter WEI compared to 0 females with -1 and +1 females intermediate (Table 3). However, the percentage of sows bred by d 7 post-weaning, the subsequent farrowing rate, and the percentage of sows culled for any reason did not differ based on previous treatment. The percentage of sows culled due to failure to conceive decreased (linear, $P = 0.038$) as initial litter size relative to functional teat count increased. Subsequent total born tended to increase (linear, $P = 0.061$) and subsequent liveborn pigs increased (linear, $P = 0.017$) as initial litter size relative to functional teat count increased. Subsequent stillbirths and

mummies did not differ based on previous treatment. Estimated PSY increased (linear, $P < 0.001$) as litter size relative to functional teat count increased.

Effect of parity category

Many of the parity category differences were typical of the differences observed among parity groups. Parity 5+ sows were heavier at entry into the farrowing house, after-farrowing, and at weaning and lost less BW during lactation and from entry to weaning ($P < 0.001$) compared to parity 1 and 2 to 4 sows (data not shown). Parity 2 to 4 sows were heavier at entry, after-farrowing, and at weaning ($P < 0.001$) compared to parity 1 sows. Parity 5+ sows had more backfat depth at entry ($P = 0.022$) compared to parity 1 sows with parity 2 to 4 sows intermediate. Parity 5+ sows had more backfat depth and a greater caliper score at weaning and lost less backfat depth and fewer caliper units ($P < 0.001$) compared to parity 1 and 2 to 4 sows. Parity 2 to 4 sows had more backfat depth and greater caliper score and lost fewer caliper units over lactation ($P < 0.001$) compared to parity 1 sows. Parity 5+ sows tended to be culled at greater rates compared to parity 1 and 2 to 4 sows. Estimated PSY was greater ($P < 0.001$) for parity 1 and 2 to 4 sows than parity 5+ sows.

Parity 1 sows had decreased litter weight on d 2, litter and pig ADG, and mean pig BW on d 2 and at weaning ($P < 0.001$) compared to parity 2 to 4 and parity 5+ sows. Litter weight at weaning was greatest in parity 2 to 4 sows ($P < 0.001$) followed by parity 5+ sows and then by parity 1 sows. Parity 2 to 4 sows had increased litter weight change from d 2 to weaning ($P < 0.001$) compared to parity 1 and parity 5+ sows. The percentage of pig mortality and percentage of combined pig removals and mortality was greater in parity 5+ sows ($P \leq 0.003$) compared to parity 1 and parity 2 to 4 sows.

DISCUSSION

The average number of liveborn pigs per litter in the United States has risen from 11.5 in 2010 to 14.3 in 2024, with the top 10% of farms achieving 15.7 liveborn pigs per litter (PigChamp, 2010; 2024). However, the average functional teat count is approximately 13.9 (Earnhardt-San et al., 2023; Obermier et al., 2023). The average functional teat count of sows utilized in this study averaged 14.7. Discrepancies in average functional teat count between studies may be driven by differences between genetic lines or definition of a functional teat as well as genetic progress over time. Recent estimates indicate that each additional functional teat increases litter size at weaning by an average of 0.3 pigs (Speckman et al., 2021). However, the benefit varies depending on the number of pigs born alive, increasing from 0.12 pigs weaned per additional teat in litters with 10 or fewer pigs to 0.38 pigs in litters with 15 or more pigs (Speckman et al., 2021).

For many years, the practice of matching the number of pigs to the number of functional teats during cross-fostering has been widely followed (Alexopoulos et al., 2018). This principle was based on studies demonstrating a high level of teat fidelity observed in nursing pigs. Teat fidelity refers to the instinct of pigs to consistently nurse from the same teat throughout lactation. De Passillé et al. (1988) observed that by d 3 of lactation, teat fidelity reached 86% and by d 10, increased to 95%. It was speculated that a high level of teat fidelity was beneficial to pigs as it reduced the number of teat disputes and thus, the chance of a pig missing nursing events (De Passillé et al., 1988). However, Clemons (2020) observed that pigs with intermediate nursing fidelity (51 to 80%) exhibited greater pre-weaning survival compared to their littermates with decreased or increased teat fidelity (≤ 50 or ≥81%). Clemmons (2020) speculated that pigs with intermediate nursing fidelity may be able to consume more milk by nursing multiple teats. The

long-standing practice of matching the number of pigs to functional teats underscores the importance historically placed on minimizing competition at the udder, although in modern production systems, litter sizes often exceed the number of functional teats, prompting alternative management strategies.

When litter size exceeds the number of functional teats, producers may either employ nurse sows or allow sows to raise surplus piglets. Nurse sows typically comprise 10 to 15% of the herd, but this can increase to 43% in some circumstances (Baxter et al., 2020). The use of nurse sows presents challenges, including reduced piglet growth due to lower milk yield, increased fighting, and delayed milk letdown of up to 8 h post-relocation (Kobek-Kjeldager et al., 2020). Nurse sows also have greater culling rates due to reproductive failure and reduce breeding efficiency by occupying non-productive stalls (Osotsi et al., 2024). Additionally, they disrupt all-in/all-out systems, increasing the risk of pathogen transmission, including PRRS and influenza A (Garrido-Mantilla et al., 2020).

Previous literature that investigated the effects of litter size relative to functional teat count on PWM has yielded variable results. In litters that were equalized based on piglet BW (light or heavy), Santos et al. (2025) observed no difference in PWM in +1 sows when compared to 0 sows but observed an increase in removals and combined removals and mortality in +1 sows when compared to 0 sows. This increase in removals and combined removals and mortality is similar to results in the current study. However, the study herein used a wider range of initial litter size relative to functional teats than Santos et al. (2025) and the largest increase in removals was between the -1 and 0 treatments with a plateau between +1 and +2 sows. Similar to results of the current study, Vande Pol et al. (2021) observed an increase in PWM in +2 sows when compared to -2 sows with 0 sows intermediate.

The increase in PWM with increasing initial litter size seen in both the current study and several previous studies is likely driven by reduced access to functional teats in the first 72 h of life when the majority of PWM occurs due to starvation and crushing (Edwards and Baxter, 2015; Muns et al., 2016). This was observed in the present study where the majority of removals and mortalities occurred during the first week after farrowing, after which litter size remained relatively stable throughout the remainder of lactation (Figure 1).

Regardless of whether PWM is affected, studies consistently show that litter size at weaning is increased when the number of initial pigs are increased relative to functional teat count (Vande Pol et al., 2021; Willard et al., 2022; 2023; Zanin et al., 2024). These findings align with the current study, which also showed a linear increase in litter size at weaning as the number of pigs relative to teat count increased. Consistent with the present study, Zanin et al. (2024) and Santos et al. (2025) observed an increase in the number of pigs weaned per functional teat in +1 sows when compared to 0 sows.

While total milk yield increases with increasing litter size, milk yield per pig declines, resulting in greater litter ADG but lower individual pig ADG (Auldist et al., 1998). The current study supports this trend, as litter ADG and weaning weight increased with more pigs relative to functional teat count, while individual pig ADG and weaning weight decreased. The reduction in piglet ADG is likely due to lower milk intake, both from decreased milk yield per pig and more missed nursing events in larger litters. Santos et al. (2025) reported that +1 litters had fewer piglets that never missed a nursing event and more that missed at least one nursing event by d 5 of lactation, compared to 0 litters. Contradictory to the results herein, Santos et al. (2025) reported no difference in pig ADG, pig weaning weight, and litter weaning weight when litter size increased from being at teat count to 1 more than teat count. Similarly, when a larger range

of litter size relative to functional teat count (-2, 0, or +2) was investigated, litter weaning weight and pig ADG were not different (Vande Pol et al., 2021). However, Vande Pol et al. (2021) observed a tendency for reduced pig weaning weight in +2 and 0 litters compared to -2 litters.

This study assessed the proportion of pigs weaned in pre-defined BW categories based on commercial relevance. We initially hypothesized that increasing litter size relative to functional teat count may increase total number of pigs weaned but shift the BW distribution toward lighter piglets. However, the relatively small differences observed in the current study in the proportion of the litter weaned across BW categories among 0, +1, and +2 sows suggests that increasing litter size relative to teat count does not significantly increase the proportion of lightweight pigs at weaning. This interpretation is supported by the lack of difference in litter CV at weaning observed in both the current study and by Santos et al. (2025) when litter size increased relative to functional teat count. In contrast, Zanin et al. (2024) observed greater litter CV on d 5 and at weaning in +1 litters compared to 0 litters. However, the difference narrowed from 6% on d 5 to 2% at weaning, and because initial CV was not reported, interpretation remains limited.

As milk production increases with litter size (Auldist et al., 1998), greater sow body condition loss is expected due to increased nutrient demands. This increase in body condition loss (BW, caliper, and backfat depth) as litter size relative to functional teat count increased was observed in the current study, though differences were modest. For example, on a percentage of BW basis, +2 sows lost only 1.4% more BW than -1 sows. Zanin et al. (2024) reported increased caliper loss but no change in backfat depth or body condition score in +1 sows compared to 0 sows. Santos et al. (2025) saw no differences in sow body condition score, caliper or backfat depth loss when +1 and 0 sows were compared, likely due to minimal differences in litter weight gain thus smaller nutrient demand differences.

Increased body condition loss during lactation can impair subsequent reproduction, particularly in parity 1 sows that are under high milk production pressure (Quesnel et al., 2007). To assess differences in response to treatment based on physiology and age, parity category was included in the statistical model as an interaction with litter size relative to functional teat count and no interactions were found for measurements of subsequent reproduction. It is unclear why in the current study, sows nursing litter matching teat count actually had the numerically longest WEI and +2 sows had the shortest. Arend et al. (2023) reported no differences in WEI, percentage of sows bred by d 7, or subsequent farrowing rate in parity 1 sows when litter size was increased from 12 to 15.5. While Arend et al. (2023) did not establish litter size relative to functional teat count, on average, sows nursing 12 pigs were 2 pigs below teat count and sows nursing 15.5 pigs were at teat count. Previous research suggests BW loss must exceed 5% in primiparous and 10% in multiparous sows to impact WEI (Thaker and Bilkei, 2005). In the present study, average losses across treatments were below these thresholds, likely explaining the lack of effect on subsequent reproduction measurements. While overall culling rates did not differ, fewer sows were culled due to failure to conceive in greater litter size treatments, counter to our original hypothesis that reproductive function might be affected by greater nutritional demand. Supporting this, Arend et al. (2023) found no impairment in fertility or hormonal function in primiparous sows nursing large litters, suggesting modern sows may be more resilient to lactational demands.

This study is the first to follow sows managed with varying litter sizes relative to functional teat count through a subsequent parity. Results showed a linear increase in subsequent liveborn and tendency for a linear increase in subsequent total born as initial litter size relative to teat count increased, though the underlying mechanisms remain unclear and warrant further

investigation. Additionally, when calculating PSY using the herd average of 2.43 litters/sow/year, PSY also increased linearly with litter size relative to teat count, consistent with the observed increase in litter size at weaning.

In conclusion, the optimal litter size relative to functional teat count depended on the response that was of greatest interest. Sows with 1 less pig than functional teats had the lowest pig mortality and sow BW loss and greatest pig weaning weight. However, sows with 2 more pigs than functional teats had the greatest number of pigs weaned per litter, litter WW, PSY, and liveborn in the subsequent litter.

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Table 5.1 Effect of initial litter size relative to functional teat count on sow lactation performance[1]

	Initial litter size +/- functional teat count[2]					*P* =		
						Treatment		
Item	-1	0	+1	+2	SEM	Linear	Quadratic	Parity
Sows, n	251	252	251	251	---	---	---	---
Parity	3.5	3.5	3.5	3.5	0.14	0.968	0.906	---
Sow BW, kg								
Entry	275.2	274.4	275.3	274.7	1.96	0.921	0.943	< 0.001
After-farrow[3]	245.0	244.9	245.5	245.2	1.94	0.868	0.966	< 0.001
Weaning	238.2	238.6	235.4	234.8	1.78	0.080	0.749	< 0.001
On-test change (after-farrow to weaning)	-6.8	-6.3	-10.0	-10.4	1.23	0.005	0.694	< 0.001
Lactation change (entry to weaning)	-36.5	-35.8	-39.8	-40.1	1.24	0.006	0.686	< 0.001
Sow backfat depth, mm								
Entry[4]	15.0	15.0	15.1	15.1	0.24	0.695	0.864	0.022
Weaning[5]	12.1	12.2	12.1	11.8	0.14	0.046	0.166	< 0.001
Lactation change (entry to weaning)[5]	-3.0	-2.9	-3.1	-3.4	0.14	0.046	0.166	< 0.001
Sow caliper score								
Entry	15.3	15.5	15.4	15.5	0.14	0.365	0.950	0.026
Weaning	13.1	13.2	12.9	12.7	0.16	0.055	0.254	< 0.001
Lactation change (entry to weaning)	-2.3	-2.3	-2.5	-2.8	0.14	0.001	0.197	< 0.001

[1]A total of 1,005 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of cross-fostering (approximately 24-h after farrowing) until weaning. Treatment and parity category and their interaction were used as fixed effects in the statistical model. Teat count category was included as a fixed effect in the model.

[2]Sows were allotted to treatment at approximately 24-h after farrowing. Sows were blocked by functional teat count and parity categories and assigned to 1 of 4 treatments: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2).

[3]Body weight loss from entry to after farrow was modeled utilizing the equations developed by Estrada et al. (2024):
Sow body weight after farrowing (kg) = pre-farrow adjusted weight (kg) - conceptus weight (kg).
Pre-farrow adjusted weight (kg) = total pigs born × 0.039 × days until farrowing from time of weighing + pre-farrow weight (kg).
Conceptus weight (kg) = 0.137 + 1.329 × total pigs born × average pig birth weight (kg).

[4]Treatment × parity, $P = 0.022$

[5]Entry backfat depth included in statistical model as a covariate.

Table 5.2 Effect of initial litter size relative to functional teat count on litter performance during lactation[1]

	Initial litter size +/- functional teat count[2]					*P* = Treatment		
Item:	-1	0	+1	+2	SEM	Linear	Quadratic	Parity
Wean age, d	21.7	22.0	22.0	21.9	0.17	0.302	0.197	0.033
Litter size, n								
d 2	13.5	14.4	15.3	16.3	0.28	< 0.001	0.914	0.900
Weaning	12.0	12.3	12.9	13.5	0.25	< 0.001	0.685	0.197
Pigs weaned per functional teat, n	0.831	0.858	0.901	0.945	0.0075	< 0.001	0.227	< 0.001
Litter weight, kg								
d 2	20.8	22.1	23.5	24.9	0.25	< 0.001	0.776	< 0.001
Weaning	78.0	78.1	80.8	83.3	1.06	< 0.001	0.227	< 0.001
Change (d 2-weaning)	57.2	56.1	57.3	58.4	1.00	0.257	0.224	< 0.001
Litter ADG, kg	2.77	2.67	2.73	2.79	0.042	0.583	0.045	< 0.001
Mean pig BW, kg								
d 2	1.54	1.53	1.53	1.53	0.016	0.417	0.850	< 0.001
Weaning	6.51	6.35	6.27	6.17	0.063	< 0.001	0.582	< 0.001
Piglet ADG, g	230	215	211	205	2.4	< 0.001	0.042	< 0.001
Litter coefficient of variation, %								
d 2	14.5	14.9	15.0	14.7	1.37	0.903	0.775	0.985
Weaning	15.5	16.1	15.5	15.3	0.69	0.654	0.550	0.866
On-test pig removals and mortalities, %								
Removals	7.6	9.9	10.4	10.6	0.55	< 0.001	0.025	0.105
Mortality	3.4	4.3	5.1	6.5	0.43	< 0.001	0.903	0.003
Removals and mortality	11.1	14.2	15.7	17.2	0.65	< 0.001	0.086	< 0.001
Litters with no removals or mortality	21.8	13.0	5.6	5.2	3.17	< 0.001	0.316	0.031

[1]A total of 1,005 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of cross-fostering (approximately 24-h after farrowing) until weaning. Treatment and parity category and their interaction were used as fixed effects in the statistical model. Teat count category was included as a fixed effect in the model.

[2]Sows were allotted to treatment at approximately 24-h after farrowing. Sows were blocked by functional teat count and parity categories and assigned to 1 of 4 treatments: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2).

Table 5.3 Effect of initial litter size relative to functional teat count on subsequent sow performance[1]

	Initial litter size +/- functional teat count[2]					*P* = Treatment		
Item:	-1	0	+1	+2	SEM	Linear	Quadratic	Parity
Culled, %	22.1	22.3	21.8	16.4	3.20	0.203	0.334	< 0.001
Culled due to non-conception, %	7.7	7.9	4.1	3.3	2.17	0.038	0.728	0.047
Wean to estrus interval, d	5.1	5.6	5.1	5.0	0.17	0.211	0.049	0.105
Bred by d 7 post wean, %	86.9	83.9	89.1	90.3	2.79	0.191	0.430	0.154
Pigs weaned/sow/year, n[3]	29.3	30.1	31.5	32.9	0.27	< 0.001	0.320	< 0.001
Farrowing rate, %	77.7	76.1	84.7	79.1	3.38	0.319	0.431	0.179
Total born, n	15.9	16.5	16.5	16.8	0.37	0.061	0.584	0.002
Liveborn, n	14.2	15.1	15.0	15.4	0.35	0.017	0.438	0.001
Stillborn, n	1.1	0.9	1.0	0.9	0.16	0.372	0.626	0.592
Mummies, n	0.5	0.5	0.5	0.5	0.09	0.513	0.653	0.972

[1]A total of 1,005 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of cross-fostering (approximately 24-h after farrowing) until weaning. Treatment and parity category and their interaction were used as fixed effects in the statistical model. Teat count category was included as a fixed effect in the model.

[2]Sows were allotted to treatment at approximately 24-h after farrowing. Sows were blocked by functional teat count and parity categories and assigned to 1 of 4 treatments: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2).

[3]An assumed litters/sow/year of 2.43 was utilized.

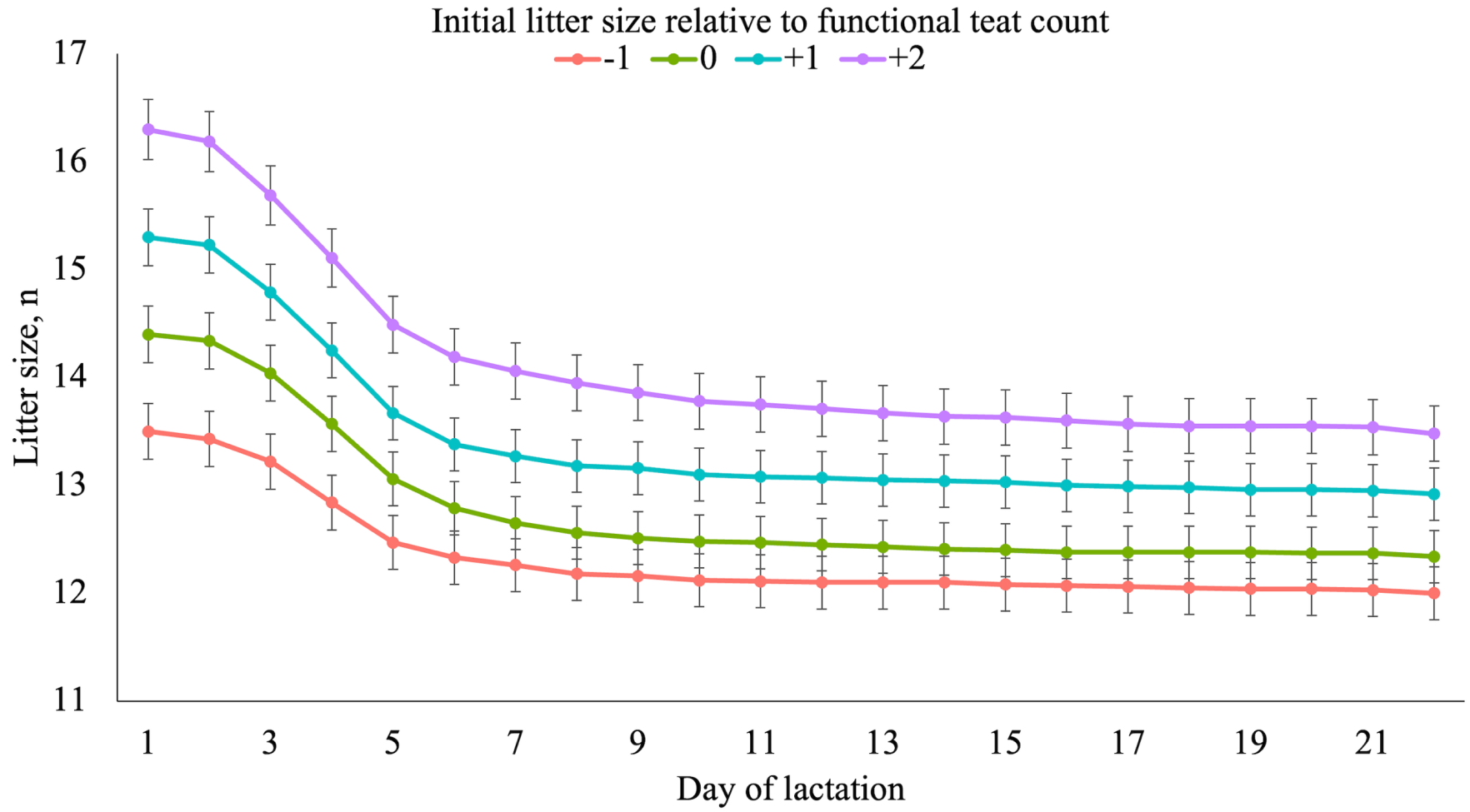

Figure 5.1 Litter size by day within treatment.
Within each initial litter size relative to functional teat count treatment, the average litter size on each day of lactation is shown. A total of 1,005 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of cross-fostering (approximately 24-h after farrowing) until weaning. Sows were blocked by functional teat count and parity categories and assigned to 1 of 4 treatments: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2). Data are expressed as means ± SE.

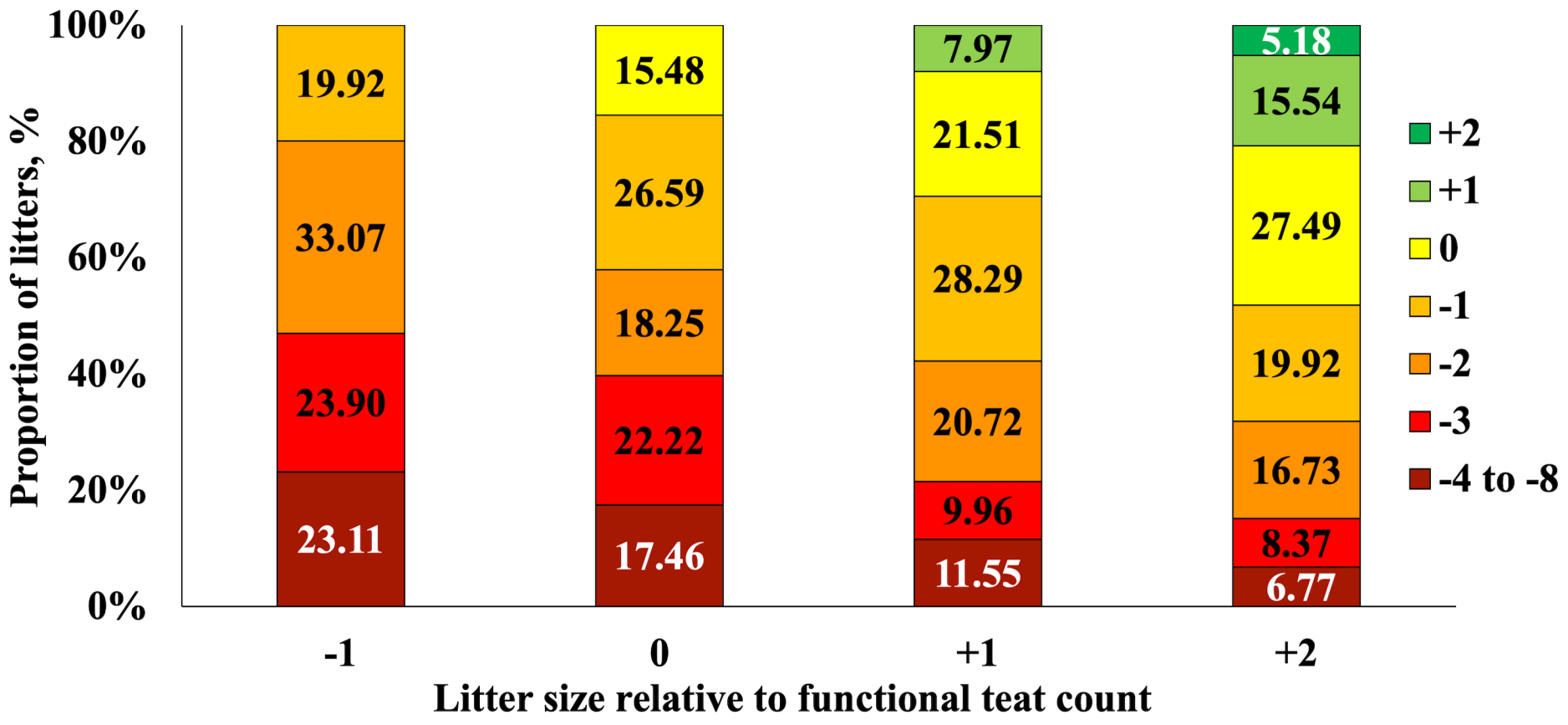

Figure 5.2 Proportion of litters weaned relative to teat count.
Within each initial litter size relative to functional teat count treatment, the proportion of litters that were weaned at different pig counts relative to functional teat counts is shown. A total of 1,005 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of cross-fostering (approximately 24-h after farrowing) until weaning. Sows were blocked by functional teat count and parity categories and assigned to 1 of 4 treatments: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2).

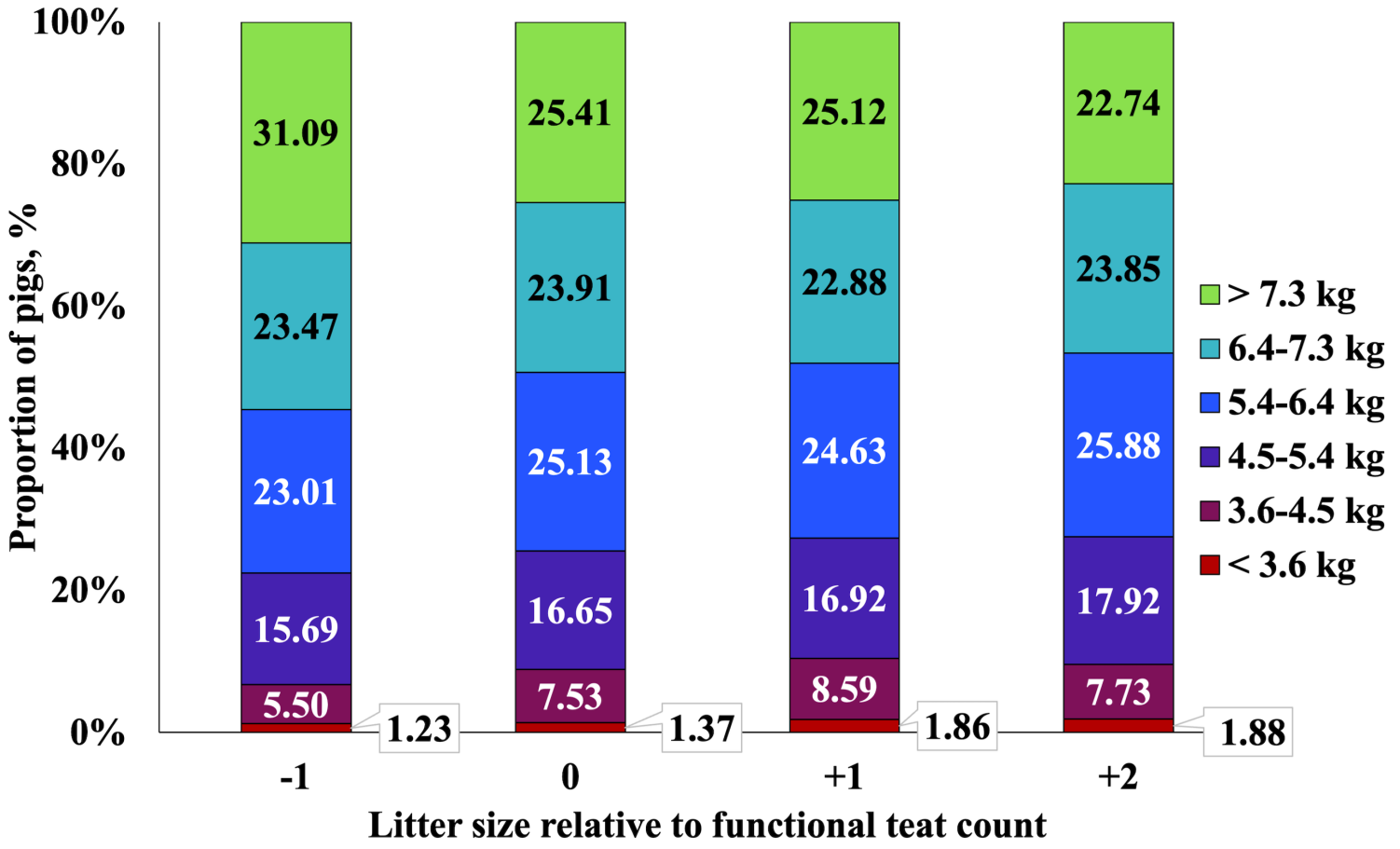

Figure 5.3 Proportion of pigs weaned in each BW category.
Within each initial litter size relative to functional teat count treatment, the average proportion of the litter that was weaned in each BW category is shown. A total of 1,005 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of cross-fostering (approximately 24-h after farrowing) until weaning. Sows were blocked by functional teat count and parity categories and assigned to 1 of 4 treatments: one less pig than functional teat count (-1), the same number of pigs as functional teat count (0), one more pig than functional teat count (+1), or two more pigs than functional teat count (+2).

Chapter 6 - Evaluating peripartum calcium administration strategies to improve sow farrowing performance and piglet livability

ABSTRACT

A total of 933 sows were used to evaluate peripartum calcium administration protocols on piglet livability and sow farrowing performance. Sows were blocked by parity and average stillbirths in previous parities then allotted to one of three treatments with 310 to 312 replications. Treatments included: 1) Control with sows receiving no intervention; 2) 25 g of a calcium chloride-based product ($CaCl_2$; TRIAD, Alltech, Inc., Nicholasville, KY) top-dressed daily each morning from approximately d 112 of gestation until farrowing; or 3) calcium gluconate injection (CaG; VetOne; Boise, ID) administered to primiparous and multiparous sows (15 or 20 mL injection, respectively), if a sow was classified as "at-risk" defined by the sow having more than 16 pigs, longer than 1 h since the birth of the last pig, 2 or more stillbirths, or farrowing duration exceeding 4 h. On a subset of sows farrowing duration, sow blood metabolites, sow urine pH, and pig immunocrit were analyzed. Parity category (P1, P2 to P4, or P5+), treatment, and their interaction were fixed effects while average past stillbirth category (< 0.5, ≥ 0.5 and ≤ 1, or > 1 stillborn/litter) was a random effect. There were no differences in total born, percentage born alive, or percentage stillborn between treatments; however, when at-risk sows were compared, administration of CaG decreased stillbirths and increased percentage of pigs born alive ($P \leq 0.006$). There was an interaction between Ca protocol and parity category for birth to cross-foster mortality ($P = 0.035$) where mortality was lowest in P1 Control sows ($P < 0.05$) compared to all other treatment × parity category combinations except for P1 CaG sows

which were intermediate. When considering all sows, sows fed $CaCl_2$ had increased blood Cl and ionized Ca ($P < 0.001$) compared to Control or CaG sows. Sows provided CaG had increased blood glucose levels ($P = 0.026$) compared to Control sows with $CaCl_2$ sows intermediate. Sows given $CaCl_2$ or CaG had decreased urine pH ($P = 0.001$) compared to Control sows. Pig immunocrit ratios tended ($P = 0.068$) to differ due to Ca protocol, with CaG offspring having a numerical increase compared to other treatments. In conclusion, in the overall population, top-dressing $CaCl_2$ before farrowing or injecting CaG peripartum altered sow metabolites but did not influence farrowing performance. However, when comparing at-risk sows, administration of CaG decreased stillbirths and increased percentage of pigs born alive.

INTRODUCTION

Among U.S. swine farms with the lowest pigs weaned per mated female per year, stillbirths account for 8.8% of total pigs born (MetaFarms, 2024). This high rate of stillbirths represents a significant economic loss for producers by reducing the number of marketable pigs and overall system productivity. Known risk factors associated with increased stillbirth incidence include older parity, prolonged farrowing duration, large litter sizes, late birth order, and lack of farrowing supervision (Le Cozler et al., 2002; Adi et al., 2024). A broad range of factors, such as nutritional management, parturition induction protocols, farrowing supervision practices, pharmacological and manual interventions, environmental conditions, and stockmanship, have been examined for their influence on the incidence of stillborn pigs (Vanderhaeghe et al., 2013). Recent findings by Craig et al. (2024) highlight a physiological contributor, showing increased stillbirth rates in sows with serum Ca level below a threshold of 2.5 mmol/L before farrowing. Calcium plays a critical role in smooth muscle contraction, including uterine contraction (Uehata

et al., 1997), which may help explain the increased incidence of dystocia and stillbirths in sows with low serum Ca levels.

Calcium chloride ($CaCl_2$) has been supplemented to sows in the feed during the transition period from entry into the farrowing house until parturition for its potential to reduce stillbirth incidence. In addition to supplying Ca, $CaCl_2$ serves to lower the dietary cation-anion difference (DCAD), which enhances Ca absorption from the small intestine and mobilization from bone in dairy cattle (Abu Damir et al., 1994). While there is less known about Ca homeostasis in sows around parturition, DeRouchey et al. (2003) observed increased ionized Ca when DCAD was reduced. Previous studies on $CaCl_2$ supplementation in the feed have reported variable effects on stillbirth rates (González-Sánchez et al., 2023; Craig et al., 2024; Ruampatana et al., 2024). Another Ca supplement used in U.S. swine production systems to support farrowing performance is injectable Ca gluconate (CaG). This compound is commonly administered in dairy cattle to treat hypocalcemia which is a condition triggered by the sudden increase in Ca demand before parturition due to the rapid increase in milk production (Oetzel, 2022). Although no controlled research trials have evaluated the use of CaG injections during farrowing in sows, case studies suggest it may alleviate dystocia symptoms (Durrell, 1942; Chutia et al., 2018; Reshma et al., 2020). Therefore, the objective of this trial was to evaluate two peripartum Ca administration protocols on piglet livability, sow performance before cross-fostering, and sow and piglet blood parameters. Our hypothesis was that increasing the amount of Ca available during parturition would improve uterine contractions, as evidenced by reduced stillbirths, shortened farrowing durations, and reduced early pig mortality.

MATERIALS AND METHODS

General

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #4915.02). This study was conducted on a commercial sow farm in northwest Texas. Sows were individually housed in an environmentally controlled and mechanically ventilated barn. Farrowing stalls were equipped with a dry *ad libitum* sow feed dispenser (SowMax, HogSlat, Newton Grove, NC) with a dry feeder and a nipple waterer placed at sow shoulder height. A nipple waterer at the base of the farrowing stall and a heat lamp was included in every farrowing stall for pig comfort.

Animals and treatment structure

A total of 933 sows (average parity 3.3, Line 1050, PIC, Hendersonville, TN) and their litters were used from May to August 2024. On approximately d 112 of gestation, sows were weighed and moved into the farrowing house from the gestation facility. Sows were blocked by parity and past average stillbirths and assigned to one of three Ca administration protocols. Parity categories were: first parity (n = 194), parity 2 to 4 (n = 489), and parities 5 and greater (n = 250). Average past stillbirth categories of multiparous sows (n = 739) were: < 0.5 (n = 326), 0.5 ≥ x ≤ 1.0 (n = 278), and > 1.0 (n = 135). Within block, sows were randomly assigned to treatment to equalize parity and average past stillbirths. Treatments included: 1) sows which sows received no intervention (Control); 2) 25 g of a calcium chloride-based product ($CaCl_2$; TRIAD, Alltech, Inc., Nicholasville, KY) top-dressed daily at the morning feeding from the time of entry (approximately d 112 of gestation) until the sow farrowed; or 3) calcium gluconate injection protocol (CaG; VetOne; Boise, ID) where primiparous and multiparous sows received a 15 or 20 mL, respectively, injection intramuscularly in the neck if a sow was termed “at-risk”.

At-risk criteria included sows with more than 16 pigs, it had been longer than 1 h since the birth of the last pig, 2 or more stillbirths in the litter, or a total farrowing duration that exceeded 4 h. At-risk sows received two injections, one on each side of the neck, using a 16-gauge × 3.8-cm needle. If more than 1 h passed after CaG administration without expulsion of a pig or placenta, a second dose was administered. In total, eight CaG sows received two doses. Sows received the $CaCl_2$ top-dress which is a proprietary blend of $CaCl_2$, *Yucca schidigera* extract, and flavors encapsulated in a lipid matrix for an average of 4.8 ± 0.14 d before farrowing. All sows were fed approximately 1.8 kg/d (0.9 kg each morning and afternoon) from d 112 of gestation until farrowing of a common sorghum-soybean meal-based lactation diet formulated to 1.05% standardized ileal digestible Lys and contained 0.88% Ca and 0.48% P. All sows were hand-fed before farrowing and had *ad libitum* access to a common lactation diet after farrowing.

Farrowing was monitored from 6:00 until 15:00 daily. All sows, regardless of farrowing timing, were included in the analysis of this study. For each sow, litter characteristics (liveborn, stillborn, and born mummified) were recorded on a sow card by a member of the farm farrowing team during each walk through the farrowing house (approximately every 15 min). If it had been longer than 30 min since the birth of the last pig or the sow appeared to be in distress, farrowing assistance (sleeving) occurred and if a pig was present in the birth canal, it was manually removed. Any instances of farrowing assistance were recorded on the sow card, regardless of whether a pig was removed or not. Oxytocin (20 IU/mL; VetOne; Boise, ID) was used sparingly in this trial and only after agreed upon by two independent parties of the research team. A total of 8 sows received oxytocin during this trial, with 4 sows from the CaG treatment and 4 sows from the $CaCl_2$ treatment. Sows were only induced to farrow if gestation length reached 118 d. A total of 28 sows were induced using prostaglandin (Lutalyse, Zoetis Inc., Kalamazoo, MI), with

13 sows from the control treatment, 8 sows from the $CaCl_2$ treatment, and 7 sows from the CaG treatment.

Any pig mortalities that occurred before cross-fostering (at approximately 24 h of age) were recorded. The cause of mortality was also recorded. Insemination date after weaning was recorded (CloudFarms system, BASF, Florham Park, NJ) and used to calculate the wean-to-estrus interval (WEI) for each sow that remained in the herd after weaning.

Blood and urine analyses

On a subset of sows (n = 74/treatment), more extensive data collection was performed, including sow blood metabolites, urine pH, and piglet immunocrit ratios. Farrowing duration was recorded for these same sows, as well as for additional sows that became available. Specifically, farrowing duration was collected on 79 control sows, 84 $CaCl_2$ sows, and 85 CaG sows. The additional sows were included when they farrowed during working hours and research personnel were present to collect the data. The beginning of farrowing was denoted as the birth of the first pig and the end of farrowing was retrospectively denoted as the birth of the last pig. The first five pigs born were marked (All-Weather Paint Stik, LA-CO Industries, Inc., Elk Grove Village, IL) for split-suckling purposes. Split suckling occurred if there were more than 14 liveborn pigs in a litter. The first five pigs born were isolated underneath the heat lamp for no longer than 90 min and then they were reintroduced to the litter. Within 4 h of the end of farrowing, blood (1 mL) was collected from a prominent auricular vein of the sow using a 21-gauge × 1.9-cm needle. Before collection, the site was cleaned with 75% alcohol. The samples were kept in a 1 mL vacutainer tube (MiniCollect Lithium Heparin, Greiner Bio-One, Kremsmünster, Austria) for a maximum of 10 min before analysis (iSTAT CHEM8+ cartridge, Zoetis, Parsippany, NJ). Sow blood metabolites including ionized Ca, glucose, blood urea nitrogen, Na, K, Cl, total carbon

dioxide, anion gap, creatinine, hematocrit, and hemoglobin were determined. Within 6 h of the end of farrowing, sow urine was collected via the method described by Nickel et al. (2017). Urine was extracted into 50 mL specimen cups and immediately analyzed for pH using a portable pH meter (Fisherbrand accumet AP115 Portable pH Meter, Fisher Scientific, Waltham, MA). At approximately 24 h after the beginning of farrowing, blood was taken for determination of immunocrit ratio from one of the first five pigs born, visually selecting the pig closest to the average birth weight of the litter. Immunocrit ratio was determined according to Vallet et al., (2013) and was utilized as an estimation of colostrum consumption. Within the CaG treatment, only sows that met the criteria for the injection were collected for sow blood metabolite, sow urine pH, and pig immunocrit analysis.

Statistical analysis

Data were analyzed using the lme4 package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a generalized randomized complete block design. Blocking structure accounted for parity and average past stillbirth record. Sow served as the experimental unit. Treatment, parity category, and their interaction were included as fixed effects. Past stillbirth category was included in the model as a random effect. Count data were analyzed using a negative binomial distribution using a log link function. Proportion data, including birth to cross-foster mortality and litter characteristics, were analyzed using a binomial distribution using a logit link function. The percentage of sows that were sleeved was analyzed using a binary distribution. Differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$. All treatment × parity interactions were $P > 0.05$ unless otherwise indicated.

Sows were also categorized into 2 subsets: at-risk sows (n = 411) or other sows (n = 522) across the three treatments for additional statistical analysis. At-risk sows were those that met the criteria to receive Ca gluconate injection (sows with > 16 pigs, > 1 h since the last birth, ≥ 2 stillbirths, or farrowing lasting > 4 h) and farrowed during hours when personnel were present (between the hours of 6:00 until 15:00). Because sows were assigned to treatment before farrowing, there were also sows assigned to the CaG treatment that were not later classified as at-risk sows and, thus, did not receive an injection of CaG. Other sows included those that farrowed during working hours (6:00 until 15:00) but did not meet the criteria for a CaG injection, as well as those that farrowed overnight when farrowing intervention was unavailable. The same statistical analyses were performed with the same statistical models, but within risk group for the 3 treatments.

RESULTS

Overall population

There was a treatment × parity interaction ($P = 0.019$) for the proportion of mummies in the litter. Within parity 1 sows there was a numeric increase in mummies in $CaCl_2$ sows compared to the other two treatments, whereas in parity 5+ sows there was a numeric increase in mummies in CaG sows compared to the other two Ca administration protocols. There was a treatment × parity interaction ($P = 0.035$) in birth to cross-foster mortality where parity 1 control sows had the lowest birth to cross-foster mortality compared to all other parity by treatment categories ($P < 0.05$) except for parity 1 CaG sows which were intermediate.

In the overall population, there was no difference in total born, the proportion of the litter born alive, or the proportion of stillbirths in the litter due to Ca administration protocol (Table 1). There were no differences in the percentage of sows sleeved, the number of times that sows

needed to be sleeved, or farrowing duration due to Ca administration protocol. Birth to cross-foster mortality was increased ($P < 0.001$) in $CaCl_2$ sows compared to control sows with CaG sows intermediate. Calcium administration protocol did not affect WEI.

Anion gap, blood urea nitrogen, creatinine, K, and Na in blood did not differ based on Ca administration protocol (Table 2). Chloride was greater ($P < 0.001$) in the blood from $CaCl_2$ sows compared to control and CaG sows. Blood glucose concentrations were greater ($P = 0.026$) in CaG sows compared to control and $CaCl_2$ sows. There was a tendency ($P \leq 0.067$) for a difference in hematocrit and hemoglobin concentrations based on Ca administration protocol where $CaCl_2$ sows had a numeric increase in both criteria, followed by CaG sows, and then by control sows. Circulating ionized calcium (iCa) concentrations were increased ($P < 0.001$) in $CaCl_2$ sows compared to both CaG and control sows. Total carbon dioxide (tCO_2) in blood was decreased ($P < 0.001$) in $CaCl_2$ sows compared to control sows with CaG sows intermediate. Urine pH was decreased ($P = 0.001$) in CaG and $CaCl_2$ treatment sows compared to control sows. There was a tendency for a difference ($P = 0.068$) in pig immunocrit ratio where pigs from CaG sows had a numeric increase in immunocrit ratio compared to pigs from control sows.

At-risk sows

In at-risk sows, percentage of stillbirths decreased ($P = 0.004$) in CaG sows compared to control and $CaCl_2$ sows (Table 3). This led to an increase ($P = 0.006$) in percentage born alive in CaG sows compared to control and $CaCl_2$ sows. The percentage of sows sleeved, the number of times sows were sleeved, and WEI interval did not differ based on Ca administration protocol. Birth to cross-foster mortality decreased ($P = 0.006$) in CaG sows compared to $CaCl_2$ sows with control sows intermediate. Blood Cl increased ($P = 0.039$) in $CaCl_2$ sows compared to control and CaG sows. Blood glucose increased ($P = 0.005$) in CaG sows compared to control and $CaCl_2$

sows. Circulating iCa concentration increased ($P = 0.004$) and total CO_2 in the blood decreased ($P = 0.011$) in $CaCl_2$ sows compared to control sows with CaG sows intermediate.

Other sows

Litter characteristics, including total born, percentage born alive, percentage stillborn, and percentage of mummies did not differ based on Ca administration protocol in all other sows. Calcium administration protocol also did not affect percentage of sows sleeved, the number of times sows were sleeved, farrowing duration, or WEI interval in these sows. Birth to cross-foster mortality was increased ($P = 0.003$) in CaG and $CaCl_2$ sows compared to control sows. Blood Cl, iCa, and tCO_2 were greater ($P \leq 0.044$) in $CaCl_2$ sows compared to control sows (Table 4). Blood Na tended to be greater ($P = 0.054$) in $CaCl_2$ sows compared to control sows.

Parity effects

As expected, parity 5+ sows were heavier ($P < 0.001$) at entry in the farrowing house compared to parity 2 to 4 sows; similarly, parity 2 to 4 sows were heavier than parity 1 sows at entry into the farrowing house (data not shown). These differences in entry body weight (BW) due to parity category were also found when sows were divided into at-risk and other sow categories ($P < 0.001$). Parity 5+ sows had decreased ($P < 0.001$) percentage of pigs born alive and greater ($P < 0.001$) percentage of stillbirths compared to parity 2 to 4 sows and parity 1 sows. Similarly, in both at-risk and other sows, stillbirths were greatest ($P \leq 0.012$) in parity 5+ sows and, in sows classified as other, parity 5+ sows had a lower percentage of pigs born alive ($P = 0.008$) compared to parity 2 to 4 and parity 1 sows. Parity 5+ sows were sleeved at the greatest frequency ($P < 0.001$), followed by parity 2 to 4 sows, and then by parity 1 sows. Birth to cross-foster mortality was lower ($P < 0.001$) in parity 1 sows compared to parity 2 to 4 sows and parity 5+ sows. A similar relationship between parity and mortality was seen in birth to cross-foster

mortality in sows classified as other ($P = 0.007$). There was a tendency for a difference ($P = 0.053$) in WEI based on parity category, where parity 5+ sows had a numeric decrease in WEI compared to parity 2 to 4 sows and parity 1 sows. Blood creatinine was greater ($P = 0.001$) in parity 5+ and parity 2 to 4 sows compared to parity 1 sows. A similar relationship between parity and blood creatinine ($P < 0.001$) was found in sows classified as other; however, in at-risk sows, blood creatinine was greater ($P = 0.001$) only in parity 5+ sows compared to parity 2 to 4 and parity 1 sows. There was a tendency for an effect ($P = 0.059$) of parity category on blood glucose level where glucose was numerically greatest in parity 1 sows and decreased as parity increased. Circulating iCa was decreased ($P = 0.001$) in parity 5+ sows compared to parity 1 and parity 2 to 4 sows. A similar relationship between parity and circulating iCa ($P \leq 0.028$) was seen in both at-risk and other sows. Blood K tended to be different ($P = 0.058$) based on parity where parity 2 to 4 sows had a numeric decrease in K compared to parity 1 and parity 5+ sows. A similar trend ($P = 0.092$) in blood K levels based on parity was seen in at-risk sows. In at-risk sows, blood Na tended to be different ($P = 0.066$) based on parity where parity 1 sows had a numeric increase in Na compared to parity 2 to 4 and parity 5+ sows.

DISCUSSION

Stillbirths have been observed in over 48% of litters (Vanderhaeghe et al., 2010) and account for 6.9 to 8.8% of the total pigs born in the United States (MetaFarms, 2024). Documented risk factors for increased stillbirth incidence include parity, farrowing duration, litter size, birth order, and level of supervision (Le Cozler et al., 2002). Fifth parity or older sows have 1.6 times greater odds of stillbirth compared to younger sows (Borges et al., 2005). This parity effect was also observed in the data herein and is likely due to reduced muscle tone affecting muscle contraction and pig expulsion (Vanderhaeghe et al., 2013). Inefficient expulsion

prolongs farrowing duration, and sows that take more than three hours to farrow have twice the odds of stillbirth (Borges et al., 2005). Larger litters (16 pigs or more) are associated with longer farrowing durations, greater maternal fatigue, and greater stillbirth incidence, particularly in the last quartile of pigs born in the litter (Adi et al., 2024).

One proposed explanation for the increased incidence of stillbirths is inadequate Ca availability. Smooth muscle contraction, including uterine contraction, is regulated by both cytosolic Ca concentration and the sensitivity of myofilaments to Ca (Uehata et al., 1997). The phosphorylation of myosin in thick filaments, which triggers smooth muscle contraction, is Ca dependent (Carsten and Miller, 1987). Inadequate Ca availability for normal physiological function is referred to as hypocalcemia and has been extensively documented in dairy cows (Reinhardt et al., 2011). Hypocalcemia results from inefficient adaptation to the abrupt increase in Ca demand for milk production (Martín-Tereso and Martens, 2014) which explains its greater incidence in cows with increased milk yield. In dairy cows, hypocalcemia can be classified as clinical or subclinical. Clinical cases involve overt disruptions in Ca homeostasis and are characterized by loss of appetite, lethargy, and in severe cases, recumbency (Arechiga-Flores et al., 2022). More commonly, subclinical hypocalcemia occurs when Ca levels are marginally low, without obvious clinical signs, yet this condition still impairs muscle contraction and immune function (Arechiga-Flores et al., 2022). Blim et al. (2022) observed that sows experiencing dystocia had reduced blood Ca concentrations before parturition, suggesting that similar subclinical disruptions in Ca metabolism may be occurring in sows.

One of the Ca supplements evaluated to reduce stillbirths in sows is $CaCl_2$ which is an acidogenic agent that causes metabolic acidosis. This compound is added to sow diets to cause a shift in electrolyte composition which decreases the DCAD or the difference between strong

positively charged cations (e.g., Na and K) and strong negatively charged anions (e.g., Cl and S). A negative DCAD results in acidification of the blood which restores tissue responsiveness to parathyroid hormone (Goff and Horst, 1998). Parathyroid hormone increases Ca resorption from bone and Ca absorption in the small intestine (Goltzman, 2018). A second Ca supplement, calcium gluconate, is a Ca salt of gluconic acid. This compound is given at a rate of 500 mL in dairy cows either intravenously or subcutaneously as the primary means of treating hypocalcemia (Oetzel, 2022). While it has been suggested in case studies that Ca gluconate can improve farrowing kinetics and hypocalcemia in sows (Durrell, 1942; Chutia et al., 2018; Reshma et al., 2020), there are currently no controlled studies that investigate it's impact on farrowing performance.

The key production indicator targeted in this study was the incidence of stillbirths. Previous research with $CaCl_2$ has led to variable results in stillbirth incidence. In general, prior research has indicated reductions in stillbirths with $CaCl_2$ administration (Elrod et al., 2015; Bents and Soto, 2023; González-Sánchez et al., 2023). However, Ruampatana et al. (2024) observed that $CaCl_2$ did not affect stillbirth rates in supervised farrowings, but reduced stillbirth rate in sows that farrowed unsupervised. Another study reported a treatment by parity interaction, where mid-parity sows (parity 2 to 5) experienced reduced stillbirth rates with $CaCl_2$ supplementation, while parity 6 or greater sows showed a numeric increase in stillbirths when provided $CaCl_2$ (DeRouchey et al., 2005). Similar to the present findings, Craig et al. (2024) observed no differences in stillbirth incidence with a $CaCl_2$ top-dress. Variability in the efficacy of $CaCl_2$ in reducing stillbirths may be partially explained by differences in baseline stillbirth rates, duration of supplementation, or supplementation dose. In the current study, all three treatments exhibited lower stillbirth rates than the control treatments observed by Bents and Soto

(2023), González-Sánchez et al. (2023), and Ruampatana et al. (2024). The duration of $CaCl_2$ supplementation has varied across studies, with the longest reported by González-Sánchez et al. (2023) at 7 d while Bents and Soto (2023), similar to the present study, initiated supplementation around d 112 of gestation. The dosage of $CaCl_2$ has also differed among studies, ranging from 25 g/d (Ruampatana et al., 2024) to 70 g/d (Elrod et al., 2015). In a pilot study by Craig et al. (2024), feed refusals were observed at doses of 80 g/d and 140 g/d, but not at 40 g/d, which was then subsequently used in their main experiment.

To our knowledge, this is the first prospective study to evaluate the effect of Ca gluconate injection on stillbirths. While only a slight numeric reduction in stillbirths was observed across the overall population, sows classified as at-risk showed a reduction in stillbirths with CaG injection. It is important to note that only sows meeting at-risk criteria and farrowing during normal working hours were included in this subgroup because they were the sows that were eligible to receive CaG. The numeric increase in stillbirths among other sows allotted to the CaG treatment appears to be driven by greater stillbirth rates during overnight farrowings. This may have contributed to the lack of overall population differences for the CaG injection protocol.

Because the incidence of stillbirths is often associated with prolonged farrowing durations, the current study evaluated farrowing duration and farrowing assistance criteria. Elrod et al. (2015) observed that sows provided $CaCl_2$ had shorter farrowing durations and improved farrowing ease scores which is indicative of reduced need for sleeving and manual pig removal. Ruampatana et al. (2024) observed no change in farrowing duration with $CaCl_2$ supplementation but reported a reduction in farrowing assistance required for the first seven pigs born. The absence of differences in farrowing duration between the control and $CaCl_2$ treatments in the current study may help explain the similar stillbirth rates observed across these groups. Notably,

at-risk sows injected with CaG had a numeric increase in farrowing duration but still had a lower incidence of stillbirths compared to at-risk control and $CaCl_2$ sows which suggests that factors beyond farrowing duration also play a critical role in determining stillbirth outcomes.

Unlike previous studies that collected blood and urine samples from sows before farrowing, the study herein collected blood and urine post-farrowing to standardize timing across treatments, as CaG sows received injections during farrowing. In agreement with previous findings in dairy cows, CaG administration in the current study led to a reduction in urine pH (Blanc et al., 2014). Similarly, prior studies in sows have reported reduced urine pH after $CaCl_2$ supplementation (DeRouchey et al., 2003; Bents and Soto, 2023; Craig et al., 2024), consistent with the present results. Urine pH is reduced because the body increases the excretion of hydrogen ions (H^+) in response to the increased dietary load of anions (such as chloride or the gluconate from calcium gluconate), as part of the kidneys' effort to maintain acid-base balance (Darriet et al., 2017).

One of the key metabolites assessed was ionized Ca (iCa), as it is the only biologically active form of Ca (Arechiga-Flores et al., 2022). Although this is the first controlled trial to examine the effects of CaG injection on farrowing performance, Hintz and Billing (2013) investigated post-farrowing CaG administration (4 h after farrowing) as a potential strategy to mitigate hypocalcemia and reported no changes in total serum Ca, similar to the lack of differences in iCa in the current study. These results also align with findings from Craig et al. (2024) and Ruampatana et al. (2024), where no differences in blood Ca were observed with a $CaCl_2$ top-dress, which may be due to the studies collecting total serum Ca instead of iCa. In contrast, both DeRouchey et al. (2003) and the present study found increased iCa and circulating Cl after $CaCl_2$ administration. DeRouchey et al. (2003) speculated that the increase in iCa was

due to the increase in bone mobilization needed as a buffer in the extracellular fluid due to the acidification of the blood. In addition, DeRouchey et al. (2003) observed a reduction in tCO_2 when $CaCl_2$ was added to the diet, which is similar to $CaCl_2$ sows in the present study. Martins et al. (2016) documented a similar impact on tCO_2 in dairy cattle fed acidogenic diets and speculated that it may reflect compensatory changes in respiration rate to maintain blood pH homeostasis.

Emerging evidence suggests that certain physiological criteria may serve as predictors of farrowing success. Craig et al. (2024) reported a reduced incidence of stillbirths in sows with serum Ca concentrations above 2.5 mmol/L and urine pH below 7.0. Additionally, Feyera et al. (2018) observed that greater arterial glucose levels one hour after the birth of the first pig was associated with shorter farrowing durations. In the present study, the only difference in sow metabolites in at-risk CaG sows when compared to at-risk control and $CaCl_2$ sows was elevated circulating glucose. This increase in glucose may be a key factor contributing to the reduction of stillbirths observed in at-risk CaG sows. However, the mechanism behind this rise in glucose is not fully understood. After administration, CaG dissociates into iCa and gluconic acid, but gluconic acid is not directly converted back to glucose in the blood. One possible explanation for the increase in glucose in CaG sows is that the rapid availability of Ca after the injection may have stimulated reactive tissues or endocrine responses, such as catecholamine release or altered insulin signaling, leading to a transient hyperglycemic state. Supporting this, Schlumbohm and Harmeyer (2003) demonstrated that hypocalcemia reduces endogenous glucose production. Previous work in dairy cattle has shown increased blood glucose concentrations 15 min after parturition in cows that received an oral bolus of $CaCl_2$ and $CaSO_4$ at the time of parturition; however, no differences in blood glucose levels were seen at the next measurement, taken 6 h

after parturition (Wilms et al., 2022). It is important to note that the type of calcium supplement, route of administration, and species differ, so these results may not be directly comparable. To the best of the authors' knowledge, no studies to date have evaluated the short-term impacts of CaG injection on glucose levels. Further research is warranted to explore the underlying mechanisms and their implications for farrowing outcomes.

In addition to increasing the risk of stillbirths, prolonged farrowing is associated with greater incidence of asphyxiated newborn pigs. Asphyxiated pigs consume less colostrum, which increases their susceptibility to hypothermia and starvation (Trujillo-Ortega et al., 2007). Based on this, we hypothesized that Ca supplementation would improve neonatal viability, thereby enhancing colostrum intake. The immunocrit procedure is an estimation of immunoglobulins in the blood which can be indicative of colostrum consumed. Interestingly, Ruampatana et al. (2024) observed that despite signs of reduced asphyxia such as lower rates of meconium staining and broken umbilical cords, colostrum intake was lower in pigs from $CaCl_2$ sows compared to pigs from control sows. In the current trial, pigs from CaG sows had the greatest immunocrit which may signify improved viability and activity. However, further research is needed to fully understand the mechanism behind this improvement in immunocrit levels.

Given that farrowing duration can influence the incidence of starvation and hypothermia, it is also associated with increased early lactation pig mortality due to crushing. This is the first controlled study to evaluate the impact of Ca administration protocols on mortality of pigs in the first 24 h after farrowing. While Bents and Soto (2023) observed no differences in farrow to weaning mortality with $CaCl_2$ supplementation, use of the same $CaCl_2$ product in the present study resulted in increased birth to cross foster mortality, particularly in parity 1 sows, when $CaCl_2$ was top-dressed. However, the authors of the current study believe that the interaction

between Ca administration protocol and parity category may be an artifact, as it appears to be driven by an unusually low birth to cross-foster mortality in parity 1 control sows.

Lastly, long farrowing durations have been linked to long WEI (Oliviero et al., 2013). Therefore, we hypothesized that Ca administration protocols could influence WEI. However, consistent with findings by DeRouchey et al. (2003) and Ruampatana et al. (2024), no differences in WEI were observed in the current trial among the treatment groups.

In conclusion, for the overall sow population, topdressing the $CaCl_2$-based product before farrowing or administering CaG injections peripartum affected sow blood and urine metabolites during farrowing but did not influence farrowing performance. However, when the sow population was further divided into at-risk (sows with > 16 pigs, > 1 h since the last birth, ≥ 2 stillbirths, or farrowing lasting > 4 h) and other sow categories, administration of a CaG injection decreased stillbirth rate in at-risk sows leading to an increase in the percentage of pigs born alive.

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Table 6.1 Effect of farrowing calcium protocol on sow performance[1]

	Farrowing protocol[2]				*P* =		
	None	Calcium chloride	Calcium gluconate	SEM	Treatment × parity	Treatment	Parity
Count, n	311	312	310	---	---	---	---
Sow entry BW, kg	269.0	269.9	268.9	2.45	0.478	0.891	< 0.001
Gestation length, d	116.1	116.1	116.0	0.08	0.310	0.679	0.362
Litter characteristics							
Total born, n	16.7	16.7	16.9	0.25	0.907	0.963	0.600
Born alive, %	90.8	90.1	91.4	0.88	0.190	0.145	< 0.001
Stillborn, %	6.7	6.7	6.1	0.87	0.462	0.226	< 0.001
Mummy, %	2.5	3.1	2.6	0.26	0.019	0.035	0.228
Farrowing characteristics							
Percentage of sows sleeved	45.0	48.7	51.3	3.32	0.278	0.381	< 0.001
Number of times sleeved	1.1	1.1	1.3	0.39	0.970	0.901	0.458
Farrowing duration, min[3]	294.6	294.2	323.7	15.07	0.951	0.194	0.350
Birth-cross foster mortality, %	5.9[b]	7.9[a]	7.1[ab]	0.42	0.035	< 0.001	< 0.001
Wean-to-estrus interval, d	4.8	5.2	4.8	0.28	0.256	0.349	0.053

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 933 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of entry into the farrowing house (approximately d 112 of gestation) until cross-fostering at approximately 24 h after farrowing.

[2]Farrowing protocol consisted of a control with sows receiving no intervention (None), 25 g of a Ca chloride based product (TRIAD, Alltech, Lexington, KY) top-dressed on the morning feeding of each sow from entry into the farrowing house until each sow farrowed (Calcium chloride), and a Ca gluconate protocol in which primiparous sows received a 15 mL injection and multiparous sows received a 20 mL injection of Ca gluconate if a litter had more than 16 pigs, it had been longer than 1 h since the birth of the last pig, the litter had 2 or more stillbirths, or farrowing duration exceeded 4 h (Calcium gluconate).

[3]Farrowing duration was collected on 79 Control sows, 84 Calcium chloride sows, and 85 Calcium gluconate sows.

Table 6.2 Effect of farrowing calcium protocol on sow blood and urine characteristics and piglet immunocrit ratio[1]

	Farrowing protocol[2]				*P* =		
	None	Calcium chloride	Calcium gluconate	SEM	Treatment × parity	Treatment	Parity
Count, n	74	74	74	---	---	---	---
Blood characteristics							
Anion gap, mmol/L	17.6	18.0	18.2	0.25	0.761	0.236	0.561
Blood urea nitrogen, mg/dL	12.6	13.0	13.6	0.71	0.232	0.401	0.405
Chloride, mmol/L	102.9[b]	104.6[a]	103.0[b]	0.39	0.973	< 0.001	0.161
Creatinine mg/dL	3.1	3.2	3.2	0.07	0.643	0.944	0.001
Glucose, mmol/L	96.5[b]	96.4[b]	102.8[a]	2.46	0.765	0.026	0.059
Hematocrit, % PCV	31.0	32.6	31.7	0.56	0.510	0.060	0.455
Hemoglobin, g/dL	10.5	11.1	10.8	0.19	0.495	0.067	0.455
Ionized Calcium, mmol/L	1.23[b]	1.29[a]	1.24[b]	0.016	0.212	< 0.001	0.001
Potassium, mmol/L	4.2	4.2	4.1	0.05	0.587	0.365	0.058
Sodium, mmol/L	142.6	143.0	142.6	0.28	0.863	0.371	0.144
Total carbon dioxide, mmol/L	27.3[a]	25.6[b]	26.5[ab]	0.34	0.452	< 0.001	0.162
Urine pH	6.96[a]	6.41[b]	6.53[b]	0.128	0.745	0.001	0.904
Piglet immunocrit ratio[3]	0.070	0.074	0.080	0.0034	0.430	0.068	0.406

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 933 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of entry into the farrowing house (approximately d 112 of gestation) until cross-fostering at approximately 24 h after farrowing.

[2]Farrowing protocol consisted of a control with sows receiving no intervention (None), 25 g of a Ca chloride based product (TRIAD, Alltech, Lexington, KY) top-dressed on the morning feeding of each sow from entry into the farrowing house until each sow farrowed (Calcium chloride), and a Ca gluconate protocol in which primiparous sows received a 15 mL injection and multiparous sows received a 20 mL injection of Ca gluconate if a litter had more than 16 pigs, it had been longer than 1 h since the birth of the last pig, the litter had 2 or more stillbirths, or farrowing duration exceeded 4 h (Calcium gluconate).

[3]Blood was taken from one of the first five pigs born in each litter at approximately 24 h after the start of farrowing.

Table 6.3 Effect of farrowing calcium protocol on sow performance for at-risk and other sows[1,2]

	At-risk sows[3]					Other sows[4]				
Farrowing protocol:	None	Calcium chloride	Calcium gluconate	SEM	Treatment, *P* =	None	Calcium chloride	Calcium gluconate	SEM	Treatment, *P* =
Count, n	139	128	144	---	---	172	184	166	---	---
Sow entry BW, kg[5,6]	271.1	272.2	272.0	4.66	0.953	267.3	268.1	265.8	3.13	0.738
Gestation length, d	116.1	116.2	116.1	0.13	0.643	116.0	115.9	115.9	0.11	0.643
Litter characteristics										
Total born, n	16.9	16.9	17.6	0.44	0.559	16.6	16.6	16.4	0.35	0.869
Total born alive, n	14.9	15.0	16.1	0.42	0.181	15.5	15.3	14.8	0.34	0.655
Stillborn, n[5,6]	1.5[a]	1.4[ab]	1.0[b]	0.16	0.039	0.8	0.9	1.0	0.18	0.865
Mummy, n	0.4	0.5	0.4	0.09	0.688	0.4	0.5	0.5	0.06	0.197
Born alive, %[6]	88.2[b]	88.6[b]	92.1[a]	0.98	0.006	92.9	91.3	91.0	1.08	0.163
Stillborn, %[5,6]	9.0[a]	8.3[a]	5.9[b]	0.91	0.004	4.8	5.6	6.1	1.09	0.772
Mummy, %[7]	2.6	3.0	2.1	0.48	0.670	2.4	2.9	2.9	0.34	0.102
Farrowing characteristics										
Percentage of sows sleeved	78.2	78.6	87.6	4.33	0.594	16.2	30.0	19.4	4.01	0.696
Number of times sleeved	2.3	2.5	2.6	0.32	0.948	0.2	0.4	0.3	0.07	0.363
Farrowing duration, min[8]	307	307	328	31.2	0.628	277	284	---	23.8	0.763
Birth-cross foster mortality, %[6]	8.1[ab]	10.1[a]	7.6[b]	0.80	0.006	4.1[b]	6.8[a]	6.7[a]	0.54	0.003
Wean to estrus interval, d	5.4	5.0	5.1	0.53	0.733	4.6	5.6	4.9	0.47	0.152

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 933 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of entry into the farrowing house (approximately d 112 of gestation) until cross-fostering at approximately 24 h after farrowing.

[2]Farrowing protocol consisted of a control with sows receiving no intervention (None), 25 g of a Ca chloride based product (TRIAD, Alltech, Lexington, KY) top-dressed each morning from entry into the farrowing house until each sow farrowed (Calcium chloride), and a Ca gluconate protocol in which at-risk primiparous sows received a 15 mL injection and at-risk multiparous sows received a 20 mL injection of Ca gluconate.

[3]At-risk sows (n = 411) were those that met the criteria to receive Ca gluconate injection (sows with > 16 pigs, > 1 h since the last birth, ≥ 2 stillbirths, or farrowing lasting > 4 h) and farrowed during working hours (6:00 until 15:00). Because sows were assigned to treatment prior to

farrowing, there were also sows assigned to the calcium gluconate treatment that were not later classified as at-risk sows and, thus, did not receive an injection of Ca gluconate.
[4]Other sows included those that farrowed during working hours (6:00 until 15:00) but did not meet the criteria for a Ca gluconate injection, as well as those that farrowed overnight when farrowing intervention was unavailable.
[5]At-risk sows: Parity, $P < 0.05$.
[6]Other sows: Parity, $P < 0.05$.
[7]At-risk sows: Parity, $P < 0.10$.
[8]Farrowing duration was collected on 79 Control sows, 84 Calcium chloride sows, and 85 Calcium gluconate sows.

Table 6.4 Effect of farrowing calcium protocol on sow blood parameters and piglet immunocrit ratio for at-risk and other sows[1,2]

	At-risk sows[3]					Other sows[4]				
Farrowing protocol:	None	Calcium chloride	Calcium gluconate	SEM	Treatment, *P* =	None	Calcium chloride	Calcium gluconate	SEM	Treatment, *P* =
Count, n	48	26	74	---	---	26	48	0	---	---
Blood characteristics										
Anion gap, mmol/L	17.7	18.0	18.2	0.49	0.428	17.4	18.1	---	0.53	0.219
Blood urea nitrogen, mg/dL	12.6	12.7	13.6	1.20	0.411	12.8	12.8	---	1.15	0.989
Chloride, mmol/L	102.9[b]	104.9[a]	103.1[b]	0.72	0.039	102.8	104.7	---	0.70	0.007
Creatinine mg/dL[5,6]	3.2	3.3	3.2	0.13	0.809	3.1	3.1	---	0.15	0.701
Glucose, mmol/L	95.6[b]	90.7[b]	102.7[a]	4.14	0.005	98.5	97.8	---	4.72	0.882
Hematocrit, % PCV	31.0	33.3	31.7	1.05	0.168	30.8	32.3	---	1.18	0.205
Hemoglobin, g/dL	10.6	11.3	10.8	0.36	0.178	10.5	11.0	---	0.40	0.212
Ionized Calcium, mmol/L[5,6]	1.22[b]	1.29[a]	1.24[ab]	0.020	0.004	1.25	1.29	---	0.019	0.044
Potassium, mmol/L[7]	4.2	4.2	4.1	0.09	0.637	4.2	4.2	---	0.08	0.647
Sodium, mmol/L[7]	142.8	143.2	142.5	0.54	0.443	142.1	143.1	---	0.53	0.054
Total carbon dioxide, mmol/L	27.6[a]	25.5[b]	26.5[ab]	0.65	0.011	27.1	25.6	---	0.73	0.026
Piglet immunocrit ratio[8]	0.072	0.073	0.080	0.0057	0.206	0.063	0.074	---	0.0078	0.131

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 933 mixed-parity sows (Line 1050, PIC, Hendersonville TN) and litters were used from the time of entry into the farrowing house (approximately d 112 of gestation) until cross-fostering at approximately 24 h after farrowing.

[2]Farrowing protocol consisted of a control with sows receiving no intervention (None), 25 g of a Ca chloride based product (TRIAD, Alltech, Lexington, KY) top-dressed each morning from entry into the farrowing house until each sow farrowed (Calcium chloride), and a Ca gluconate protocol in which at-risk primiparous sows received a 15 mL injection and at-risk multiparous sows received a 20 mL injection of Ca gluconate.

[3]At-risk sows (n = 411) were those that met the criteria to receive Ca gluconate injection (sows with > 16 pigs, > 1 h since the last birth, ≥ 2 stillbirths, or farrowing lasting > 4 h) and farrowed during working hours (6:00 until 15:00). Because sows were assigned to treatment prior to farrowing, there were also sows assigned to the calcium gluconate treatment that were not later classified as at-risk sows and, thus, did not receive an injection of Ca gluconate.

[4]Other sows included those that farrowed during working hours (6:00 until 15:00) but did not meet the criteria for a Ca gluconate injection, as well as those that farrowed overnight when farrowing intervention was unavailable.

[5]At-risk sows: Parity, $P < 0.05$.

[6]Other sows: Parity, $P < 0.05$.

[7]At-risk sows: Parity, $P < 0.10$.
[8]Blood was taken from one of the first five pigs born in each litter at approximately 24 h after the start of farrowing.

Appendix A - Chapter 1 Supplemental Tables

Appendix Table A.1 Diet composition (as-fed basis)

	Exp. 1			Exp. 2	
	Dietary phase[1]			Dietary phase[2]	
Item	1	2	3	1	2
Ingredient, %					
Corn	39.35	56.95	65.50	47.15	60.60
Soybean meal, 46.5% CP[3]	17.65	25.95	28.30	20.30	28.15
Spray-dried whey	25.00	10.00	---	17.50	---
Corn DDGS[4]	5.00	---	---	5.00	7.50
Fermented soybean meal[5]	2.00	---	---	5.00	---
Fish meal	9.00	2.50	---	---	---
Choice white grease	3.00	1.00	2.00	1.50	---
Monocalcium P, 21.5% P	0.40	0.60	1.10	1.08	0.95
Calcium carbonate	0.50	0.75	0.75	0.61	0.81
Sodium chloride	0.30	0.50	0.60	0.35	0.55
Vitamin premix with phytase	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
L-Lys-HCl	0.48	0.48	0.55	0.48	0.52
DL-Met	0.20	0.20	0.21	0.23	0.17
L-Thr	0.18	0.21	0.23	0.18	0.21
L-Trp	0.05	0.04	0.05	0.04	0.05
L-Val	0.10	0.15	0.16	0.10	0.11
Choline chloride, 60%	0.04	---	---	0.04	---
Vitamin E, 20,000 IU	0.05	---	---	0.05	---
Sodium metabisulfite	0.25	---	---	---	---
Viligen[6]	0.15	---	---	---	---
Phytase[7]	0.02	---	---	---	---
Cerdanase[8]	---	0.01	---	---	---
All-Bind HD[9]	---	---	0.15	---	---
Zinc oxide[10]	0.39	0.25	---	+/-	---
Copper sulfate[11]	---	---	---	+/-	+/-
Antimicrobial[12]	---	---	---	+/-	+/-
Total	100	100	100	100	100
Calculated analysis					
Standardized ileal digestible (SID) amino acids					
Lys, %	1.38	1.35	1.29	1.35	1.30
Ile:Lys	57	54	50	59	57
Leu:Lys	111	109	107	120	124
Met:Lys	37	37	36	38	35
Met and Cys:Lys	58	57	56	57	57
Thr:Lys	65	64	62	64	64
Trp:Lys	19	18	19	20	19

Val:Lys	69	72	71	70	70
His:Lys	30	31	32	35	38
Net energy, kcal/kg	2,537	2,554	2,642	2,529	2,420
CP, %	20.0	19.9	19.4	21.3	21.3
Ca, %	0.86	0.77	0.65	0.70	0.67
P, %	0.68	0.60	0.59	0.67	0.61
STTD P, %[13]	0.62	0.49	0.48	0.58	0.48

[1]Pigs were fed a common diet in a 3-phase feeding program. Phase 1 diet was fed from d 0 to 10, phase 2 diet was fed from d 10 to 21, and phase 3 diet was fed from d 21 to 42.
[2] Diets were fed in a 2-phase feeding program. Phase 1 experimental diets were fed from d 0 to 22 and phase 2 experimental diets were fed from d 22 to 43.
[3] CP, crude protein
[4]DDGS, Distillers dried grains with solubles
[5]MEPRO (Prairie AquaTech, Brookings, SD)
[6] Viligen (Alltech, Lexington, KY)
[7]Quantum Blue 5G (AB Vista Inc., Plantation, FL)
[8]Cerdanase (Alltech, Lexington, KY)
[9]All-Bind HD (Alltech, Lexington, KY)
[10]Zinc from ZnO was provided in the diet at 110 or 2,414 mg/kg during dietary phase 1.
[11] Copper from $CuSO_4$ was provided in the diet at 17 or 168 mg/kg during dietary phases 1 and 2.
[12]Mecadox 2.5 (carbadox; Phibro Animal Health, Teaneck, NJ) was added at 0 or 10 g/kg during dietary phases 1 and 2.
[13]STTD P, standardized total tract digestible phosphorus.

Appendix Table A.2 Evaluation of carbadox and pharmacological levels of zinc and copper on growth performance of nursery pigs[1,2]

Item	Control[3]	Supplemented control[4]	SEM	*P* =
BW, kg				
d 0	5.6	5.6	0.16	0.900
d 22	9.8	11.9	0.16	< 0.001
d 43	22.1	25.9	0.24	< 0.001
d 0 to 22 (Phase 1)				
ADG, kg	0.19	0.28	0.012	< 0.001
ADFI, kg	0.26	0.33	0.008	< 0.001
G:F	0.72	0.85	0.024	< 0.001
d 22 to 43 (Phase 2)				
ADG, kg	0.59	0.67	0.006	< 0.001
ADFI, kg	0.89	0.99	0.010	< 0.001
G:F	0.66	0.68	0.004	0.072
d 0 to 43 (Overall)				
ADG, kg	0.38	0.47	0.006	< 0.001
ADFI, kg	0.56	0.65	0.007	< 0.001
G:F	0.68	0.72	0.006	< 0.001
Injections per 1,000 pig days				
Total injections	35.70	28.75	0.091	0.020
Enrofloxacin[5]	30.05	24.90	0.098	0.060
Penicillin	5.64	3.84	0.240	0.114

[1]A total of 360 barrows (DNA 200 × 400, initial BW 5.6 ± 0.16 kg) were used in a 43-d experiment with 5 pigs per pen and 36 replications per dietary treatment.
[2]ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio.
[3]Control diets were formulated to meet NRC (2012) requirements for Zn (110 mg/kg) and Cu (17 mg/kg) with no addition of an antimicrobial.
[4]Supplemented control diets contained pharmacological levels of Zn (2,414 ppm from ZnO) in Phase 1 diet and Cu (168 ppm from $CuSO_4$) in both Phase 1 and Phase 2 diets. Both phases contained carbadox (50 g/ton; Mecadox, Phibro Animal Health, Teaneck, NJ).
[5]Baytril 100 (Elanco, Indianapolis, Indiana)

Appendix Table A.3 Evaluation of different allotment strategies on growth performance of nursery pigs[1,2]

Item	Random[3]	BW distribution[4]	BW grouping[5]	SEM	*P* =
Exp. 1					
BW, kg					
d 0	6.2	6.2	6.2	0.12	0.997
d 21	11.7	11.9	11.8	0.37	0.775
d 42	24.4	25.1	24.7	0.41	0.309
d 0 to 21					
ADG, kg	0.26	0.27	0.26	0.014	0.645
ADFI, kg	0.32	0.32	0.32	0.011	0.685
G/F	0.82	0.83	0.82	0.019	0.738
d 21 to 42					
ADG, kg	0.61	0.63	0.62	0.009	0.430
d 0 to 42 (Overall)					
ADG, kg	0.43	0.45	0.44	0.008	0.320
Exp. 2					
BW, kg					
d 0	5.6	5.6	5.6	0.17	1.000
d 22	10.8	10.8	10.9	0.19	0.910
d 43	23.7	24.0	24.3	0.30	0.293
d 0 to 22					
ADG, kg	0.23	0.23	0.24	0.012	0.593
ADFI, kg	0.30	0.29	0.29	0.009	0.578
G/F	0.77[b]	0.79[ab]	0.80[a]	0.024	0.013
d 22 to 43					
ADG, kg	0.61[b]	0.63[ab]	0.64[a]	0.008	0.055
ADFI, kg	0.92	0.93	0.95	0.012	0.218
G/F	0.66	0.67	0.67	0.005	0.448
d 0 to 43 (Overall)					
ADG, kg	0.42	0.42	0.43	0.007	0.109
ADFI, kg	0.60	0.60	0.61	0.009	0.340
G/F	0.69	0.70	0.71	0.007	0.065

[a-c]Means within row with different superscripts differ ($P < 0.05$).

[1]A total of 720 barrows (DNA 200 × 400, initially 6.20 ± 0.12 kg) were used in two 42-d experiments with 5 pigs per pen and 24 replications (n=24) per allotment strategy. No interactions between allotment strategy and dietary treatment were observed.

[2]ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio.

[3]Pigs were allotted to pens in a completely randomized design.

[4]Pigs were sorted by BW into five groups. Within each BW group, pigs were randomized to pen such that each pen consisted of 1 random pig from each BW group to ensure that distribution of BW within pen was relatively consistent across pens.

[5]Pigs were sorted into three groups by BW (light, medium, heavy). Within each BW group, pigs were randomized to pen such that each pen consisted of pigs from a single BW group (pens of light pigs, pens of medium pigs, pens of heavy pigs).

Appendix Table A.4 Evaluation of different allotment strategies on population coefficient of variation[1,2]

Item	Random[3]	BW distribution[4]	BW grouping[5]
Exp. 1			
d 0	13.5	13.5	13.2
d 3	13.6	13.5	13.4
d 10	14.8	14.1	13.2
d 14	15.7	14.2	13.1
d 21	16.9	14.1	12.5
d 42	13.7	12.0	12.5
Exp. 2			
d 0	15.0	14.7	14.9
d 8	16.3	17.1	16.1
d 15	17.6	19.0	17.9
d 22	19.2	19.0	18.4
d 29	20.2	19.5	18.1
d 36	18.4	17.3	15.6
d 43	17.3	15.5	14.0

[1]A total of 720 barrows (DNA 200 × 400, initially 6.20 ± 0.12 kg) were used in two 42-d experiments with 5 pigs per pen and 24 replications (n=24) per allotment strategy.
[2]Calculated by dividing standard deviation of body weight of all pigs within allotment strategy by mean body weight of all pigs within that allotment strategy.
[3]Pigs were allotted to pens in a completely randomized manner.
[4]Pigs were sorted by BW into five groups. Within each BW group, pigs were randomized to pen such that each pen consisted of 1 random pig from each BW group to ensure that distribution of BW within pen was relatively consistent across pens.
[5]Pigs were sorted into three groups by BW (light, medium, heavy). Within each BW group, pigs were randomized to pen such that each pen consisted of pigs from a single BW group (pens of light pigs, pens of medium pigs, pens of heavy pigs).