Nutritional strategies to optimize growth performance in nursery pigs

by

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B.S., Universidad Nacional de Colombia, 2017
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AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

Optimizing dietary strategies in pigs is crucial to enhance growth performance, nutrient utilization, and economic efficiency while minimizing environmental impact. A total of nine experiments (5,423 pigs) and a meta-regression analysis structured in 5 chapters were used to evaluate the effect of Zn levels and sources under different formulation strategies, nursery feed budgets, and a functional fiber ingredient on growth performance, fecal dry matter (DM), mineral excretion, and economic outcomes. In chapter 1, a meta-regression analysis utilizing data from 51 published papers was conducted to identify dietary factors associated with Zn excretion in pigs and develop predictive models for Zn excretion. Principal component analysis indicated that nutrient intake variables, including minerals, fiber, nitrogen, as well as body weight and feed intake, accounted for a significant portion of the variability in Zn excretion. Overall, Zn intake was the primary determinant of fecal Zn excretion, with meta-regression models predicting that 61% of Zn intake is excreted when diets meet the requirement and up to 82% when Zn exceeds requirement estimates. In Chapter 2, two experiments utilizing 2,268 pigs were conducted to evaluate the effects of Zn source (ZnO, Zn hydroxychloride, $ZnSO_4$, or chelated Zn) and inclusion level on growth performance, fecal DM, fecal Zn concentration, and carcass characteristics. In nursery pigs, increasing dietary Zn led to higher fecal Zn concentrations independent of the Zn source, although overall growth performance and fecal DM were unaffected by Zn source or level. In finishing pigs, Zn source did not affect growth performance, carcass weight, or carcass yield, with only marginal effects observed on backfat depth and lean percentage in favor of Zn hydroxychloride. Chapter 3 utilized 360 nursery pigs to evaluate the impact of dietary acid-binding capacity to pH 4 (ABC-4) and Zn levels on growth performance, fecal DM, and Zn excretion. At the same dietary Zn concentration, low ABC-4 diets enhanced Zn absorption, apparent total tract digestibility of Zn,

and fecal DM at early stages, although overall growth performance was not significantly affected by dietary ABC-4. Increasing Zn in low ABC-4 diets improved ADG and ADFI during the experimental period; however, these differences did not translate into a better overall performance. Furthermore, d 24 fecal Zn excretion and serum Zn concentration increased with dietary Zn, validating the results from chapter 1, where the main determinant of fecal Zn excretion was the dietary Zn concentration. Chapter 4 was composed of two experiments that utilized a total of 1,800 pigs to evaluate the effect of nursery feed budget on growth performance and economics. Experiment 1, which was conducted in university research facilities, demonstrated that reducing the budget of complex phase 1 and 2 diets did not affect overall pig performance but improved economic outputs. To confirm these findings, a second experiment was conducted in a commercial research facility. While pigs provided a reduced feed allowance and less complex diets tended to grow slower in the early nursery period, no differences in growth performance were observed by d 63 after weaning. Collectively, these findings suggest that reducing early nursery feed budgets could be a strategy to improve economic outcomes without compromising long-term pig growth. Chapter 5 was composed of three experiments utilizing a total of 995 pigs to evaluate the effect of ValoproWin (VLPW), a functional fiber ingredient composed primarily of insoluble, poorly fermentable fiber, on growth performance and fecal DM in nursery pigs. Within three experiments, sub-objectives were to determine how the response was impacted by: (1) VLPW feeding duration and dietary ABC-4; (2) VLPW inclusion level; and (3) potential interactions between VLPW inclusion level and dietary formulation strategies (nutrient dilution vs. nutrient adjustment). Considering the results of the three experiments collectively, the inclusion of VLPW had minimal effects on growth performance; however, it may serve as a nutritional strategy to promote intestinal health and increase fecal DM.

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Optimizing dietary strategies in pigs is crucial to enhance growth performance, nutrient utilization, and economic efficiency while minimizing environmental impact. A total of nine experiments (5,423 pigs) and a meta-regression analysis structured in 5 chapters were used to evaluate the effect of Zn levels and sources under different formulation strategies, nursery feed budgets, and a functional fiber ingredient on growth performance, fecal dry matter (DM), mineral excretion, and economic outcomes. In chapter 1, a meta-regression analysis utilizing data from 51 published papers was conducted to identify dietary factors associated with Zn excretion in pigs and develop predictive models for Zn excretion. Principal component analysis indicated that nutrient intake variables, including minerals, fiber, nitrogen, as well as body weight and feed intake, accounted for a significant portion of the variability in Zn excretion. Overall, Zn intake was the primary determinant of fecal Zn excretion, with meta-regression models predicting that 61% of Zn intake is excreted when diets meet the requirement and up to 82% when Zn exceeds requirement estimates. In Chapter 2, two experiments utilizing 2,268 pigs were conducted to evaluate the effects of Zn source (ZnO, Zn hydroxychloride, $ZnSO_4$, or chelated Zn) and inclusion level on growth performance, fecal DM, fecal Zn concentration, and carcass characteristics. In nursery pigs, increasing dietary Zn led to higher fecal Zn concentrations independent of the Zn source, although overall growth performance and fecal DM were unaffected by Zn source or level. In finishing pigs, Zn source did not affect growth performance, carcass weight, or carcass yield, with only marginal effects observed on backfat depth and lean percentage in favor of Zn hydroxychloride. Chapter 3 utilized 360 nursery pigs to evaluate the impact of dietary acid-binding capacity to pH 4 (ABC-4) and Zn levels on growth performance, fecal DM, and Zn excretion. At the same dietary Zn concentration, low ABC-4 diets enhanced Zn absorption, apparent total tract digestibility of Zn,

and fecal DM at early stages, although overall growth performance was not significantly affected by dietary ABC-4. Increasing Zn in low ABC-4 diets improved ADG and ADFI during the experimental period; however, these differences did not translate into a better overall performance. Furthermore, d 24 fecal Zn excretion and serum Zn concentration increased with dietary Zn, validating the results from chapter 1, where the main determinant of fecal Zn excretion was the dietary Zn concentration. Chapter 4 was composed of two experiments that utilized a total of 1,800 pigs to evaluate the effect of nursery feed budget on growth performance and economics. Experiment 1, which was conducted in university research facilities, demonstrated that reducing the budget of complex phase 1 and 2 diets did not affect overall pig performance but improved economic outputs. To confirm these findings, a second experiment was conducted in a commercial research facility. While pigs provided a reduced feed allowance and less complex diets tended to grow slower in the early nursery period, no differences in growth performance were observed by d 63 after weaning. Collectively, these findings suggest that reducing early nursery feed budgets could be a strategy to improve economic outcomes without compromising long-term pig growth. Chapter 5 was composed of three experiments utilizing a total of 995 pigs to evaluate the effect of ValoproWin (VLPW), a functional fiber ingredient composed primarily of insoluble, poorly fermentable fiber, on growth performance and fecal DM in nursery pigs. Within three experiments, sub-objectives were to determine how the response was impacted by: (1) VLPW feeding duration and dietary ABC-4; (2) VLPW inclusion level; and (3) potential interactions between VLPW inclusion level and dietary formulation strategies (nutrient dilution vs. nutrient adjustment). Considering the results of the three experiments collectively, the inclusion of VLPW had minimal effects on growth performance; however, it may serve as a nutritional strategy to promote intestinal health and increase fecal DM.

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Dedication

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Chapter 1 - Dietary factors influencing zinc excretion in pigs: A meta-regression analysis

Abstract

Although several nutritional tables report Zn requirements for pigs, dietary Zn levels that exceed recommendations are commonly used to improve performance in nursery pigs. However, excessive Zn supplementation is a practice that has been recently regulated in different countries. Understanding the factors influencing Zn excretion is important for optimizing Zn utilization in swine production and mitigating regulations of its use. This review compiled data from 51 published studies to identify factors associated with Zn excretion using a principal component analysis and developed predictive models for fecal Zn excretion utilizing a meta-regression analysis. Because urine Zn excretion was negligible (around 2% of the total Zn excretion) fecal and total Zn excretion did not differ. The first two principal components (PC) accounted for 35.9% and 19.3% of the total database variance, respectively. Principal component 1 was primarily influenced by nutrient intake variables, including fiber, NE, nitrogen, and minerals such as P, Ca, and Fe, as well as body weight and feed intake. Principal component 2 was dominated by Zn-related variables including Zn intake, fecal Zn excretion, the difference between Zn intake and Zn requirements estimates from NRC (2012), and added dietary Zn. Variables such as Mn, Cu, and lactose intake moderately contributed to both components, whereas the use of antibiotics, exogenous enzymes other than phytase, and acidifiers had a low impact on the variation explained by both components. Stepwise selection indicated that when pigs were fed at or below their Zn requirement estimate, Zn, P, and Fe intake significantly influenced fecal Zn excretion. However, when Zn intake exceeds the requirement estimate, Zn sources and Zn intake were the most influential variables. Regardless of the scenario, Zn intake

was the primary determinant of fecal Zn excretion. The meta-regression analysis indicated that when pigs are fed at or below their Zn requirement, 61% of Zn intake is excreted in feces, increasing to 82% when Zn intake exceeded requirements. In conclusion, while Zn excretion is affected by multiple dietary factors, their contribution under practical conditions is relatively low and Zn intake remains the primary determinant of fecal Zn excretion.

Introduction

Zinc is an essential micromineral for pigs, playing a key role in various physiological processes, including protein synthesis, immune function, cellular proliferation and differentiation, and bone development. The NRC (2012) estimates dietary Zn requirements concentrations ranging from 100 mg/kg for 5 kg pigs to 50 mg/kg for 135 kg pigs. Additionally, the use of pharmacological levels of Zn as a preventive strategy to reduce the negative impact of post-weaning diarrhea on nursery performance has been a common practice in the swine industry (Poulsen and Larsen, 1995; Bonetti et al., 2021).

Following the repletion of Zn pools in the body after weaning, Zn homeostasis in pigs is regulated through a saturable absorption process in the small intestine, with excess Zn excreted when intake exceeds physiological requirements (Maares and Haase, 2020). When Zn supplied via manure exceeds the crop requirement, most of the excreted Zn accumulates in soils, potentially leading to public health and environmental concerns including antimicrobial resistance (Qian et al., 2018; Duan et al., 2019), plant toxicity (Kaur and Garg, 2021), and water pollution (Noulas et al., 2018). These issues have led the European Union to regulate the dietary levels of total Zn in nursery pigs and sows to a maximum of 150 mg/kg and in finishing pigs to 120 mg/kg (EC regulation 1334/2003 and 2016/1095).

In response to regulations limiting the use of Zn in swine diets, research efforts focused on evaluating more sustainable approaches for the use of Zn, such as improving endogenous Zn utilization, have increased over the past decade (Nielsen et al., 2024). However, no consensus has been reached regarding the effects of these strategies on pig growth performance or mineral excretion, likely due to the numerous variables and interactions influencing the response. Whereas several literature reviews have summarized the impact of Zn on performance (Sales, 2013; Luise et al., 2024), to our knowledge, only one meta-analysis (Hansen et al., 2023) has focused on Zn excretion in pigs. However, this review was limited to nursery pigs and the resulting model may not be applicable to growing and finishing pigs. Furthermore, there are limited literature reviews that comprehensively describe the factors influencing Zn excretion from pigs. Therefore, the first objective of this study was to identify and describe factors associated with Zn excretion in pigs, and the second objective was to develop a meta-regression analysis model to predict Zn excretion in pigs from weaning to 135 kg. It was hypothesized that Zn excretion in pigs is influenced by multiple dietary factors, including nutrient intake, feed additives, and feed intake, and that multivariate statistical approaches could identify the factors contributing most to Zn excretion, ultimately enabling the development of predictive models to estimate Zn excretion in pigs.

Materials and Methods

Data collection

A systematic literature review was conducted in accordance with PRISMA flow charts and guidelines (Liberati et al., 2009) to identify studies for inclusion in this meta-analysis. The literature search utilized the online databases Google Scholar, CABI Digital Library, PubMed, Scopus, Web of Science, and the literature review assistant Litmaps. Searches were limited to

titles and abstracts and included combinations of the following keywords: “Zinc or Zn”, “digestibility or ATTD or excretion or absorption”, “pigs or piglets or swine”, “mineral excretion”, “zinc status”. A total of 827 publications were identified. After removing duplicated publications, 334 unique publications remained. In the initial screening, studies were excluded if they met any of the following criteria: a) literature reviews, theses, or book chapters, b) full articles not available, c) estimates of mineral excretion based on mathematical models instead of direct measurement, d) evaluation of different species rather than pigs, e) studies conducted with gestating and lactating sows, or f) written in a language other than English. Following this process, 108 publications were retained. To be included in the meta-analysis, studies were required to report diet composition (ingredients and inclusion levels), analyzed dietary Zn, and either Zn balance or apparent total tract digestibility (**ATTD**) coefficient for Zn. Ultimately, 51 publications were selected (Supplemental figure 1) that allowed for 376 datapoints for fecal Zn excretion and 149 datapoints for urinary Zn excretion (23 studies; Table 1). The information extracted from each study is presented in Supplemental Material 1.

Statistical analysis

Fecal vs urine Zn excretion

Data from the 23 studies that reported urinary and fecal Zn excretion were compiled to evaluate if urinary Zn excretion has a significant effect on total Zn excretion (urine + fecal Zn). To ensure model assumptions were met, data were natural logarithm transformed. A mixed model was used for the analysis utilizing the lmer function in the lme4 package in RStudio (version 4.4.1, R Core Team, R Foundation for Statistical Computing, Vienna, Austria), where the fixed effects included the Zn excreted in urine, feces, or total excretion. Zn intake was used

as a covariate, and the study code was treated as a random effect. The model was weighted based on the number of replications within each study.

Factors affecting fecal Zn excretion

A principal component analysis (**PCA**) was conducted to identify dietary (nutrient intake, use of dietary enzymes, antibiotics, acidifiers, and the type of supplemented Zn) and animal factors (body weight and feed intake) associated with Zn excretion (mg/d) in weanling to 135 kg pigs. The analysis was performed using the prcomp library from R (version 4.4.1, R core team, Vienna, Austria). Categorical variables (use of antibiotics and exogenous enzymes other than phytase) were converted to a binary scale. All variables were scaled and centered to ensure comparability and eliminate bias associated with the different units from each variable. Scaling involved standardizing each variable with a mean of 0 and a standard deviation of 1.

Eigenvalues were calculated to determine the proportion of total variance explained by each principal component (**PC**). Principal components with eigenvalues greater than 1 were retained, and the first two PCs were visualized to identify key relationships among variables. Loading scores were used to interpret the contributions of individual variables to each PC, with higher absolute values indicating stronger associations.

For graphical representation, a biplot was generated to display the relationships between individual variables and PCs. Variables strongly associated with Zn excretion were highlighted, and their contributions were overlaid using a color gradient. Interpretations focused on identifying clusters of variables with similar loading directions and magnitudes that indicate potential associations between the evaluated variables with Zn excretion.

The PCA results guided further analysis, with variables selected based on their contribution to the PCA. Papers focusing on specific variables were used to develop models to

provide additional context and validate the observed associations in the PCA. Analyses were performed using the lmer function from RStudio. In all cases, the study was included as a random effect, Zn intake as a covariate, and the models were weighted based on the number of replicates within each study.

The effect of added phytase on fecal Zn excretion was evaluated utilizing 8 studies that were designed for this purpose. Within each study, only the negative control (low P diet) and the negative control + phytase were considered for the analysis. To ensure that model assumptions were met, data were log (x) transformed. The model included added phytase as a binary variable (Yes/No), Zn requirement status (below or above the NRC (2012) requirement estimate), and their interaction as fixed effects.

To evaluate the effect of Zn source on fecal Zn excretion, Zn was categorized as chelated when more than 50% of the added Zn came from an chelated source, and inorganic otherwise. To ensure that model assumptions were met, data were log (x) transformed.

The relationship between Zn intake and Zn excretion was established by fitting a regression line between both variables using the complete database (376 data points). The analysis was performed in R using the lm function from the stats library.

General prediction model

The variables with the greatest contribution to PC1 and PC2, as determined by the PCA, were selected for prediction model analysis. To reduce the multicollinearity among predictors, a correlation analysis was performed using the cor function in RStudio (version 4.4.1, R Core Team, R Foundation for Statistical Computing, Vienna, Austria). When two variables exhibited a correlation coefficient greater than 0.70, one variable was excluded from the analysis based on biological relevance and model performance (Akoglu, 2018).

The database, consisting of 376 data points, was subdivided based on daily Zn intake relative to the NRC (2012) requirement estimate. The first subset (n = 120) included data points where the difference between daily Zn intake and the NRC (2012) requirement estimate was less than 3 mg/d, where in 94% of the cases, Zn intake was below the NRC (2012) recommendations. The second subset (n = 256) comprised data points where Zn intake exceeded the requirement by more than 3 mg/d. This classification allowed for a more detailed evaluation of Zn excretion patterns in pigs fed under/at or over their Zn requirement.

The Kennard-Stone (**KS**) algorithm (Kennard and Stone, 1969) was used to divide each database into calibration (80%) and validation (20%) subsets. The KS model ensures uniform coverage of the data space by selecting samples iteratively based on their Euclidian distances in a multivariate space, thereby maximizing the diversity within the calibration data set.

A backward stepwise selection process was used to identify the most appropriate model for predicting Zn excretion using the calibration database from each scenario (under or at versus over the Zn requirement). The model development began with inclusion of linear terms, followed by additions of linear and quadratic terms, and was subsequently expanded to include linear, quadratic, and all possible interaction terms. Due to the low number of observations included in the calibration database when pigs were fed at or below the Zn requirement (n = 96), the model including all possible interactions was not evaluated, because the number of potential predictors (n = 106) exceeded the number of observations. Mixed-effects models were fit using the lmer function in the lme4 package in RStudio. Study was considered the experimental unit, and individual observations within studies were treated as observational units. Predictor variables were included as fixed effects, whereas maintaining the same random effect, covariate, and weighting structure as described for the phytase and Zn source analysis. The best-fitting model

was selected based on Akaike Information Criteria (**AIC**), which provided a balance between model complexity and goodness of fit (Agiakloglou and Tsimpanos, 2023).The model with the lowest AIC value was chosen as the best statistical representation of the data.

In addition to the model resulting from the stepwise selection, a model was fit with Zn intake as a single predictor. Validation of the models was conducted using the designated database. The evaluation of the model performance was based on the R^2, which estimates model precision by comparing predicted and observed Zn excretion values. Additionally, the standard error of prediction (**SEP**) and bias (average differences between predicted and observed values) were used to assess model accuracy. An analysis of variance was performed using the lm function from the stats package in RStudio (version 4.4.1, R Core Team, R Foundation for Statistical Computing, Vienna, Austria) to compare the observed value with the predictions obtained from the stepwise model and Zn intake as a unique predictor. Differences between predictions were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Urinary Zn excretion

The amount of Zn excreted in urine accounted for only 2% of the Zn excreted in feces ($P < 0.001$). However, no differences were observed between Zn excreted in feces or total Zn excretion (urine + feces), indicating that the urinary Zn excretion was negligible, and that fecal Zn excretion could be utilized as a reliable indicator of total Zn excretion (Table 2). Regression analysis between Zn intake and Zn excretion in feces (Figure 1a) and total Zn excretion (Figure 1b) also indicated that urinary Zn excretion represented approximately 2% of the total Zn excretion. Therefore, subsequent analysis will focus on fecal Zn excretion as a representative measure of total Zn excretion.

Factors influencing fecal Zn excretion

Principal component analysis is a statistical technique that reduces the dimensionality of large datasets by transforming correlated variables into a smaller set of uncorrelated principal components that capture most of the variation of the database (Ringnér, 2008). This approach enhances data interpretation, facilitates the selection of the most relevant variables, and helps to identify interactions between variables. Figure 2 presents the PCA evaluating the relationships among variables associated with Zn excretion in pigs. The first two principal components accounted for 35.9% and 19.3% of the total variance, respectively, explaining 55% of the variability in the database. The contribution to Zn excretion is highlighted in a pink-to-orange color scale with the orange shade representing a greater contribution to the observed variance. The PC-1 (horizontal axis) was primarily influenced by variables related to nutrient intake, including neutral detergent fiber (**NDF**), NE, nitrogen (**N**), and minerals such as P, Ca, and Fe. Additionally, body weight (**BW**) and average daily feed intake (**ADFI**) were significant contributors to PC-1, likely indicating the interdependence between BW, ADFI, and nutrient supply. The PC-2 (vertical axis) was dominated by Zn-related variables, including Zn intake, fecal Zn excretion, the difference between Zn intake and estimated Zn requirements from NRC (2012), and dietary added Zn. The percentage of added Zn that was in an inorganic form also contributed positively to PC-2, indicating a moderate influence of the Zn form (chelated or inorganic) on fecal Zn excretion. Variables such as Mn intake, Cu intake, and lactose contributed moderately to both components, whereas the use of antibiotics, exogenous enzymes other than phytase, acidifiers, and days on feed had a low impact on the variation explained by both components. Strong collinearity was observed among closely clustered variables. In PC-1, these included NDF intake, NE intake, P intake, Ca intake, BW of the pigs, and ADFI while in PC-2,

collinearity was evident among Zn intake, fecal Zn excretion, and the difference between Zn intake and estimated Zn requirements from the NRC (2012). This pattern suggests interdependence between the variables within each PC.

Mineral interactions

The influence of other minerals on Zn excretion was anticipated, as it is well established that minerals can interact in the gastrointestinal tract (Sampath et al., 2023). To maintain homeostasis, multiple physiological mechanisms regulate mineral absorption and/or excretion (Maares and Haase, 2020). Minerals that share regulatory pathways often have antagonistic interactions (Berdanier et al., 2015), reducing the absorption of one or more minerals. This reduction may lead to increased excretion not only of the affected mineral but also of others that share the same mechanisms of absorption and regulation (Broom et al., 2021).

Interactions between Zn and divalent minerals (Cu^{+2}, Fe^{+2}, Cd^{+2}, and Ca^{+2}), as well as other dietary components such as phytase, P, fiber, and N compounds, have been properly documented (Lönnerdal, 2000).

Zinc and Cu use different transporters in the small intestine (CRT1 for Cu and ZIP4 for Zn; Wang et al., 2011; Maares and Haase, 2020); however, after they are absorbed, intracellular transporters located on the basolateral membrane (metallothionein) are used for both minerals (Oestreicher and Cousins, 1985). Despite the effect of Zn on Cu absorption is the most evaluated interaction (Dalto et al., 2023), there is evidence that the antagonism can occur in the opposite direction; however, wide dietary imbalances between both minerals are needed to observe a significant impact of Cu on Zn excretion (Cousins, 1985). Although metallothionines have higher affinity to Cu than Zn, the gene expression regulating metallothionein synthesis within the enterocyte is more responsive to Zn than Cu levels (Oestreicher and Cousins, 1985). Thus, when

dietary Cu is elevated while Zn remains at requirement levels, Cu binding to the available metalloproteins may not significantly impair Zn absorption from the intestinal lumen. However, in an in vitro model using brush border membrane vesicles, Bertolo et al. (2001) observed that high dietary Cu levels (exceeding those typically consumed by human infants) reduced Zn uptake at the brush border membrane. In a study with nursery pigs, Veum et al. (2004) determined the effect of Cu source and level on Zn excretion. They observed that when pigs were fed a corn-soybean meal-based diet containing 165 mg/kg of added Zn (ZnSO4), addition of 250 mg/kg of Cu ($CuSO_4$) increased daily Zn excretion compared with a diet containing 16.5 mg/kg of Cu; however, no differences in daily fecal Zn excretion were observed when the basal diet (16.5 mg/kg of Cu) was supplemented with 50 or 100 mg/kg of Cu from copper-propionate. Similarly, in a study by Apgar and Kornegay (1996), the addition of 200 mg/kg of Cu as $CuSO_4$ or Cu-Lys, in a diet containing 300 mg/kg of Zn and a basal Cu level of 36 mg/kg, did not affect daily fecal or urine Zn excretion. These observations indicate that a substantial imbalance is required to initiate a measurable effect of Cu on Zn excretion, and results of the current PCA that indicated that Cu intake has a moderate impact on Zn excretion support these observations.

The interaction between Fe and Zn in the intestine is primarily antagonistic, as both minerals utilize membrane transporters such as ZIP type (Cousins et al., 2006) and divalent metal iron transporter-1 (DMT1; Iyengar et al., 2012). The exact physiology underlying this interaction has not been fully clarified (Kondaiah et al., 2019), and the interactions between these minerals may not occur at the intestinal level (Kordas and Stoltzfus, 2004). Increasing added Fe from 0 to 150 mg/kg in nursery pigs fed a basal corn-soybean meal-based diet containing 224 mg/kg Fe and 1,947 mg/kg of Zn (analyzed values) did not change Zn retention (% of Zn intake), indicating no differences in Zn excretion (Rincker et al., 2005). However, Middleton et al.

(2021) fed growing pigs diets containing 300 mg/kg of Zn and either 500 or 3,000 mg/kg of Fe from $FeSO_4$. At the end of the experiment (d 60), Zn concentration in muscle was lower in pigs that received 3,000 mg/kg of Fe, indicating a possible reduction in Zn retention due to the high dietary Fe levels. The discrepancy in the results between these studies may be related to the Fe:Zn ratio. As demonstrated in humans, a wide Fe:Zn ratio (25:1) is required to negatively impact intestinal Zn absorption (Lönnerdal, 2000). In the U.S industry, the added Fe levels range from 113.1 to 61.3 mg/kg from weaning to 135 kg (Faccin et al., 2023), indicating that significant effects on Zn excretion are unlikely. The high contribution of the Fe intake to PC-1 may be a result of 24 observations from 3 experiments (Brugger et al., 2014; Veum et al., 2014; Gowalock et al., 2016) where the Fe:Zn ratio was greater than 14, primarily due to low Zn diets (below requirements) combined with adequate or high Fe levels.

In swine diets, Ca and P are formulated on a ratio basis (NRC, 2012). This interdependence explains the strong correlation in direction and magnitude of vectors observed between Ca and P intake in PC-1. Calcium and P absorption in pigs occurs primarily in the small intestine, through both active transport (vitamin D mediated) and passive diffusion (Fuchs and Peterlik, 1979; Bronner, 2003). Because Ca, P, and Zn do not share common absorption pathways, it is logical to assume minimal direct interaction between these minerals during the absorption process (Spencer et al., 1965). However, in pigs, increasing the total amount of Ca and P intake by 54% while maintaining a constant Ca:P ratio (1.2:1) in a corn-soybean meal diet, resulted in a 30% reduction in the ATTD of Zn (Jolliff and Mahan, 2013). This observation indicates that dietary Ca and P can interact with other dietary components, ultimately reducing Zn absorption and leading to increased Zn excretion.

Phytic acid and phytase

Phytic acid ($C_6H_{18}O_{24}P_6$) is one of the most extensively studied antinutritional factors in non-ruminants (Selle and Ravindran, 2007), as it serves as the primary storage form of P in cereal grains (Eeckhout and De Paepe, 1994). Due to its electronic configuration, phytic acid has a strong capacity to chelate other dietary components, particularly positively charged minerals such as Ca, Zn, Cu, and Fe, rendering them unavailable for absorption (Selle et al., 2009; Madrid et al., 2013). The use of exogenous phytase to release the phytic-P and mitigate the negative effects of the phytic acid is a common practice in the swine industry (Walk et al., 2016). Interactions between Ca, phytic acid, and Zn have been reported (Fordyce et al., 1987). High dietary Ca induces the formation of insoluble complexes between the 3 molecules (Lönnerdal, 2000), reducing phytase activity, and ultimately limiting the release of Zn bound to phytic acid, resulting in increased mineral excretion (Klein et al., 2023). Although the physiological mechanism underlying the interactions between Ca, phytic acid, phytase, and Zn is well understood, the response in pigs is not always consistent. Walk et al. (2015) evaluated added Zn, phytase, and the Ca:P ratio on mineral digestibility in pigs. Results indicated that the ATTD of Zn was not affected by STTD P level or the phytase inclusion. However, Adeola et al. (1995) observed that the use of 1,500 FTU/kg of phytase improved Zn absorption, which implies a reduction in fecal Zn excretion. The lack of consistency in the results when comparing studies could be related to differences in dietary composition, Ca:P ratios, Zn status of the pigs, or differences associated with the phytase source.

Despite scientific evidence suggesting that phytase may reduce the Zn excretion, the PCA indicated that this variable has a minimal impact in PC1 or PC2. The lack of response may be attributed to the limited number of observations in the database that include phytase. Eight studies in the database were designed to evaluate the effect of the phytase on Zn excretion (Table

1). Results comparing Zn excretion with and without phytase addition, using only these eight studies, are presented in Table 3. Two data points from Martínez et al. (2005) were removed from the analysis, as residual analysis identified them as outliers, likely because these points were the only ones that used pharmacological levels of Zn (4,000 mg/kg). No significant effect ($P = 0.571$) of added phytase was observed for fecal Zn excretion, which supports the low effect of added phytase on Zn excretion observed in the PCA.

The effect of phytase on Zn excretion seems to be dependent on the dietary Zn level. Meta-analysis has shown that phytase improves ATTD of Zn only when added Zn levels are at or below the requirement (Bikker et al., 2012; Philippi et al., 2023; Ketata et al., 2024a). These findings suggest that when Zn requirements are met through exogenous sources, the additional Zn released by phytase has limited potential to further contribute to Zn status. In our database, the low number of studies feeding Zn levels below the requirement may contribute to the low importance of phytase in the PCA. Additionally, the variability in fecal Zn excretion at high Zn intake suggests that other factors beyond added phytase influence Zn excretion.

Fiber

In pigs, the effect of fiber on Zn utilization seems to be dependent on the fiber characteristics (Metzler-Zebeli et al., 2010; Barszcz et al., 2019; Saliu et al., 2024) and the presence of chelating agents such as phytic acid (Lönnerdal, 2000). High fiber levels in swine diets are commonly associated with the inclusion of industrial by-products (Lindberg, 2014) and anti-nutritional factors, such as phytic acid (Humer et al., 2015). Although the physiological mechanism underlying the effect of fiber on Zn utilization in pigs is not completely clear, reduction in the retention time within the small intestine, the major site of Zn absorption, and

changes in digesta viscosity are thought to be the key factors contributing to the negative impact on Zn utilization (Ravindran et al., 1984).

Protein

Zinc is typically associated with the protein fraction of feed ingredients due to its capacity to form complexes with nucleic acids which play a key role in cellular structure. The Zn concentration in feed ingredients varies widely (NRC, 2012) due to the differences intrinsic to the production process including origin (animal or plant protein source), processing conditions, and agronomic managements (Cakmak and Kutman, 2018). Animal protein sources exhibit greater Zn variability than plant protein sources. For example, according to the NRC (2012), Zn concentration in common protein sources used in nursery diets, such as blood plasma, blood meal, fish meal, meat meal, and whey powder range from 13 to 94 mg/kg. In contrast, Zn concentrations in plant protein sources including soybean meal, canola meal, wheat, and corn distiller dried grains with solubles range from 47 to 55 mg/kg. The main cereals used in swine diets in North America have lower Zn concentrations, with corn at 16.5 mg/kg or sorghum at 15 mg/kg. These observations suggest that an increase in protein levels in swine diets could be related to an increase in Zn concentration, which aligns with the observed high contribution of N intake to the PC-1.

Feed intake and body weight

Feed intake increases as body weight increases due to greater nutrient demand (NRC, 2012). This physiological response explains the observed collinearity between ADFI, NE intake, and weight of the pigs in the PC-1. Previous studies have described the relationship between feed intake and Zn excretion (Walz and Pallauf, 2002; Martínez et al., 2005; Metzler-Zebeli et al., 2010; Oh et al., 2022), demonstrating that, at a constant Zn concentration, increased feed intake

associated with greater BW leads to greater Zn excretion in feces. The current substantial contribution of ADFI and BW to PC-1 also supports previous observations.

Zinc sources

Zinc sources in swine diets can be classified as inorganic and chelated forms, differing in their chemical structure, bioavailability, and excretion rates. Inorganic Zn sources, such as ZnO and ZnSO4, are the most used due to their cost-effectiveness and widespread availability (Nielsen et al., 2022). However, high soluble forms of some inorganic Zn sources rapidly release reactive free ions in the intestinal lumen (Villagómez-Estrada et al., 2020). These ions can interact with other dietary compounds, reducing their availability for absorption (Thomaz et al., 2015). In contrast, chelated Zn sources consist of Zn ions chelated or complexed with organic molecules, including amino acids, peptides, or polysaccharides (Luise et al., 2024). Complexing minerals with an organic molecule may increase their solubility in aqueous and lipid media and reduce interactions with other dietary components (Byrne and Murphy, 2022), enhancing digestibility compared to inorganic Zn sources (Choi et al., 2025).

From our database, 16 publications evaluated the effect of various Zn sources on fecal Zn excretion, resulting in 132 data points. The sources included ZnSO4, ZnO, and Zn chelated with amino acids, protein, polysaccharides, or their combinations. Analysis from these studies indicated that no differences ($P = 0.417$) were observed between chelated and inorganic sources on fecal Zn excretion (Table 4). These results agree with the conclusions of the meta-analysis conducted by Hansen et al. (2023), who did not observe a significant effect of Zn source on fecal Zn excretion in weanling pigs fed diets with added Zn levels ranging from 0 to 4,000 mg/kg. However, from a meta-regression analysis conducted by Ketata et al. (2024a) it was concluded

that pigs fed diets containing Zn levels below 165mg/kg Zn ATTD improved (suggesting reduction in fecal Zn excretion) with the utilization of chelated Zn sources.

Antibiotics

The physiological interactions between the use of antibiotics and mineral digestibility are not fully understood; however, they appear to be associated with changes in microbial activity (Skrypnik and Suliburska, 2018) and reductions in passage rate which may extend digestion and absorption times, potentially enhancing mineral absorption (Ravindran et al., 1984). Specifically for Zn, Wang et al. (2005) supplemented a nursery corn-soybean basal diet with 100 mg/kg of either cyadox or olaquindox and reported an improvement in the ATTD of Zn with cyadox whereas no effect was observed with olaquindox. These observations indicate that the effect of antibiotics on Zn utilization is dependent on the active ingredient, as was previously reported for P digestibility (Lindemann et al., 2010). Despite the positive effect of antibiotics on the ATTD of Zn reported in previous studies, the limited number of studies evaluating the effect of antibiotics on Zn excretion in pigs may explain the low contribution of the antibiotic intake to the PCA.

Lactose

In the database, only 39% of the data points included diets with lactose, among these, 77% contained Zn levels that exceeded the physiological requirements. This indicates that the effect of lactose intake on PC-1 and PC-2 may have been associated with high Zn levels. Based on our knowledge, no in vivo studies have evaluated the impact of lactose on Zn utilization in pigs. However, human studies summarized by Shkembi and Huppertz (2021) suggest that consumption of dairy products, which are rich in lactose, can enhance intestinal Zn absorption by increasing its solubility and bioavailability in the gastrointestinal tract. Similarly, In vitro studies using brush border membrane vesicles from pigs indicate that lactose increased Zn absorption by

240 to 255% relative to the control treatment (Bertolo et al., 2001). However, further research is needed to validate this effect and elucidate the underlying mechanism.

Acidifiers

Although the use of acidifiers did not present a significant contribution to PC-1 or PC-2, individual studies have reported their positive effect on reducing Zn excretion in pigs. The lack of detectable responses in the PCA could be attributed to the limited number of studies (n = 3) in our database focused on the evaluation of the impact of dietary acidification on Zn excretion. Dietary acidification enhances mineral solubility in the small intestine by lowering digesta pH, increasing ionization, reducing mineral complexation with dietary antagonists, reducing stomach emptying rate, and optimizing the performance of the digestive enzymes (Jongbloed et al., 2000; Van Der Aar et al., 2017), thereby facilitating mineral release and absorption. However, the effect of dietary acidification on Zn excretion remains inconclusive. While some studies report a positive effect of the use of acidifiers on Zn absorption (Blank et al., 2012; Arroyave et al., 2024), others have shown no effect or increased Zn excretion (Blank et al., 2012; Kristoffersen et al., 2021). These inconsistencies may be attributed to differences in acidifiers, basal diet characteristics, phytase inclusion, dietary Zn levels, and Zn status of the pigs.

Zinc intake

Zinc uptake primarily occurs in the small intestine through a saturable process mediated by the ZIP-4 transporters. In pigs fed Zn below their requirement, ZIP-4 transporters are upregulated to enhance Zn absorption from the intestinal lumen. However, when dietary Zn exceeds the nutritional requirements, ZIP-4 transporters are downregulated, reducing Zn uptake from the small intestine (Wan and Zhang, 2022). Additionally, ZnT-5B transporters are upregulated at the brush border to facilitate Zn efflux from enterocytes into the intestinal lumen

(Maares and Haase, 2020). Given that Zn homeostasis is primarily regulated in the small intestine, it is logical to conclude that most of the excess dietary Zn will be excreted in feces. The regression between Zn intake and Zn excretion in feces indicated that 97% of the variation in Zn excretion is explained by Zn intake (Figure 3), which supports the high contribution of Zn intake to PC-2 and suggests that Zn intake is a good estimator for Zn excretion. Similarly, in agreement with the current results, a meta-regression analysis conducted by Hansen et al., (2023) concluded that there is a linear relationship between Zn intake and Zn excretion in nursery pigs.

Zinc feeding duration

Although not included in the PCA due to a lack of published data, independent studies suggest that Zn excretion follows a temporal pattern. Brugger et al., (2014) reported that after an adequate Zn intake, pigs fed a Zn-deficient diet required approximately 10 d for signs of Zn deficiency to manifest. This timeframe aligns with a theoretical model in which a 3% daily increase in BW results in 1.5% daily dilution of whole-body Zn, progressively depleting the Zn pool, which is estimated to represent 15% of total body Zn. We hypothesized that this model can be applied inversely and estimated that at least 10 d are required to increase the Zn pools in the body; during this period, Zn excretion would be expected to remain low despite high dietary Zn levels. Supporting this, Rincker et al. (2005) observed that weaned pigs fed diets with no added Zn or supplemented with 2,000 mg/kg of Zn from either ZnO or Zn methionine exhibited similar fecal Zn excretion during the first week post-weaning. However, from d 7 to 14 (phase 2), Zn excretion increased significantly in the supplemented groups. From their graphical reported data, it appears that fecal Zn excretion in the supplemented groups began to diverge from the control group around d 9, supporting the hypothesis that approximately 10 d are needed to fully replenish Zn pools. These observations indicate that feeding pharmacological levels of Zn during

the first week post-weaning may not substantially increase Zn excretion. However, given the limited available data, further research is warranted to better characterize the temporal dynamics of Zn excretion and inform the development of more sustainable Zn feeding strategies.

Modeling fecal Zn excretion

Based on the PCA, days on feed, use of exogenous enzymes other than phytase, and use of antibiotics were not included in the stepwise selection due to the low contribution to PC-1 and PC-2. Additionally, added Zn and the differences between Zn intake and the NRC (2012) requirement were also not included because they showed a correlation greater than 0.87 with Zn intake, which was retained in the model. Additionally, ADFI was not included as it exhibited a correlation greater than 0.70 with NE, Ca, P, NDF intake, and BW.

Models for pigs fed with diets containing Zn levels at or below their Zn requirements

Similar R^2, SEP, and bias (0.97, 3.47, and -1.38, respectively) were observed across all models. The AIC favored the linear model (AIC = 605) over the L + Q model (AIC = 609), indicating that the addition of quadratic terms did not substantially improve model fit, and the linear terms brought the best statistical fit to the calibration database.

The three terms included in the linear stepwise regression model were Zn, P, and Fe intake (Table 5). Among these variables, Zn intake was the determining factor of Zn excretion, with a significant and positive coefficient ($P < 0.001$ and $\beta = 0.6539 \pm 0.0277$). Phosphorus intake was positively associated with Zn excretion ($P = 0.007$, $\beta = 1.210111 \pm 0.438334$), whereas Fe intake had a negative effect ($P < 0.001$, $\beta = -0.047559 \pm 0.009339$). The regression analysis between predicted and observed values using the calibration and validation databases is presented in Figure 4. A strong relationship was observed, with data points closely aligning with

the 45° line of perfect agreement, indicating that the model selected through the stepwise selection accurately predicted fecal Zn excretion.

Given that Zn intake is the primary predictor of Zn excretion, the model including only Zn intake as a unique predictor was included in Table 6. This model had a significant and positive slope ($P < 0.001$, $\beta = 0.6139 \pm 0.0287$) and performed similarly to the model with the lowest AIC. The mean predicted values from both models (18.3 mg/d) were comparable to the observed mean (19.7 mg/d). No significant differences ($P = 0.949$) between the predicted and observed values were observed, indicating that Zn intake could be accurately utilized to predict fecal Zn excretion in pigs fed diets at or below their Zn requirement.

Models for pigs fed with diets containing Zn levels over their Zn requirement estimates

The model including only linear terms had the lowest AIC, SEP, and BIAS (2,138, 37.73, and -6.02, respectively) and the highest R^2 (0.96), indicating strong predictive ability while maintaining model parsimony. The addition of quadratic terms resulted in slightly higher AIC (2,156) without improving model fit, suggesting limited benefit in including nonlinear effects. The most complex model incorporating linear, quadratic and interactive terms had the highest AIC (2,379) and the lowest R^2 (0.95), indicating overfitting. The model that only considered Zn intake as a unique prediction variable had a nearly identical R^2 and SEP to the best stepwise model highlighting the strong influence of Zn intake on fecal Zn excretion.

The variables selected in the linear stepwise model were Zn intake and the proportion of added Zn from inorganic sources as significant predictors of fecal Zn excretion (Table 6). This model indicated that for each mg that the Zn intake increases, fecal Zn excretion increased by 0.823 mg ($P < 0.001$). The proportion of added Zn coming from inorganic sources (as opposed to

chelated sources) also had a significant but relatively small effect on the model ($P = 0.0372$). This supports the results found in the PCA analysis, where there was no significant difference in the fecal Zn excretion between chelated and inorganic sources. As an example, at the same dietary Zn concentration, changing the diet from 100% of the Zn from an inorganic source to 100% from an chelated source is predicted to decrease Zn excretion by 19 mg/d. The model that only used Zn intake as a predictor performed similarly to the model selected through the stepwise selection, with an estimated zinc excretion coefficient of 0.8246 ($P < 0.001$).

The stepwise model was validated using 51 independent samples from the calibration database. Results indicated a strong relation between predicted and observed values, with data points closely following the 45° lines (Figure 5). Furthermore, the performance of the models was also evaluated by comparing the observed with the predicted values utilizing the validation dataset. The observed mean for fecal Zn excretion was 156 mg/d, while both the stepwise and Zn intake models predicted an average of 150 mg/d. No significant differences ($P = 0.982$) were observed between observed and predicted values, which indicated that both models adequately predict Zn excretion in pigs.

Model comparison

In pigs fed diets at or below their Zn requirements, variables other than Zn intake, especially P and Fe intake, were identified as significant factors influencing fecal Zn excretion. In contrast, when pigs were fed diets exceeding their Zn requirement, only the percentage of added Zn that is in an inorganic form was a significant predictor of Zn excretion in addition to Zn intake. The differences between variables in the two scenarios may be attributed to the physiological regulation of Zn absorption. Under conditions of low Zn intake, the homeostasis mechanism enhances Zn absorption through increased expression of Zn transporters in the brush

border membrane (Maares and Haase, 2020), and the interaction between Zn and other minerals may play a more prominent role in modulating Zn absorption. As previously indicated, multiple interactions between P, Ca, and phytase could result in the formation of Zn-phytate complexes that are not available for absorption, thus increasing Zn excretion (Selle et al., 2009; Madrid et al., 2013). Conversely, the antagonism between Fe and Zn during intestinal absorption could explain the impact of Fe on fecal Zn excretion (Cousins, et al., 2006). When Zn intake exceeds the physiological requirements, mechanisms involved in the intestinal Zn absorption are downregulated. Consequently, Zn form becomes more important likely because Zn sources chelated with organic molecules such as amino acids or proteins utilize alternative absorption routes (Byrne and Murphy, 2022) leading to greater absorption of Zn.

Although the models suggest that additional variables may influence fecal Zn excretion, in both scenarios (under or over the physiological Zn requirement), models based solely on Zn intake provide a more straightforward and practical approach for estimating Zn excretion while maintaining accuracy, making them useful for field applications. As previously indicated, a meta-regression analysis conducted by Hansen et al. (2023) utilized Zn intake to predict fecal Zn excretion in nursery pigs feeding diets containing added Zn levels ranging from 0 to 3,000 mg/kg, reporting a slope of 0.85. This value is slightly higher but comparable with the slope of 0.82 observed in our meta-regression analysis for pigs fed diets exceeding their Zn requirement. The differences between values could be explained by the broader scope of our study, which was not limited to a single productive stage but included data from nursery, growing, and finishing pigs. Combining results, when pigs are fed diets containing Zn levels that exceed their physiological requirements, 82 to 85% of the Zn is excreted in feces. However, for pigs fed Zn diets at or below their Zn requirement, approximately 61% of the Zn intake is excreted in feces.

The estimated slope of 0.61 is comparable with the average ATTD of Zn of 39% (61% of the Zn intake excreted in feces) reported in a literature review by Stein (2024) for a corn-soybean meal diet supplemented with approximately 100 mg/kg of Zn from ZnSO4.

As previously demonstrated, Zn excretion in pigs is a complex process dependent on factors related to nutrient intake, Zn feeding duration, and Zn status of the pigs. Although prediction models identify Zn intake as the primary factor affecting Zn excretion, evidence from independent studies suggests that Zn feeding duration and metabolic adaptations to dietary Zn levels also play a critical role. However, further research is needed to clarify the interactions between Zn feeding duration, dietary Zn concentration, and Zn excretion. Additionally, improving our understanding of the bioavailable Zn content in feed ingredients could allow for more precise diet formulation, reducing overall Zn inclusion while maintaining the performance and minimizing Zn excretion.

Conclusion

This paper evaluated factors influencing Zn excretion in pigs and developed predictive models to estimate fecal Zn excretion in wean-to-finish pigs. Although fecal Zn excretion is affected by mineral interactions, nutrient intake (N, Lactose, and fiber), other dietary factors such as exogenous enzymes other than phytase, acidifiers, and the Zn feeding duration, Zn intake was the main contributor to fecal Zn excretion. When pigs are fed at or below their Zn requirement, 61% of the Zn intake is excreted in feces. However, when Zn intake exceeds the requirement, fecal Zn excretion increases to 82% of the Zn intake. While managing Zn levels in the diet is critical to reducing Zn excretion, future research should focus on accurately characterizing the Zn present in feed ingredients (amount and digestibility) and metabolic adaptations to dietary Zn levels. By improving our understanding of the bioavailable Zn content in the ingredients and the

interaction between Zn feeding duration and Zn excretion, diets can be formulated more precisely, ultimately optimizing overall Zn inclusion while maintaining pig performance

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Table 1.1 Summary of average body weight, Zn intake, fecal Zn excretion, and urine Zn excretion[1]

Study	Observations, n	Average BW, kg	Range of Zn intake, mg/d	Range of fecal Zn excretion, mg/d	Range of urine Zn excretion, mg/d
Näsi, 1990[2]	3	98.0	187.0 to 190.0	155.0 to 184.0	10.6 to 13.3
Adeola et al., 1995[2]	4	16.8	23.0 to 118.0	12.0 to 83.0	--
Poulsen and Larsen, 1995	10	43.5	44.4 to 317.0	35.9 to 273.0	0.6 to 1.0
Apgar and Kornegay, 1996	3	76.5	615.9 to 647.5	495.7 to 525.8	0.3 to 2.6
Wu et al., 2001	8	25.6	226.0 to 2542.7	195.0 to 2108.6	3.0 to 44.0
Dourmad et al., 2002	7	14.0	22.8 to 43.6	16.7 to 30.1	0.4 to 0.9
Case and Carlson, 2002	5	8.1	127.0 to 2052.0	137.0 to 2157.0	0.5 to 2.7
Walz and Pallauf, 2002[2]	12	53.3	90.0 to 137.0	89.0 to 128.6	--
Meyer et al., 2002	6	10.5	43.0 to 2081.0	28.0 to 1704.0	--
Brady et al., 2003[2]	6	50.0	227.2 to 330.4	170.2 to 246.1	--
Walz and Pallauf, 2003	3	92.2	161.0 to 178.0	126.1 to 148.3	--
Veum et al., 2004	4	24.5	269.4 to 290.4	195.0 to 239.2	3.0 to 4.1
Carlson et al., 2004	16	14.2	83.3 to 2542.0	74.4 to 2109.0	3.5 to 44.0
Wang et al., 2005	3	20.9	158.3 to 168.1	103.2 to 132.8	--
Rincker et al., 2005	5	8.2	443.9 to 595.8	259.5 to 352.8	0.2 to 0.6
Buff et al., 2005	5	14.4	164.4 to 2269.1	144.8 to 1660.5	4.8 to 53.8
Martínez et al., 2005[2]	13	10.5	50.0 to 3822.0	27.0 to 2777.0	0.3 to 5.4
Zacharias et al., 2007	8	76.0	142.6 to 260.3	112.5 to 214.5	--
Veum et al., 2009	7	20.7	29.8 to 143.2	21.9 to 129.1	0.7 to 1.8
Burkett et al., 2009	32	60.6	54.5 to 392.2	42.0 to 351.0	--
Metzler-Zebeli et al., 2010	4	41.4	162.0 to 206.0	151.0 to 321.0	12.0 to 62.0
Deng et al., 2010	2	11.7	42.8 to 132.4	21.9 to 79.3	2.5 to 5.5
Paulicks et al., 2011	10	24.5	38.8 to 110.4	25.0 to 68.4	--
Blank et al., 2012	8	37.5	44.0 to 49.5	28.6 to 38.8	--
Carlson et al., 2012	5	43.3	7.3 to 57.5	9.4 to 50.8	0.3 to 0.6
Jolliff and Mahan, 2013	6	53.4	42.8 to 265.3	29.5 to 225.5	0.8 to 1.8
Madrid et al., 2013[2]	3	47.8	348.6 to 363.9	190.3 to 210.7	3.2 to 4.7
Veum et al., 2014	26	6.7	0.3 to 67.0	0.2 to 43.1	0.0 to 0.4
Lebel et al., 2014	10	20.6	17.4 to 362.2	9.8 to 160.6	0.5 to 2.3

Table 1.1 continued. Summary of average body weight, Zn intake, fecal Zn excretion, and urine Zn excretion[1]

Study	Observations, n	Average BW, kg	Range of Zn intake, mg/d	Range of fecal Zn excretion, mg/d	Range of urine Zn excretion, mg/d
Liu et al., 2014	6	40.2	13.6 to 87.5	7.7 to 45.4	2.7 to 6.2
Brugger et al., 2014	8	13.4	6.4 to 39.2	6.1 to 37.3	--
Walk et al., 2015	9	16.3	69.5 to 1974.0	80.8 to 1826.6	--
Zeng et al., 2016[2]	5	19.3	77.4 to 95.5	54.3 to 73.8	--
Gowalock et al., 2016	8	46.5	39 to 270.6	24.3 to 220.0	0.6 to 2.1
Milani et al., 2017	5	10.3	77.4 to 1625.3	27.1 to 1322	--
Barszcz et al., 2019	4	26.5	155.1 to 218.7	99.6 to 158.1	--
Arredondo et al., 2019[2]	7	13.6	133.7 to 230.2	63.8 to 97.5	6.0 to 7.8
Zhang et al., 2020	5	19.0	28.0 to 2087.0	44.0 to 1788.0	--
Kristoffersen et al., 2021	2	31.9	201.4 to 238.0	167.1 to 197.5	--
H.J. Oh et al., 2021	12	18.5	52.1 to 1495.6	47.6 to 1290.7	--
H. Oh et al., 2021	8	6.9	23.3 to 663.6	21.3 to 612.1	--
H.J. Oh et al., 2022	12	8.4	34.0 to 427.5	29.2 to 381.1	--
Nielsen et al., 2022	8	10.0	43.0 to 393.0	45.9 to 358.8	--
H. Oh et al., 2022	12	12.5	28.2 to 1823.4	25.2 to 1577.2	--
De Mille et al., 2022	2	12.8	211.0 to 1804.0	135.0 to 1046.0	15.0 to 23.0
Arroyave et al., 2024	6	13.6	159.2 to 1147.3	125.9 to 878.1	--
Ribeiro et al., 2024b	4	14.2	140.4 to 171.2	96.1 to 110.3	--
Ribeiro et al., 2024a	4	12.2	129.4 to 143.5	71.6 to 81.0	--
Saliu et al., 2024	4	35.3	174.6 to 246.6	154.5 to 210.3	--
Ketata et al., 2024b	4	49.9	231.7 to 865.6	200.6 to 437.1	--
Choi et al., 2025	7	26.6	9.0 to 76.9	5.0 to 47.8	--

[1]The literature search identified 51 studies, yielding 376 data points. Observation represents the number of data points collected from an individual study. In studies using repeated measurements, data from each time point were considered as an independent observation. Zinc intake, as well as fecal and urinary Zn excretion, are summarized by representing the minimum and maximum values within each study.
[2]Studies that evaluated the effect of phytase on Zn excretion.

Table 1.2 Comparison of fecal, urinary, and total Zn excretion[1]

Item	Feces	Urine	Total	SEM	*P* =
Zn excretion, mg/d	60.28[a]	1.41[b]	62.87[a]	1.117	< 0.001

[1]The analysis was conducted using Zn balance data from 23 published studies, which resulted in 149 data points. The model included the study as a random effect and Zn intake as a covariate. Studies were weighted based on the number of replicates within each study.

[a,b] means with different superscripts indicates statistical differences at a confidence level of $P < 0.05$.

Table 1.3 Effect of phytase on fecal Zn excretion[1]

	Added phytase			
Item	Yes	No	SEM	P = [2]
Zn excretion, mg/d	103.2	107.5	10.54	0.571

[1]The analysis was conducted using Zn balance data from 8 published studies, which resulted in 40 data points. The model included added phytase as a dichotomic variable (Yes/No), Zn requirement status (below or above the NRC (2012) Zn requirement), and their interaction as fixed effects. Study was included as a random effect and Zn intake as a covariable. Studies were weighted based on the number of replicates within each study.
[2]No significant interaction (P = 0.491) was observed between phytase and Zn requirement status.

Table 1.4 Effect of mineral source on fecal Zn excretion[1]

	Mineral Source[2]			
	Chelated	Inorganic	SEM	*P* =
Zn excretion, mg/d	185	196	13.4	0.417

[1]The analysis was conducted using Zn balance data from 16 published studies, which resulted in 132 data points (69 points for inorganic sources and 63 for chelated sources). The model included Zn source (chelated or inorganic) as a fixed effect. Study was included as a random effect and Zn intake (mg/d) as a covariate. Studies were weighted based on the number of replicates within each study.
[2]If more than 50% of the added Zn came from an chelated source, the data point was considered in the chelated category, otherwise it was considered inorganic

Table 1.5 Models to predict fecal Zn excretion in pigs fed diets at or below the dietary Zn requirement estimate[1]

	Stepwise model[2]		
	Value	SE	*P* =
Intercept	7.587053	2.872634	0.011
Zn intake, mg/d	0.653867	0.027706	< 0.001
P intake, g/d	1.210111	0.438334	0.007
Fe intake, mg/d	-0.047559	0.009339	< 0.001
	Zn intake model [3]		
Intercept	8.0735	2.5117	0.002
Zn intake, mg/d	0.6139	0.0287	< 0.001

[1]The model was based on 96 data points from 27 publications, where the difference between Zn intake and the NRC (2012) requirement estimate was lower than 3 mg/d.

[2]The stepwise model corresponds to the model with the lower AIC in Table 4.

[3]The model only includes total Zn intake as a unique predictor.

Table 1.6 Models to predict fecal Zn excretion in pigs fed diets over the dietary Zn requirement[1]

	Stepwise model[2]		
	Value	SE	*P* =
Intercept	-26.388799	12.237416	0.0333
Inorganic Zn, %[3]	0.19383	0.092272	0.0372
Zn intake, mg/d	0.823291	0.006883	< 0.001
	Zn intake model[4]		
Intercept	-10.546543	9.600192	0.277
Zn intake, mg/d	0.824622	0.006936	< 0.001

[1]The model was based on 197 data points from 45 publications, where the difference between Zn intake and the NRC (2012) requirement estimate was greater than 3 mg/d.

[2]The stepwise model corresponds to the model with the lower AIC in Table 7.

[3]Percentage of the added Zn that is supplemented in an inorganic form.

[4]The model only includes total Zn intake as a unique predictor.

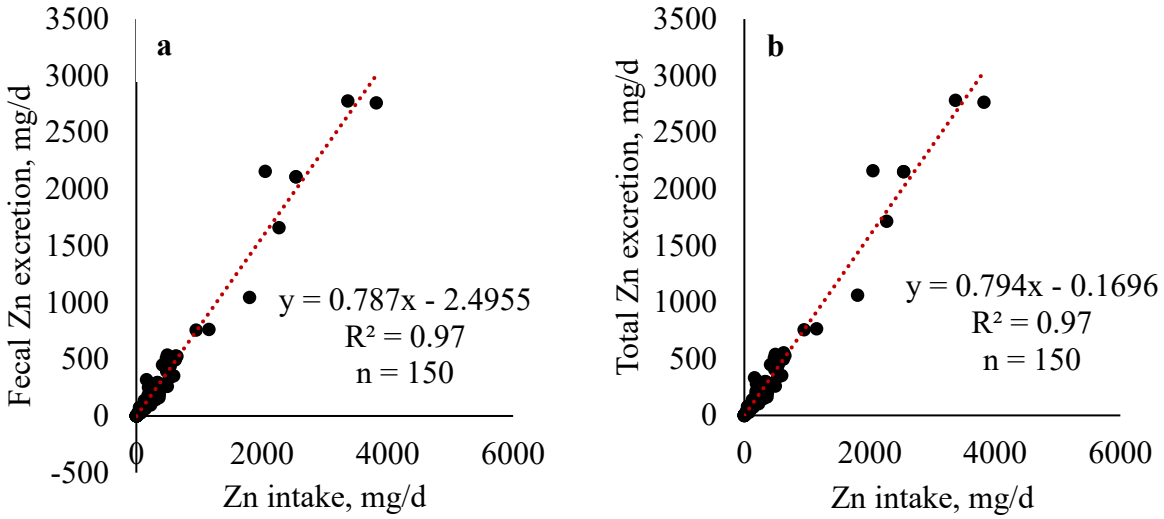


Figure 1.1 Relationship between Zn intake and fecal Zn excretion (a) and total fecal excretion (b). In both cases, data were derived from the same experiments, meaning that only studies reporting both fecal and urinary excretion were included in this analysis. Black dots represent individual observations, and the dotted red line indicates the regression line.

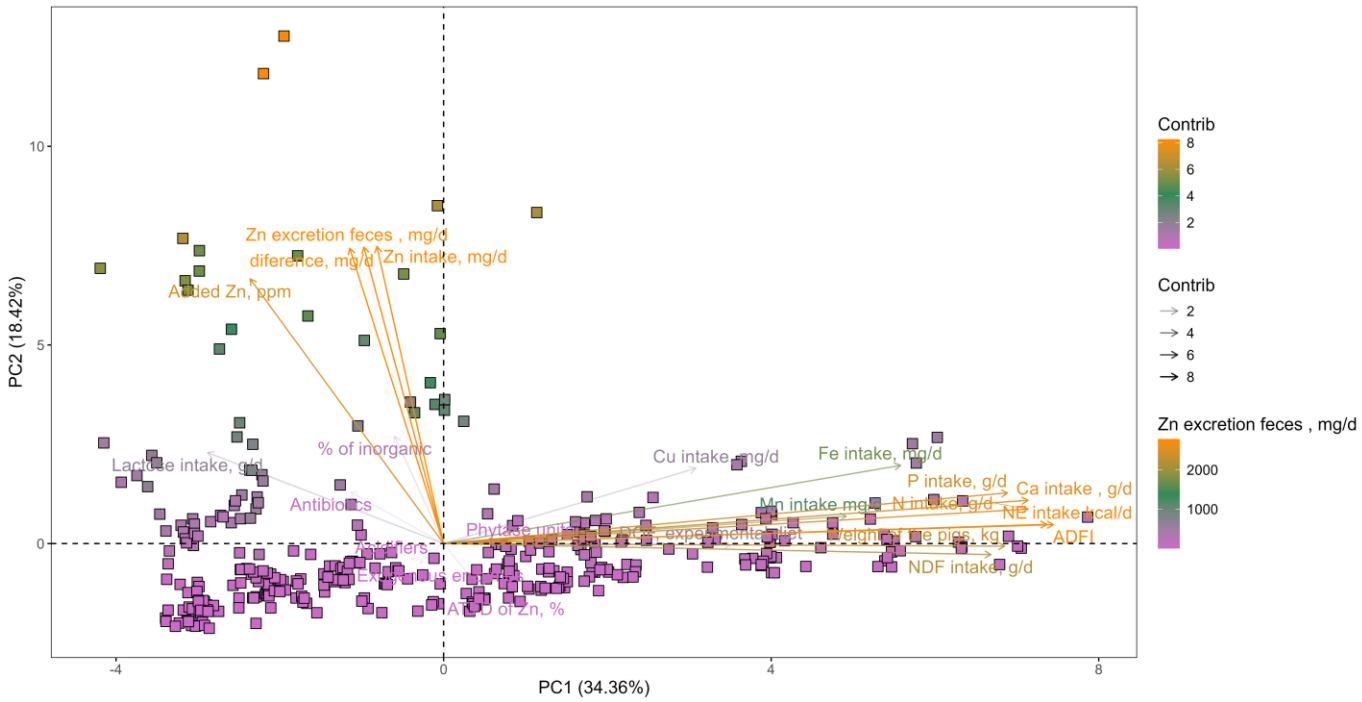


Figure 1.2 Principal component analysis of factors influencing Zn excretion in pigs. The first two principal components explained 55% of the total variance, with PC-1 (horizontal axis) mainly associated with nutrient intake (e.g., NDF, NE, N, P, Ca, Fe, BW, and ADFI) and PC-2 (vertical axis) driven largely by Zn-related variables (Zn intake, fecal Zn excretion, dietary Zn above NRC requirements, and form of added Zn). The color scale (pink to orange) indicates the relative contribution of each variable to the explained variance, with darker orange reflecting greater influence. The length of the vectors represents the strength of the variable contribution to the component, whereas the angle between vectors illustrates the degree of correlation, with smaller angles denoting stronger positive associations.

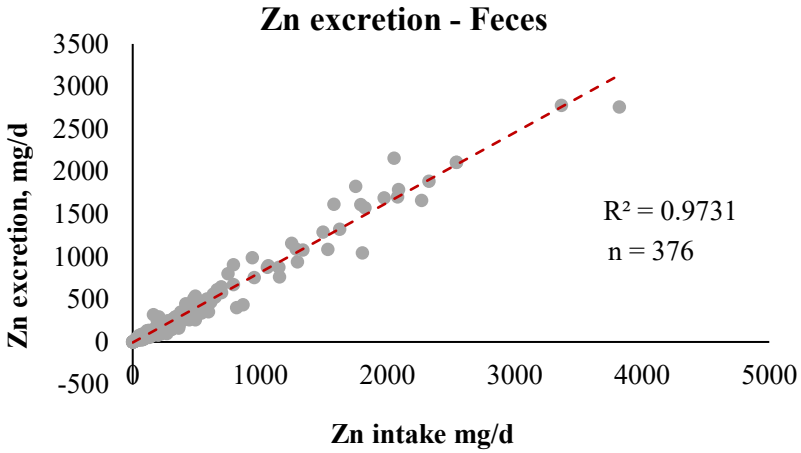


Figure 1.3 Regression line between Zn intake and fecal Zn excretion using the complete database (376 data points). The red dotted line indicates the regression line. Results indicate that 97% of the variation in fecal Zn excretion is explained by Zn intake.

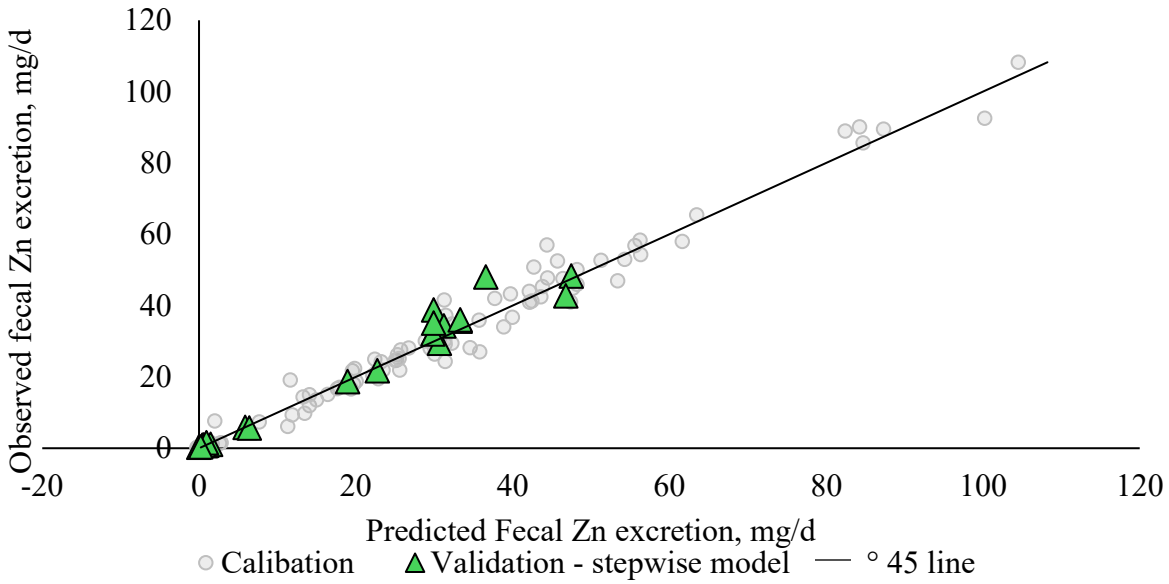


Figure 1.4 Regression analysis of the relationship between predicted and observed Zn excretion in pigs fed diets under their Zn requirement, based on the prediction model selected through the stepwise selection. Gray circles represent data points used for model calibration (n = 93), while green triangles denote data points used for model calibration (n = 24). The solid black line represents the 45° line (predicted = observed).

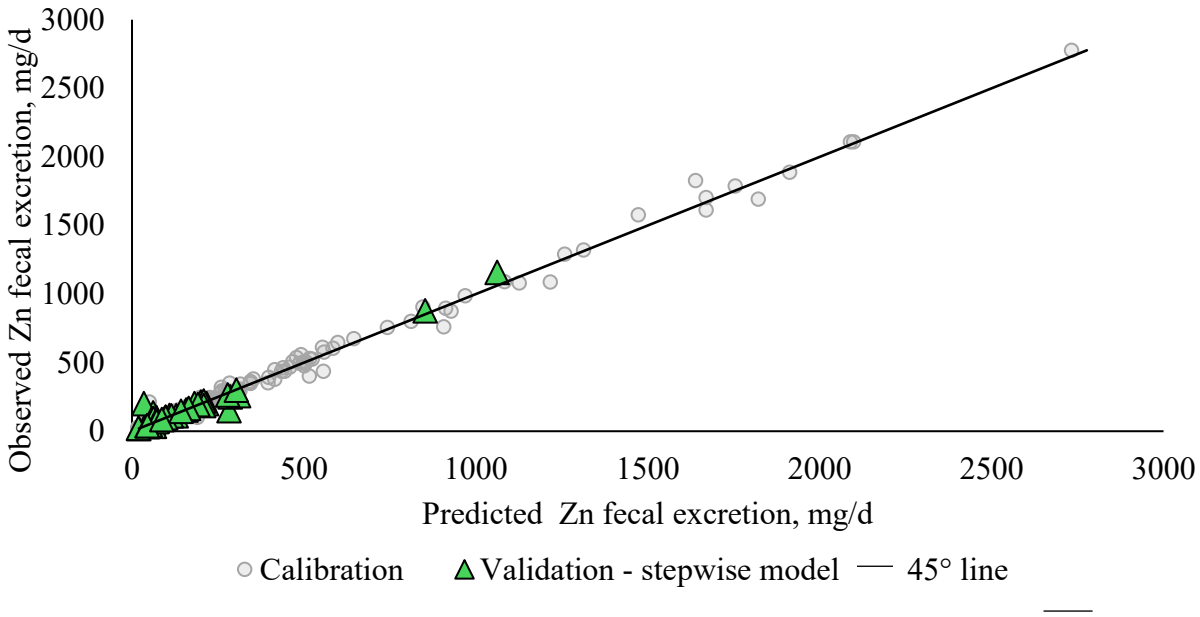


Figure 1.5 Regression analysis of the relationship between predicted and observed Zn excretion in pigs fed diets over the dietary Zn requirement estimate (NRC, 2012), based on the prediction model selected through the stepwise selection. Gray circles represent data points used for model calibration (n =197), while green triangles denote data points used for model calibration (n = 51). The solid black line represents the 45° line (predicted = observed).

Supplemental Material

Supplemental material 1. Information extracted from each paper

From the 51 selected papers, the following information was extracted and compiled in an Excel file:

1. General information including title, authors, publication year, country where the experiment was conducted, days on feed (DOF), number of replications per treatment, and methodology used to determine the Zn excretion.
2. Each study was assigned a unique code for identification during the statistical analysis.
3. Added Zn level (mg/kg), Zn sources (as indicated in the paper), and the percentage of added Zn that came from an inorganic source.
4. Body weight (BW) of the pigs at the beginning of the collection period.
5. Feed intake (g/d) and Zn intake. If feed intake was not reported, it was estimated using Equation 1. When Zn intake was not reported, it was estimated using ADFI (equation 2), in cases where the ADFI was not reported, the Zn intake was calculated using the ATTD coefficients and Zn excretion (equation 3).

$$ADFI, g/d = \frac{Daily\ Zn\ intake,\ mg/d}{Analyzed\ dietary\ Zn,\ mg/kg} * 1000 \qquad \text{(Eq. 1)}$$

$$Zn\ intake, mg/d = ADFI, kg/d\ *\ Analyzed\ dietary\ Zn, mg/kg \qquad \text{(Eq. 2)}$$

$$Zn\ intake, mg/d = \frac{Fecal\ Zn\ excretion,\ mg/d}{100 - ATTD\ of\ Zn} * 100 \qquad \text{(Eq. 3)}$$

6. Zinc requirement estimates for the corresponding BW range. NRC (2012) values were used: 5 to 7 kg = 26.6 mg/d; 7 to 11 kg = 46.8 mg/d; 11 to 25 kg = 72.4 mg/d; 25 to 50 kg = 90.2 mg/d; 50 to 75 kg -= 105.9 mg/d; 75 to 100 kg = 125.3 mg/d; > 100 kg = 139.4 mg/d.

7. Difference (mg/d) between Zn intake and Zn requirement according to the NRC (2012) (estimated on point 6)
8. Fecal Zn excretion in mg/d. In papers where the Zn excretion was not reported it was estimated using equation 4.

$$Fecal\ Zn\ excretion, mg/d = Zn\ intake, mg/d * (\frac{100 - ATTD\ of\ Zn}{100}) \quad \text{(Eq. 4)}$$

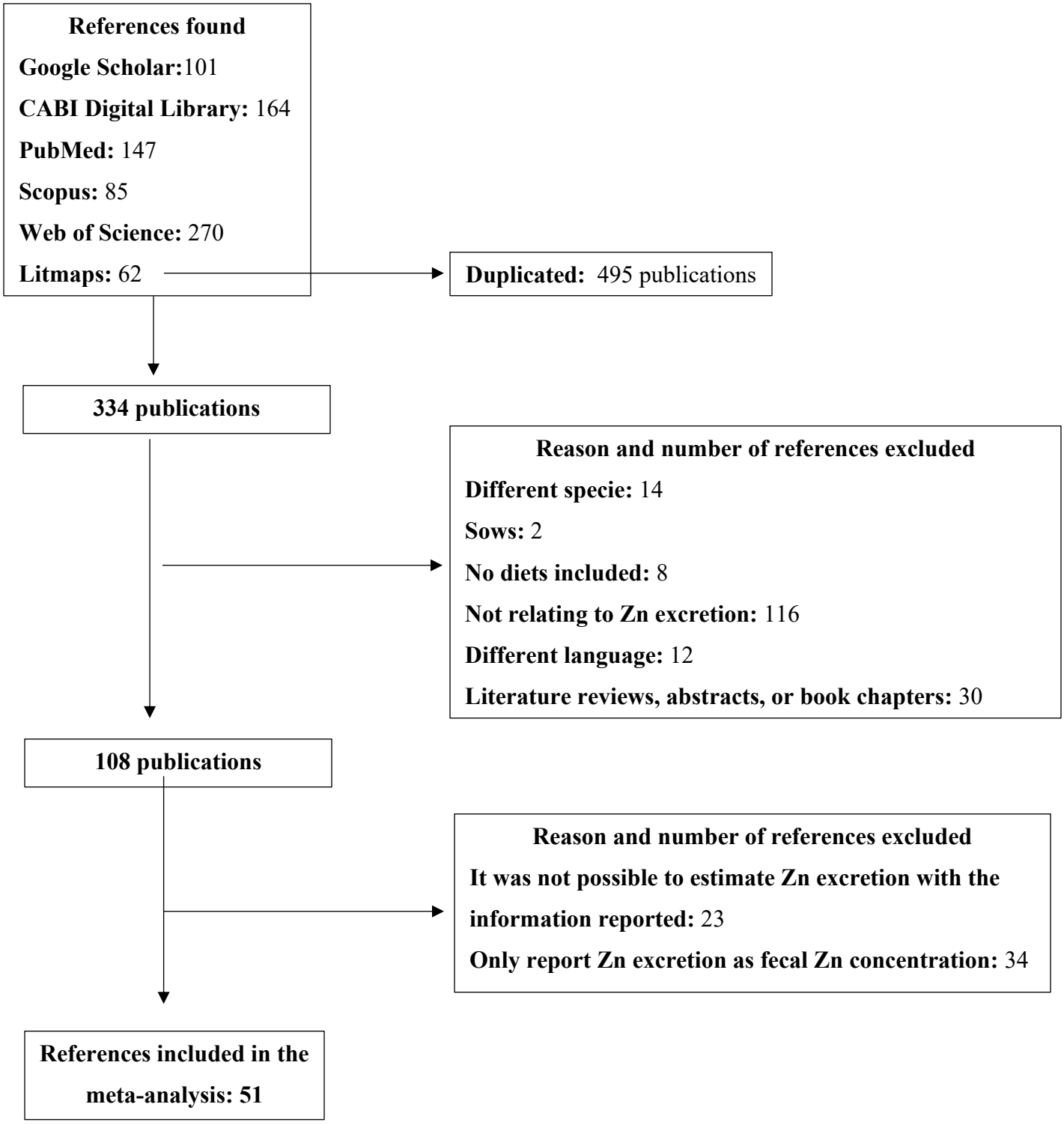
9. Apparent total tract digestibility (ATTD) coefficient. In papers that did not report the ATTD, it was estimated using equation 5.

$$ATTD\ of\ Zn, \% = \left(\frac{Zn\ intake, mg/d - Fecal\ Zn\ excretion, mg/d}{Zn\ intake, mg/d}\right) * 100 \quad \text{(Eq. 5)}$$

10. Zinc absorption (mg/d) was calculated as the differences between Zn intake and Zn excreted in feces.
11. If the paper reported urinary Zn excretion, it was recorded in mg/d. Total Zn excretion was calculated as the sum of the fecal and urinary Zn.
12. Diet characteristics including:
    - Use of antibiotics (Yes / No)
    - Phytase units
    - Use of acidifiers (Yes / No)
    - Exogenous enzymes rather than phytase (Yes / No)
13. The chemical composition of diets included total Ca, P, Cu, Mn, Fe, N or CP, lactose, NDF, and NE. If a paper reported analyzed values for any of these components, they were used. Otherwise, all diets were reformulated based on the NRC (2012) ingredient database. If additional nutrient values were required, they were obtained from the Brazilian tables from poultry and swine (Rostagno et al., 2024), Feedipedia (available at

Feedipedia: An on-line encyclopedia of animal feeds | Feedipedia), ingredient composition reported within the publication, or the Hans H. Stein Monogastric Nutrition Laboratory (available at https://nutrition.ansci.illinois.edu/static/ feed_database.html). Nutrient values were expressed as daily nutrient intake, calculated by multiplying the nutrient concentration by reported ADFI.

Supplemental Figure 1. Flow chart for publications included in the meta-analysis.



Chapter 2 - Effect of zinc feeding programs in nursery and finishing pig diets on growth performance, fecal zinc concentration, and carcass characteristics under commercial conditions

Abstract

Two experiments were conducted to evaluate the effects of dietary Zn source and level on growth performance, fecal dry matter (DM), fecal Zn concentration, and carcass characteristics in nursery and finishing pigs. In experiment 1, a total of 2,268 pigs (337 × 1050, PIC; initially 5.7 ± 0.31 kg) were allotted to one of seven dietary treatments arranged in a 3 × 2 + 1 factorial, with main effects of Zn source (ZnO and two Zn hydroxychloride sources) and Zn level (1,500 followed by 1,000 mg/kg or 1,000 mg/kg followed by 500 mg/kg in phase 1and 2, respectively). The seventh treatment contained 3,000 and 2,000 mg/kg Zn from ZnO in phases 1 (d 0 to 11) and 2 (d 11 to 25), respectively. After the experimental period (dietary phases 1 and 2), all pigs were fed a common phase 3 diet containing 100 mg/kg Zn from $ZnSO_4$ for 14 d. Fecal samples were collected on d 7 and 21 for DM and Zn analysis. Overall, no Zn source × level interactions or main effects of Zn source or increasing level were observed for growth performance or fecal DM. For d 7 fecal Zn concentration, a Zn source × level interaction ($P = 0.009$) was observed, where fecal Zn concentration increased with increasing Zn from ZnO or Zn hydroxychloride source B but not for hydroxychloride source A. On d 21, fecal Zn concentration increased ($P < 0.001$) as dietary Zn increased, regardless of source. In experiment 2, a total of 2,363 pigs (337 × 1050, PIC; initially 26.9 ± 0.61 kg) were allotted in a randomized complete block design to one of four Zn sources ($ZnSO_4$, Zn Hydroxychloride source A and B, and a chelated Zn), each added

at 100 mg/kg of complete feed. No differences between Zn sources were observed for growth performance, mortality, removals, total removals and mortality, hot carcass weight, carcass yield, and loin depth. A marginal Zn source response was observed for backfat depth ($P = 0.095$) and percentage lean ($P = 0.072$), but without means separation. In conclusion, Zn source and level had no effect on overall nursery growth performance or fecal DM; however, lower dietary Zn levels reduced fecal Zn concentration. In finishing pigs, Zn source did not affect performance or carcass characteristics, with only marginal effects observed for backfat depth and lean percentage.

Introduction

Zinc is an essential micromineral playing a key role in various physiological processes, including protein synthesis, immune function, and cellular proliferation and differentiation (Kambe et al., 2015). The daily Zn requirements suggested by NRC (2012) increases as body weight (BW) increases, beginning at 27 mg/d for pigs weighing 5 kg and increases to 139 mg/d for pigs weighing 135 kg. However, the use of pharmacological levels of Zn, typically from ZnO in nursery diets is a common practice in the swine industry (Faccin et al., 2023) to reduce the incidence of post-weaning diarrhea (PWD) and improve growth performance (Luise et al., 2024).

Due to increasing environmental concerns regarding Zn use in swine diets (Duan et al., 2019; Kaur and Garg, 2021), the interest in more bioavailable Zn sources and strategies to optimize their use in swine diets has increased (Lei and Kim, 2018; Bonetti et al., 2021). Zinc hydroxychloride (ZnHyd), produced through the reaction between Zn, water, and hydrochloric acid (Leisure et al., 2014), along with amino acid-chelated Zn are some Zn alternatives, potentially offering greater Zn availability and decreased Zn excretion compared to $ZnSO_4$ and ZnO (Villagómez-Estrada et al., 2020; Stein, 2024).

The effects of alternative Zn sources on growth performance and carcass characteristics in pigs remain inconsistent. In finishing pigs Van Kuijk et al. (2019) reported improvement in feed efficiency (F:G), average daily gain (ADG), and carcass characteristics with the use of ZnHyd, particularly during the last phase of the finishing period. However, Cemin et al., (2018) observed a tendency for lower growth performance of nursery pigs fed ZnHyd (1,000 mg/kg) compared to pharmacological levels of ZnO (3,000 mg/kg). Other studies have not demonstrated performance or carcass improvements associated with alternative Zn sources such as ZnHyd or amino acid chelated Zn in nursery and finishing pigs (Cemin et al., 2019; Xiong et al., 2023; Soto et al. 2024). Therefore, the objectives of these studies were: 1) to evaluate the effects of Zn level and source on growth performance, fecal Zn concentration, and fecal DM in nursery pigs; and 2) to evaluate the effects of Zn source on growth performance and carcass characteristics in finishing pigs raised under commercial conditions.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments (IACUC # 4847). Experiment 1 was conducted in two independent rooms at a commercial nursery research site (Hord Farms West, Pipestone, MN). Each pen (2.3 × 3.7 m) provided approximately 0.32 m^2 per pig and was equipped with a 6-hole dry feeder and a drinker pan. Experiment 2 was conducted in two independent barns at a commercial finishing research site (Hord Farms West, Pipestone, MN). The barns were naturally ventilated and double-curtain-sided with totally slatted floors. Each pen (3.0 × 5.6 m) provided approximately 0.63 m^2 per pig and was equipped with a 5-hole dry feeder and a cup drinker. In both facilities, daily feed additions were recorded using a computerized feeding system

(FeedPro; Feedlogic Corp., Willmar, MN). Pigs were provided ad libitum access to feed and water throughout both experiments.

Animals and diets

For Exp. 1, a total of 2,268 pigs (Line 337 × 1050, PIC, Hendersonville, TN) initially 5.7 ± 0.31 kg were used in a 39-d study with 27 pigs per pen and 12 pens per treatment. At placement, pens of pigs were divided into 6 BW blocks and then randomly assigned to one of six dietary treatments in a randomized complete block design. The 2 identical rooms had an equal representation of dietary treatments and BW categories. Pigs were fed experimental diets for the first 2 dietary phases, lasting 11 and 14 d, respectively. In the remaining 14 d of the experiment, all pigs were fed a common phase 3 diet. Phase 1 diets were manufactured at Hubbard Feeds in Mankato, MN. Phase 2 and 3 diets were manufactured at the Hord Farms West feed mill located in Pipestone, MN. The first phase was fed in pellet form, and the remaining two phases were fed in meal form. All diets were formulated to meet or exceed the NRC (2012) requirement estimates for amino acids relative to Lys and vitamins and minerals. Dietary treatments were arranged in a 3 × 2 + 1 factorial, with the main effects of Zn source and level. The Zn sources included ZnO (Zinc Nacional, Monterey, Mexico) and two sources of Zn hydroxychloride (Hydroxy Zn, SAM Nutrition, Bloomington, MN and Intellibond Z, Selko, Indianapolis, IN). The total dietary Zn levels included 1,500 followed by 1,000 mg/kg or 1,000 mg/kg followed by 500 mg/kg in phase 1 and 2, respectively. The seventh treatment contained 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively (Table 1). Pigs and feeders were weighed on d 0, 11, 25, and 39 to determine ADG, average feed intake (ADFI), and G:F. Fecal samples were collected via rectal palpation from three pigs per pen on d 7 and 21 of the study to determine fecal DM. Samples were dried at 55°C for 48 h and loss of weight was used to determine the percentage of fecal

DM. Due to multiple reports of *E. coli* in other nurseries filled with the same sow farm origins, all the piglets were vaccinated against *E. coli* in the second-week post placement (Entero Vac, Arco Laboratories, Jewell, Iowa).

For Exp. 2, a total of 2,363 pigs (Line 337 × 1050, PIC, Hendersonville, TN) initially 26.9 ± 0.61 kg in two barns were used in a 120-d study with 27 or 26 pigs per pen and 22 pens per treatment. At placement, pens of pigs were randomly assigned to one of four dietary treatments in a randomized complete block design. The two identical barns had an equal representation of dietary treatments and BW categories. Dietary treatments were corn-soybean meal-based and contained 110 mg/kg of Zn from zinc sulfate ($ZnSO_4$), two sources of Zn hydroxychloride (Hydroxy Zn, SAM Nutrition, Bloomington, MN and Intellibond Z, Selko, Indianapolis, IN), or a chelated Zn (B-Traxim 2C Zn-260, Pancosma, Rolle, Switzerland). Diets were fed in four phases from 27 to 50, 50 to 74, 74 to 100 kg, and 100 kg BW to marketing. All treatment diets were formulated to meet or exceed NRC (2012) requirement estimates for finishing pigs for their appropriate weight ranges (Table 2). All diets were fed in meal form and manufactured at the Hord Farms West feed mill located in Pipestone, MN. Every 2 weeks, pens of pigs were weighed and feed disappearance was measured to determine ADG, ADFI, G:F, removals, and mortality.

The three heaviest pigs per pen were visually identified, weighed, and marketed 25 d before the end of the experiment. These pigs were included in the growth performance analysis but were excluded from the carcass data collection. On the last day of the experiment, final pen weights were obtained, and pigs were tattooed with a pen identification number and transported to a U.S. Department of Agriculture-inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth,

backfat depth, and percentage lean. The percentage lean calculations were obtained from a proprietary equation used by the packer. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight obtained at the farm.

Chemical analysis

For Exp. 1, mineral concentration in feed and dry fecal samples was analyzed using inductive coupled plasma (ICP-OES) at the K-State Research and Extension Soil Testing Lab. Before the analysis, the feed and dried feces were ashed and then digested in 4 M nitric acid at 90°C for 4 h (adapted method from Sposito et al., 1982). A feed sample per dietary treatment and phase was analyzed for moisture (AOAC 930.15), crude protein (AOAC 990.03), either extract (AOAC 2003.05), ash (AOAC 942.05), and acid detergent fiber (ANKOM Technology Method) at Midwest Laboratories, Omaha, NE. For Exp. 2, mineral premixes were analyzed in triplicate (Cumberland Valley Analytical Services, Inc., Waynesbro, PA) for Zn, Ca, Mg, K, Cu, Fe, and Mn (AOAC, 2000, method 985.01). Feed samples were collected weekly from at least 10 feeders per treatment and were analyzed for Zn, Ca, Mg, K, Cu, Fe, Mn, and S in duplicate (K-State Research and Extension Soil Testing Lab) following the same methodology described for Exp. 1.

Statistical Analysis

Both experiments were analyzed using RStudio [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria]. For Exp. 1, data were analyzed as a randomized complete block design with pen serving as the experimental unit. For performance data, the model utilized treatment as a fixed effect and BW block and barn as random effects. For fecal DM, in addition to the previous model components, the pen was used as a random effect to account for the subsampling associated with multiple individual pigs analyzed from each pen. Specific contrasts were conducted to compare the effect of Zn source,

level, and their interaction on the response variables. Linear and quadratic responses to ZnO concentration were evaluated. Contrast coefficients were established based on the Zn concentration fed within a specific dietary phase or the weighted average of Zn concentration based on feed intake when considering multiple dietary phases.

For Exp. 2, data were analyzed as a randomized complete block design with pen serving as the experimental unit. The performance data model utilized dietary treatment as a fixed effect and the BW block nested within barn as a random effect. Additionally, the proportion of barrows within each pen at d 0 was used as a covariate. For carcass yield and HCW, data were analyzed on a pen basis using the same fixed and random effects as the analysis of growth performance data. For carcass composition (backfat depth, loin depth, and percentage lean), data were analyzed using individual carcasses as the observational unit. The model included treatment as a fixed effect, pen and weight block within barn as random effects, and HCW as a covariate.

For both experiments, mortality, removals, and total mortality and removals were analyzed using a binomial distribution. The proportion of dead or removed pigs over the initial pigs placed per pen was used as the response variable for mortality and removals. Results were considered significant with $P \leq 0.05$ and marginally significant with $P \leq 0.10$.

Results

Experiment 1

The proximate composition and mineral concentration from the experimental diets were generally consistent with the formulated values, within the expected analytical variation (AAFCO, 2018), with the exception of Zn. The analyzed Zn concentrations for the ZnO treatments and ZnHyd source B closely matched the formulated levels in both phases. However, for ZnHyd source A, the analyzed Zn level in the treatment formulated with 1,000 mg/kg was

1,882 mg/kg (84% more than expected) while the treatment formulated to 1,500 mg/kg the analyzed value was lower than expected (1,453 mg/kg). Although these discrepancies raise concerns about potential manufacturing or sampling error, there is no evidence that the treatments were reversed. . In contrast, the analyzed values for phase 2 diets were closely aligned with the formulated concentrations across all treatments (Table 3).

For growth performance, mortality, removals, mortality and removals, total mortality, and fecal DM, no significant interactions or main effects were observed between the Zn sources and level, except for a marginal response ($P = 0.073$) to Zn level for ADFI in phase 2, where pigs fed diets containing 1,000 mg/kg had increased feed intake compared with those fed 500 mg/kg, resulting in greater ($P = 0.023$) ADG (Table 4). Furthermore, there were no linear or quadratic responses based on ZnO level for growth performance or survivability. For fecal Zn concentration, on d 7, a significant ($P = 0.009$) Zn source × level interaction was observed, where greater ($P < 0.05$) fecal Zn concentration was observed as the dietary Zn inclusion increased when ZnO or Zn hydroxychloride source B were used, but no differences between levels were observed in the Zn hydroxychloride source A. On d 21, no significant interaction ($P = 0.748$) between Zn source × level was observed. For the main effects, there was no effect of Zn source on d 7 or 21 fecal Zn concentration. However, a marginal effect of Zn level was detected on d 7 ($P = 0.065$), and a significant effect was observed on d 21 ($P < 0.001$), where fecal Zn concentration decreased as dietary Zn levels decreased. Furthermore, fecal Zn concentration decreased (linear, $P = 0.001$) with decreasing ZnO level.

Experiment 2

The mineral concentration of experimental diets was consistent with formulated values considering normal analytical variation (AAFCO, 2018; Table 5).

No differences were observed ($P > 0.10$) in growth performance, mortality, removals, total removals, HCW, carcass yield, and loin depth between pigs fed different Zn sources (Table 6). A marginal response was observed for backfat depth ($P = 0.095$) and percentage lean ($P = 0.072$). Although no mean separation among Zn sources was detected, pigs fed Zn hydroxychloride source B had numerically greater backfat depth and lower percentage lean than pigs fed the other Zn sources.

Discussion

The increasing regulation of Zn use in swine diets has heightened the need to identify alternative Zn sources with greater bioavailability, enabling reductions in Zn excretion without compromising growth performance (Brugger and Windisch, 2015). Zinc hydroxychloride is one alternative, characterized by a crystalline structure formed through covalent bonds between Zn ions, hydroxyl groups, and the chloride ions (Leisure et al., 2014). This structure results in low solubility in water but high solubility in acidic environments, such as the stomach, which reduces the interactions with other dietary components compared to more reactive sources like $ZnSO_4$ (Van Kuijk et al., 2019; Villagómez-Estrada et al., 2020). Similarly, the use of minerals chelated with organic compounds (amino acids, peptides, or proteins) has been proposed as an alternative to increase Zn absorption from the gastrointestinal tract and reduce its excretion, as these mineral forms utilize alternative absorption routes associated with the chelated agent (Byrne and Murphy, 2022).

The effect of ZnHyd on nursery growth performance has been previously investigated. Soto et al. (2024) evaluated the effect of $ZnSO_4$ and two sources of ZnHyd at two dietary levels (125 or 250 mg/kg of Zn). Although the Zn levels were lower than in the present study, they reported no significant interaction or main effects between sources and levels. In contrast to the

current results, Cemin et al., (2018) observed that nursery pigs fed ZnHyd (1,000 mg/kg of Zn) tended to have decreased ADG, primarily due to reduced ADFI, compared to those fed pharmacological levels of ZnO (3,000 mg/kg of Zn). However, direct comparisons with the current study are limited due to differences in the dietary Zn levels and sources.

The beneficial effect of pharmacological levels of ZnO in reducing the incidence of PWD and improving performance in nursery pigs has been widely demonstrated (Oh et al., 2021; Huang et al., 2023). However, the optimal dietary inclusion level of ZnO to maximize growth performance remains uncertain. Hansen et al. (2022), found a quadratic response for both ADG and ADFI to Zn levels from ZnO, with maximal responses occurring between 950 and 1,450 mg/kg of added Zn. These findings may explain the lack of performance differences observed in the present study, as the dietary ZnO levels evaluated were within the range associated with the maximum growth response reported by Hansen et al. (2022). Conversely, the positive effect of pharmacological levels of Zn on growth performance has been previously reported in nursery pigs (Mavromichalis et al., 2000; Hill et al., 2001). However, many of these studies included negative control diets (no added Zn) or Zn levels at the nutritional requirement, which differs from the current study. In the present experiment, all dietary treatments exceeded the estimated Zn requirements for nursery pigs (NRC, 2012) to be reflective of current industry feeding practices (Faccin et al., 2023). A treatment group formulated to meet only the requirement or serve as a negative control was not included, as such diets are not commonly used in commercial nursery settings and would not provide practical insights for industry application. However, this design limits direct comparison with other studies evaluating pharmacological levels of Zn.

In the present study, feeding 1,000 mg/kg Zn improved ADG during phase 2 compared with 500 mg/kg Zn. However, this improvement was not sustained during the common period.

These findings are consistent with those of De Mille et al. (2022), who reported a reduction in growth performance when pigs were transitioned from a diet containing 3,000 mg/kg Zn to a diet with no added Zn. The authors suggested that this decline may be related to a reduction in plasma ghrelin concentrations associated with the withdrawal of pharmacological Zn. This reduction in growth when transitioning away from pharmacological levels of ZnO is commonly observed (Carlson et al., 1999; Stas et al., 2025).However, moreresearch should be performed to understand the mechanism further and develop strategies to limit the negative impact observed.

Reviews by Bonetti et al. (2021) and Tang et al. (2024) suggest that the mode of action of pharmacological levels of ZnO to improve performance may be attributed to its ability to modulate gastrointestinal microbiome as well as improve nutrient absorption and intestinal morphology. However, to our knowledge, similar properties have not been reported for ZnHyd, which could contribute to the lack of response in performance observed in the present study. Additionally, the low level of enteric challenge and the use of vaccination against *E.coli* could also contribute to the lack of response in performance between Zn sources and levels.

Developing strategies to reduce the environmental impact of animal production is a critical objective to ensure long-term food security and promote sustainable agricultural systems (Shurson et al., 2022). Zinc homeostasis is tightly regulated at the intestinal level, where, once body Zn pools are saturated, fecal Zn excretion linearly increases with Zn intake (Rincker et al., 2005; Hansen et al., 2023). This increase is attributed to the downregulation of Zn transporters in the brush border membrane and enterocytes (Maares and Haase, 2020). Consistent with the present findings, several studies have reported a decrease in fecal Zn concentration as dietary Zn levels decreased in nursery pigs (Creech et al., 2004; Wang et al., 2010; Zhang et al., 2021). When comparing fecal Zn concentration between Zn sources, the current findings are consistent

253 with those of Villagómez-Estrada et al. (2020), who reported no differences in fecal Zn
254 concentration between Zn sources in finishing pigs when Zn was provided as either ZnHyd or
255 $ZnSO_4$. The significant interaction between Zn source and inclusion level observed for fecal Zn
256 concentration on d 7 may be attributed to an error during diet preparation. Specifically, the
257 analyzed Zn concentration in the phase 1 diet containing ZnHyd form source A did not match the
258 formulated value and appeared to be reversed between level treatments. However, fecal Zn
259 concentrations were consistent with the analyzed dietary Zn levels, suggesting that the analyzed
260 values accurately reflected the actual Zn content of the diets and that the discrepancy likely
261 resulted from a mixing or labeling error in the feed bins of the farm.
262 The effects of various dietary Zn sources on growth performance in finishing pigs have
263 been evaluated in numerous studies, yielding mixed results. In a meta-analysis conducted by van
264 Kuijk et al. (2019), data from six studies were analyzed to compare ZnHyd and $ZnSO_4$ at the
265 same dietary levels on performance. The authors reported that supplementation with 80 mg/kg of
266 Zn from ZnHyd improved ADG and G:F by 3.9% during the late finishing phase compared to
267 the same level of Zn from $ZnSO_4$. However, no significant differences in growth performance
268 between Zn sources were observed during the grower phase or overall. Similarly, Villagómez-
269 Estrada et al. (2020) evaluated the effects of feeding 20 or 80 mg/kg of Zn from $ZnSO_4$ or
270 ZnHyd to finishing pigs (19 to 93 kg of BW) and concluded that the use of ZnHyd tended to
271 improve ADG in the last portion of the experiment (d 85 to 105), although overall performance
272 was unaffected. In agreement with the current study, Carpenter et al. (2016) and Cemin et al.
273 (2019) observed no changes in growth performance in finishing pigs fed diets containing 50,
274 100, or 150 mg/kg of Zn from ZnHyd or $ZnSO_4$. Additionally, Corso et al., (2025) observed no
275 differences in overall growth performance among finishing pigs (22 to 135 kg) fed diets

276 containing 100 mg/kg from ZnHyd, ZnO, or $ZnSO_4$. Furthermore, consistent with the present
277 findings, no differences in growth performance have been reported between inorganic and
278 organic Zn sources in finishing pigs when diets were formulated to contain the same Zn levels
279 (101 mg/kg Zn from 20 to 50 kg and 79 mg/kg Zn from 50 to 80 kg; Ma et al., 2012) or when
280 $ZnSO_4$ was partially replaced with amino acid chelated Zn at 30, 45, or 60% of ZnSO4 level
281 (Xiong et al., 2023).

282 The use of hydroxychloride minerals in finishing pigs appears to positively influence
283 meat quality and carcass yield, as the mineral form may affect the ratio of protein to fat
284 deposition and reduce oxidative stress, potentially through enhanced stability of vitamin E in the
285 feed, and improve antioxidant status in the animal (Van Kuijk et al., 2019). However, the results
286 across studies remain inconsistent. While the meta-analysis conducted by van Kuijk et al. (2019)
287 indicated an improvement in lean percentage when finishing pigs were fed 80 mg/kg of Zn from
288 ZnHyd compared to $ZnSO_4$, other studies showed no differences in carcass characteristics
289 associated with the use of ZnHyd , these reports align with the present study where ZnHyd from
290 source B but not source A numerically increased backfat and reduced lean percentage compared
291 with the other Zn sources. In contrast with the current results, improvements in carcass yield,
292 primarily driven by increases in HCW, have been associated with the use of ZnHyd instead of
293 $ZnSO_4$ in pigs (Cemin et al., 2018; Villagómez-Estrada et al., 2020). For organic chelated Zn,
294 Tian et al. (2025) reported increased loin muscle area and carcass yield in pigs fed diets
295 containing glycinate-chelated Zn and Fe compared with sulfate forms, which contrasts with the
296 findings observed in the present study. However, other studies have reported no differences in
297 carcass characteristics associated with the use of organic mineral sources (Burkett et al., 2009;
298 Xiong et al., 2023), which aligns with the results of the present study.

In conclusion, for nursery pigs, the source and level of dietary Zn did not affect growth performance or fecal DM. However, using lower dietary Zn levels independent of the source resulted in lower fecal Zn concentration. In finishing pigs, Zn sources evaluated did not affect finishing pig growth performance or carcass characteristics, except for a marginal difference between Zn sources for backfat and lean percentage.

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Table 2.1 Diet composition, Exp. 1 (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn[2]	43.53	58.60	56.55
Soybean meal, 47% CP	16.68	28.81	29.95
Corn distillers dried grains with solubles	5.00	7.50	10.00
Spray-dried whey	25.00	---	---
Microbial enhanced soybean meal[3]	5.00	---	---
Corn oil	1.00	---	---
Limestone	0.45	0.77	0.72
Monocalcium phosphate	1.10	1.35	0.70
Salt	0.15	0.45	0.29
L-Lys-HCl	0.48	---	---
DL-Met	0.26	0.24	0.18
L-Thr	0.19	---	---
L-Trp	0.03	0.02	---
L-Val	0.12	0.13	0.05
Thr[4]	---	0.30	0.21
Liquid lysine, 55%	---	0.80	0.64
Trace mineral premix[5]	0.15	0.15	---
Vitamin premix[6]	0.25	0.25	---
Vitamin and trace mineral premix[7]	---	---	0.13
Choline chloride 60%	0.04	---	---
Phytase[8]	0.08	0.08	0.04
Sodium metabisulfite	0.50	---	---
Feed additive[9]	---	0.55	0.55
Total	100.00	100.00	100.00
Calculated analysis			
SID amino acids, %			
Lys	1.35	1.35	1.30
Ile:Lys	57	55	60
Leu:Lys	119	120	132
Met:Lys	39	39	37
Met & Cys:Lys	60	60	60
Thr:Lys	65	65	65
Trp:Lys	20	20	21
Val:Lys	70	69	70
His:Lys	34	37	40
Total Lys, %	1.49	1.51	1.47
NE, kcal/kg	2,520	2,379	2,394
SID Lys:NE, g/Mcal	5.36	5.68	5.43
CP, %	20.43	21.82	22.68
Ca, %	0.71	0.76	0.63
P, %	0.69	0.69	0.57
STTD P, %	0.62	0.56	0.44

[1] Phase 1 was fed for 10 d in a pellet form and phase 2 for 14 d in a mash form. A common phase 3 was fed to all treatments for 14 d in mash form.

[2] The total dietary Zn was adjusted according to the experimental treatments using ZnO (72% Zn; Zinc Nacional, Monterey, Mexico), Hydroxy Zn (58% Zn; SAM Nutrition, Bloomington, MN), or Intellibond Z (55% Zn; Selko, Indianapolis, IN) at the expense of corn.

[3] ME-PRO (Aquatech, Brookings, SD).

[4] Thr Pro (CJ America).

[6] Provided per kilogram of feed: 110 mg of Zn as zinc sulfate; 17 mg of Cu as copper sulfate; 33 mg of Mn as manganese oxide; 110 mg of Fe as ferrous sulfate; 0.30 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite.

[6] Provided per kilogram of feed: 4,134 IU of vitamin A; 1,653 IU of vitamin D; 44 IU of vitamin E; 3.31 mg of vitamin K3; 33 μg of vitamin B_{12}; 8 mg of riboflavin; 28 mg of pantothenic acid; 50 mg of niacin.

[7] Provided per kilogram of feed: 110 mg of Zn as zinc sulfate; 18 mg of Cu as copper sulfate; 27 mg of Mn as manganese oxide; 110 mg of Fe as ferrous sulfate; 0.40 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite; 3,307 IU of vitamin A; 1,309 IU of vitamin D; 35 IU of vitamin E; 3 mg of vitamin K3; 27 μg of vitamin B_{12}; 6 mg of riboflavin; 22 mg of pantothenic acid; 44 mg of niacin.

[8] Phases 1 and 2: Ronozyme Hiphos 2700 (dsm-firmenich, Parsippany,NJ) provided 2,000 FTU/kg and an estimated release of 0.14% STTD P. Phase 3: Optiphos Plus 2500 G (Huvepharma, Sofia, Bulgaria) provided 1,000 FTU/kg and an estimated release of 0.13% standardized total tract digestible (STTD) P.

[9] Feed Aid (Elanco Animal Health, Greenfield, IN).

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

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %				
Corn	57.94	63.20	68.13	85.60
Soybean meal, 47.7% CP	19.10	14.00	9.35	12.20
Corn DDGS[2]	20.00	20.00	20.00	--
Limestone	1.27	1.23	1.16	0.82
Monocalcium phosphate	0.21	0.20	0.06	0.23
Salt	0.35	0.35	0.35	0.35
Liquid lysine 55%	0.67	0.63	0.60	0.44
DL-Met	0.05	0.03	0.00	0.03
Thr	0.13	0.10	0.08	0.09
L-Trp	0.03	0.04	0.03	0.02
Phytase[3]	0.04	0.04	0.04	0.04
Vitamin and trace mineral premix[4]	0.20	0.20	0.20	0.20
TOTAL	100.00	100.00	100.00	100.00
Calculated analysis				
SID amino acids				
Lys, %	1.10	0.95	0.82	0.72
Ile:Lys	59	60	60	60
Leu:Lys	147	158	170	153
Met:Lys	31	31	31	31
Met & Cys:Lys	57	59	60	59
Thr:Lys	62	62	62	62
Trp:Lys	19	19	19	19
Val:Lys	69	71	73	70
Lys:NE, g/Mcal	4.58	3.90	3.32	2.82
NE, kcal/kg	2,403	2,434	2,467	2,557
CP, %	20.1	18.1	16.2	13.3
Ca, %	0.61	0.58	0.52	0.43
STTD P, %	0.39	0.37	0.33	0.28

[1] Phases 1, 2, 3, and 4 were formulated to be fed from 27 to 50, 50 to 74, 74 to 100, and 100 kg to marketing, respectively.

[2] DDGS = dried distiller's grains with solubles.

[3] Optiphos 2500 (Huvepharma Inc. Peachtree City, GA) provided 1,000 FTU/kg of diet with an assumed release of 0.12% STTD P.

[4] Dietary treatments were based on a vitamin-trace mineral premix that contained either zinc sulfate, one of 2 sources of Zn Hydroxychloride (Hydroxy Zn SAM Nutrition, Bloomington, MN or Intellibond Z (55% Zn; Selko, Indianapolis, IN) , or a chelated Zn source (B-Traxim 2C Zn-260, Pancosma, Rolle, Switzerland). All premixes were formulated to provide the following per kilogram of feed: 110 mg of Zn (Source changed according to the dietary treatment); 150 mg of Cu as Tribasic copper chloride; 30 mg of Mn as manganese hydroxychloride; 110 mg of Fe as iron sulfate; 0.3 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite; 2,400 IU of vitamin A; 600 IU of vitamin D; 12 IU of vitamin E; 1 mg of vitamin K3; 11 µg of vitamin B_{12}; 23 mg of riboflavin; 8 mg of pantothenic acid; 23 mg of niacin.

Table 2.3 Chemical composition of experimental diets, Exp. 1 (as-fed basis)[1,2]

	Dietary Zn, mg/kg						
Source	ZnO			Zn hydroxychloride[3]			
				A		B	
Phase 1	3,000	1,500	1,000	1,500	1,000	1,500	1,000
Phase 2	2,000	1,000	500	1,000	500	1,000	500
Phase 1							
DM, %	88.13	87.92	88.55	88.84	89.00	87.78	87.66
CP[4], %	19.6	20.10	19.20	19.60	18.10	19.70	19.40
Ether extract, %	4.86	4.63	4.32	5.14	4.92	4.83	4.92
Ash, %	5.52	6.08	5.79	6.18	6.05	5.92	5.85
ADF[4], %	1.70	1.80	1.80	2.80	2.90	2.90	3.40
P, %	0.63	0.66	0.68	0.67	0.65	0.64	0.66
Ca, %	0.83	0.85	0.77	0.81	0.74	0.71	0.79
Zn, mg/kg	2,675	1,562	1,138	1,453	1,882	1,866	1,244
Mg, mg/kg	1,631	1,605	1,531	1,557	1,547	1,488	1,513
Cu, mg/kg	34	28	28	29	37	24	25
Fe, mg/kg	427	339	309	282	326	302	293
Mn, mg/kg	106	70	58	60	54	69	53
Na, %	0.41	0.34	0.35	0.34	0.36	0.35	0.35
K, %	1.18	1.17	1.11	1.09	1.13	1.09	1.06
Phase 2							
DM, %	85.92	86.24	85.71	86.29	86.69	86.89	86.96
CP, %	20.60	22.50	20.4	20.20	19.2	20.60	20.80
Ether extract, %	3.75	2.64	2.74	2.42	3.08	2.95	2.94
Ash, %	5.48	5.41	5.45	5.82	5.37	5.18	5.55
ADF, %	3.40	4.20	4.0	3.60	3.7	4.00	3.70
P, %	0.60	0.64	0.62	0.70	0.70	0.63	0.70
Ca, %	0.51	0.56	0.63	0.83	0.88	0.58	0.78
Zn, mg/kg	1,425	883	439	1,014	635	982	599
Mg, mg/kg	1,831	1,835	1,667	1,705	1,670	1,749	1,783
Cu, mg/kg	33	19	8	12	59	20	15
Fe, mg/kg	307	279	272	337	354	286	296
Mn, mg/kg	50	43	47	50	63	64	55
Na, %	0.23	0.28	0.37	0.46	0.32	0.26	0.37
K, %	0.97	0.96	0.87	0.83	0.76	0.80	0.79

[1] For proximate analysis, the values represent one sample per phase. For mineral analysis, the value represents the mean of 2 samples per dietary phase.

[2] Phase 3 analyzed composition; DM: 85.71, CP: 21.80, Fat: 3.20, Ash: 4.33, and NDF: 4.00. All values are expressed as a % of the final feed on an as-fed basis.

[3] Source A: Hydroxy Zn, SAM Nutrition, Bloomington, MN; Source B: Intellibond Z, Selko, Indianapolis, IN.

[4] CP: Crude protein; ADF: Acid detergent fiber

Table 2.4 Effect of Zn sources and levels on nursery performance, fecal DM, and fecal Zn concentration, Exp. 1[1]

	Dietary Zn, mg/kg										
Source	ZnO			Zn hydroxychloride[2]							
				A		B					
Phase 1	3,000	1,500	1,000	1,500	1,000	1,500	1,000		$P =$[3,4]		
Item: Phase 2	2,000	1,000	500	1,000	500	1,000	500	SEM	Source × level	Source	Level
BW, kg											
d 0	5.6	5.6	5.7	5.7	5.6	5.6	5.7	0.31	0.617	0.778	0.703
d 11	6.9	6.9	7.1	6.9	6.8	6.9	7.0	0.19	0.512	0.674	0.501
d 25	13.2	12.8	12.9	13.1	12.5	12.8	12.8	0.32	0.203	0.998	0.536
d 39	21.8	214	21.8	21.8	21.3	21.4	21.4	0.45	0.316	0.659	0.980
Phase 1 1 (d 0 to 11)											
ADG, g	113	114	128	113	107	108	115	16.8	0.370	0.835	0.333
ADFI, g	152	151	153	156	154	156	152	6.1	0.895	0.734	0.702
G:F, g/kg	737	746	840	713	670	692	749	86.4	0.401	0.727	0.176
Phase 2 (d 11 to 25)											
ADG, g	412	400	396	410	378	403	387	18.8	0.382	0.901	0.023
ADFI, g	523	507	506	521	484	513	496	15.5	0.399	0.883	0.073
G:F, g/kg	787	791	782	787	781	785	781	20.8	0.875	0.922	0.467
Experimental period (d 0 to 25)											
ADG, g	272	268	273	270	254	268	261	21.8	0.516	0.703	0.356
ADFI, g	350	343	345	351	333	350	338	14.6	0.693	0.794	0.164
G:F, g/kg	776	778	791	768	761	764	773	33.3	0.433	0.662	0.515
Common period (d 25 to 39)											
ADG, g	616	612	628	619	622	611	615	11.5	0.931	0.400	0.303
ADFI, g	890	877	906	913	896	902	897	24.6	0.743	0.767	0.885
G:F, g/kg	776	778	791	768	761	764	773	33.3	0.433	0.662	0.515
Overall (d 0 to 39)											
ADG, g	388	386	396	389	380	387	383	18.2	0.752	0.902	0.848
ADFI, g	533	527	539	541	526	541	530	15.1	0.852	0.849	0.586

G:F, g/kg	729	733	736	718	723	715	723	22.1	0.828	0.877	0.338
Mortality, %	0.93	0.31	0.00	0.31	0.31	0.93	0.62	0.532	0.809	0.286	0.999
Removals, %	11.29	8.92	8.33	11.58	9.51	7.45	9.21	3.044	0.228	0.164	0.890
Removals and mortality, %	12.24	9.27	8.67	12.24	9.86	8.38	9.86	3.645	0.246	0.246	0.762
Total mortality, %[5]	2.75	2.22	1.49	2.75	1.74	1.49	1.99	1.450	0.289	0.508	0.498
Fecal DM, %[6]											
d 7	23.3	22.2	22.7	23.6	25.8	24.0	23.6	1.70	0.425	0.609	0.581
d 21	19.5	18.6	18.6	18.9	19.5	18.4	20.0	1.50	0.534	0.994	0.229
Fecal Zn, mg/kg DM basis											
d 7[7]	11,339	6,535	4,734	5,879	7,014	6,988	4,899	2,286.2	0.009	0.404	0.065
d 21	8,699	5,400	3,297	4,868	2,864	5,101	2,988	166.4	0.748	0.293	<0.001

[1] A total of 2,268 pigs (Line 337 × 1050, PIC, Hendersonville, TN) initially weighing 5.7 ± 0.31 kg were used in a 39-d growth study with 27 pigs per pen and 12 pens per treatment. Treatments were assigned in a 3 × 2 factorial with the main effects of Zn source and levels. An additional treatment was included as a positive control (pharmacological levels of ZnO in phases 1 and 2).

[2] Source A: Hydroxy Zn, SAM Nutrition, Bloomington, MN; Source B: Intellibond Z, Selko, Indianapolis, IN

[3] *P*-value associated with source and level compares the three sources (ZnO and hydroxy chloride A and B) at 1,500 or 1,000 ppm in phase 1 and 1,000 or 500 ppm in phase 2.

[4] Linear and quadratic effects of the ZnO level were tested. No significant responses ($P > 0.10$) were observed for any period or performance parameters, except for fecal Zn concentration, where at d 7 and 21 fecal Zn concentration linearly decreases ($P < 0.001$) as dietary Zn concentration decreases.

[5] Total mortality = mortality in pen + removals mortality

[6] Three pigs per pen were sampled at the end of the second and third post-placement week; the weighted average days on feed were 7 and 21, respectively.

[7] For the significant interaction ($P = 0.009$) between the Zn source × level a significant difference ($P < 0.05$) between levels was observed when ZnO or Zn hydroxychloride source B were used, but no differences ($P > 0.10$) between levels were observed in the Zn hydroxychloride source A.

Table 2.5 Chemical composition of experimental premixes and diets, Exp. 2 (as-fed basis)[1]

	Zn, mg/kg	Ca, %	Mg, %	K, %	Cu, mg/kg	Fe, mg/kg	Mn, mg/kg	S, %
Premix								
$ZnSO_4$	60,291	9.37	0.15	0.26	75,585	62,088	16,542	--
Zn hydroxychloride, A[2]	49,346	9.00	0.13	0.20	70,891	59,584	17,074	--
Zn hydroxychloride, B[2]	51,394	9.23	0.14	0.22	74,934	60,001	13,268	--
Chelated Zn	63,389	8.72	0.13	0.23	76,427	61,893	17,342	--
Complete diets[3]								
$ZnSO_4$	171	0.57	0.16	0.33	127	178	47	0.10
Zn hydroxychloride, A	158	0.53	0.16	0.30	109	159	45	0.09
Zn hydroxychloride, B	181	0.50	0.16	0.30	126	200	43	0.09
Chelated Zn	145	0.55	0.16	0.29	144	182	45	0.10

[1]Feed samples by treatment were collected weekly and analyzed for mineral content in duplicate at the K-State Research and Extension Soil Testing Lab. A composite sample from each premix was analyzed in triplicate at Cumberland Valley Analytical Services, Inc.

[2]Source A: Hydroxy Zn, SAM Nutrition, Bloomington, MN; Source B: Intellibond Z, Selko, Indianapolis, IN.

[3] Values represent a composite including all dietary phases using a weighted average based on the feed budget for each dietary phase.

Table 2.6 Effect of Zn source on finishing pig performance and carcass characteristics, Exp. 2[1]

	Zn Source					
		Zn hydroxychloride[2]				
Item	$ZnSO_4$	Source A	Source B	Chelated Zn[3]	SEM	*P* =
BW, kg						
d 0	26.9	26.9	26.9	26.9	0.61	0.985
d 42	65.4	65.0	65.0	65.0	1.26	0.493
d 120	131.9	131.4	131.2	132.3	1.06	0.187
Grower period, (d 0 to 42)						
ADG, kg	0.91	0.90	0.90	0.90	0.018	0.673
ADFI, kg	1.89	1.90	1.89	1.89	0.057	0.849
G:F	0.48	0.48	0.48	0.48	0.006	0.146
Finishing period, (d 42 to 120)						
ADG, kg	0.86	0.86	0.86	0.87	0.011	0.634
ADFI, kg	2.61	2.62	2.62	2.63	0.020	0.782
G:F	0.33	0.33	0.33	0.33	0.004	0.713
Overall, (d 0 to 120)						
ADG, kg	0.89	0.89	0.89	0.89	0.005	0.706
ADFI, kg	2.32	2.32	0.32	0.33	0.021	0.878
G:F	0.38	0.38	0.38	0.38	0.002	0.661
Mortality and removals, %						
Mortality	1.89	2.24	1.89	1.55	0.614	0.900
Removals	4.69	4.68	4.67	5.71	1.013	0.800
Total	6.46	6.80	6.45	7.16	1.092	0.999
Carcass characteristics						
HCW, kg	98.7	97.7	98.0	98.9	0.78	0.397
Carcass yield, %	74.42	73.75	74.24	74.07	0.361	0.615
Backfat depth, mm[4]	16.23	16.34	17.05	16.72	0.274	0.095
Loin depth, mm[4]	69.78	69.80	69.26	69.85	0.364	0.612
Lean, %[4]	57.36	57.31	56.78	57.09	0.183	0.072

[1]A total of 2,363 pigs (PIC 337 × 1050, Hendersonville, TN) initially 26.9 ± 0.61 kg were used in a 120-d study. There were 26 or 27 pigs per pen and 22 pens per treatment. All Zn sources were included at 100 mg/kg of diet.

[2]Source A: Hydroxy Zn, SAM Nutrition, Bloomington, MN; Source B: Intellibond Z, Selko, Indianapolis, IN.

[3]B-Traxim 2C Zn-260, Pancosma, Rolle, Switzerland.

[4]Adjusted using hot carcass weight as a covariate.

Chapter 3 - Effects of increasing zinc in low acid binding capacity diets on nursery pig performance, fecal dry matter, zinc digestibility, and plasma zinc

ABSTRACT

A total of 360 barrows (initially 6.0 kg and 21 d of age) were used to evaluate the effects of added Zn in low acid-binding capacity at a pH of 4 **(ABC-4)** diets on nursery pig growth performance, fecal dry matter (**DM**), plasma Zn, and apparent total tract digestively (**ATTD**) of Zn. At weaning, pigs were divided into two body weight categories and then randomly assigned to dietary treatments in a generalized random block design. There were 5 pigs per pen and 12 pens per treatment. All diets contained 110 mg/kg of Zn from zinc sulfate in the trace mineral premix. The control treatment was a low ABC-4 diet (200 and 250 meq/kg from d 0 to 10 and 10 to 24 after weaning, respectively). In the next four diets, Zn (from zinc oxide; **ZnO**) was added at 500, 1,000, 2,000, and 3,000 mg/kg in phase 1, and 333, 666, 1,332, and 2,000 mg/kg in phase 2. The sixth treatment diet was formulated without considering ABC-4 (441 and 430 meq/kg from d 0 to 10 and 10 to 24, respectively) with 3,000 and 2,000 mg/kg of Zn from d 0 to 10 and 10 to 24 after weaning, respectively. In all periods, no differences were observed between pigs fed the low and high ABC-4 diets with 3,000 mg/kg Zn in phase 1 and 2,000 mg/kg Zn in phase 2, except for d-10 fecal DM, where pigs fed the low ABC-4 diet had increased ($P = 0.002$) fecal DM. From d 0 to 24, average daily gain **(ADG)** and average daily feed intake **(ADFI)** increased (linear, $P < 0.05$) with increasing dietary Zn, with no differences in gain:feed ratio. However, for the overall 46 d period, no response to dietary Zn was observed for any performance criteria.

Zinc intake, absorption, and excretion increased quadratically ($P < 0.001$) and ATTD of Zn tended to linear increase ($P = 0.074$) with increasing dietary Zn. Pigs fed the low ABC-4 diet had greater ($P < 0.05$) Zn intake, absorption, and ATTD of Zn than pigs fed the high ABC-4 diet at the same Zn concentration. No difference was observed for fecal Zn excretion between ABC-4 formulation strategies. Day 24 serum Zn concentration increased quadratically ($P < 0.001$) as dietary Zn increased, and a marginal increase ($P = 0.095$) in the low ABC-4 diet was observed between formulation strategies. In conclusion, low ABC-4 diets containing 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively, increased d 10 fecal DM, Zn absorption, and ATTD of Zn. Increasing Zn in low ABC-4 diets improved ADG and ADFI during the experimental period, but not for the overall study.

INTRODUCTION

Zinc is an essential micromineral playing a key role in structural components, catalytic factors, and signaling mediators (Kambe et al., 2015). The Zn requirement estimates for nursery pigs range between 26.6 and 72.4 mg/d (NRC, 2012). However, using pharmacological concentrations of Zn, mainly from zinc oxide (**ZnO**), during the first two to three weeks after weaning has been a common strategy in the U.S. swine industry (Faccin et al., 2023) to minimize the incidence of post-weaning diarrhea (**PWD**) and improve growth performance (Hansen et al., 2022; Landero et al., 2024).

Zinc oxide is poorly digested with apparent total tract digestibility (**ATTD**) ranging from -6.82 to 11.9 %; (Oh et al., 2021; Nielsen et al., 2022), and, therefore, Zn excretion increases as dietary Zn concentration increases (Hansen et al., 2023). Feeding pigs high levels of Zn has been associated with bacterial resistance at the intestinal level (Ciesinski et al., 2018). Moreover, in

492 soils, when Zn input through manure application exceeds Zn removal by crops, the excess Zn
493 can accumulates in soils (Qian et al., 2018) potentially leading to issues related to bacterial
494 resistance (Duan et al., 2019) and plant toxicity (Kaur and Garg, 2021). Regulations restricting
495 the use of pharmacological concentrations of Zn in many countries have intensified research
496 efforts to identify suitable alternatives. These include alternative Zn sources (Lei and Kim, 2018;
497 Dong et al., 2019; Pei et al., 2019), providing low-protein diets (Heo et al., 2010; Lynegaard et
498 al., 2021), increasing dietary fiber (Molist et al., 2011; Fernandes et al., 2020), adding dietary
499 phytogenics (Papadomichelakis et al., 2023), and lowering the dietary acid-binding capacity
500 (Landero et al., 2024; Stas et al., 2025).
501 Dietary acid binding capacity to a pH of 4 **(ABC-4)** is the amount of acid required to
502 reduce 1 kg of an ingredient or complete feed to a pH of 4 (Lawlor et al., 2005). Feeding low
503 ABC-4 diets has been proposed as a nutritional strategy to maintain the acidic conditions of the
504 gastrointestinal tract in the weanling pig (Lawlor et al., 2005; Stas et al., 2022) and improve
505 mineral digestibility in diets containing phytase (Kristoffersen et al., 2021) as the acidification of
506 the gastrointestinal tract may increase ionization and reduces complexation of minerals with
507 dietary antagonists that at end will result in greater mineral absorption (Jongbloed et al., 2000;
508 Van Der Aar et al., 2017). Recent studies have observed that when pharmacological
509 concentrations of Zn (2,000 to 3,000 mg/kg of Zn from ZnO) are not used, feeding low ABC-4
510 diets (200 to 250 meq/kg) improves growth performance compared to high ABC-4 diets
511 (Landero et al., 2024; Stas et al., 2025). However, these studies also observed no growth
512 performance benefits from dietary acidification when pharmacological concentrations of Zn were
513 included in the diet. This raises an important question regarding whether low ABC-4 diets can

support a reduction in pharmacological concentrations of Zn while maintaining growth performance.

Therefore, the objectives of this study were to evaluate the effects of increasing dietary Zn in low-ABC-4 diets and to assess the effect of dietary ABC-4 at specific dietary Zn concentrations. Both objectives were evaluated for their effects on nursery growth performance, fecal DM, plasma Zn, and fecal Zn excretion. It was hypothesized that using low ABC-4 diets would enable a reduction in pharmacological concentrations of Zn without negatively affecting growth performance or fecal DM. For Zn status, it was anticipated that Zn excretion would increase with increasing dietary Zn, but the use of low ABC-4 diets might increase Zn digestibility, resulting in less excretion.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment (IACUC-4943). The experiment was conducted in two identical barns located at the Kansas State University Segregated Early Weaning facility in Manhattan, KS, between February and May 2024. Each pen (1.2 × 1.5 m) provided approximately 0.36 m^2/pig and was equipped with a 4-hole dry feeder and a cup drinker to provide *ad libitum* access to feed and water. Both barns have metal tri-bar flooring, were mechanically ventilated with room temperatures set at 32.2°C during the first 10 d and then gradually reduced by 0.28°C daily until the set point reached 25°C. No environmental enrichment was utilized during the experiment.

Animals and diets

A total of 360 barrows (Line 200 × 400, DNA, Columbus, NE) initially 6.0 ± 0.74 kg were used in a 46-d study. The pigs were weaned at 21± 2 d of age and divided into two body

weight (BW) categories, light or heavy. The pigs were then randomly assigned to pens within BW category and pens were allotted to one of six dietary treatments. Each pen had five pigs and there were 12 pens per treatment. The two identical barns had an equal representation of dietary treatments and BW categories.

Pigs were fed experimental diets during phase 1 (d 0 to 10) and phase 2 (d 10 to 24 after weaning). From d 24 to 46 after weaning, all pigs were fed a common phase 3 diet. All diets contained a basal Zn level of 110 mg/kg from zinc sulfate (**$ZnSO_4$**) provided by the trace mineral premix, (Table 1). The Zn concentration of the ingredients was not considered in diet formulation.

The dietary treatments consisted of a control diet formulated to an ABC-4 capacity of 200 and 250 meq/kg in phase 1 and phase 2, respectively. Crystalline lactose and microbial-enhanced soybean meal (ME-PRO, Prairie Aquatech, Brookings, SD) were used as lactose and protein concentrate sources, along with fumaric acid to lower the ABC-4 value. The next four diets were the control diet with increasing added Zn concentrations of 500, 1,000, 2,000, and 3,000 mg/kg in phase 1, and 333, 666, 1,332, and 2,000 mg/kg in phase 2 using ZnO (containing 72% Zn). The sixth treatment was a diet formulated without consideration of ABC-4 value with 3,000 and 2,000 mg/kg added Zn in phases 1 and 2, respectively. In this diet, crystalline lactose and fermented soybean meal (HP300, Hamlet Protein, Findlay, OH) were used as lactose and specialty soy sources, respectively, resulting in ABC-4 values of 441 and 430 meq/kg in phase 1 and 2, respectively. For the common phase 3, diet was formulated with corn and soybean meal as the base, without the inclusion of soy specialty ingredients or lactose sources.

All treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The first phase was fed in pellet form, and the

560 remaining two phases were fed in meal form. Samples of complete diets were collected during
561 the bagging of experimental diets, with a subsample (approximately 100 g) collected from every
562 other bag in phase 1, every 3 bags in phase 2, and every 5 bags in phase 3. Feed samples were
563 pooled into one composed sample per treatment and dietary phase.

564 Pigs and feeders were weighed on d 10, 24, and 46 to determine average daily gain
565 **(ADG)**, average daily feed intake **(ADFI)**, and gain:feed ratio **(G:F)**. Individual fecal samples
566 were collected via rectal stimulation from the same three pigs per pen on d 10 and 24 of the
567 study. Fecal samples (10 to 20 g/pig) were collected into 20.3 × 17.8 cm plastic zipper bags and
568 stored at -10ºC until fecal dry matter **(DM)** analysis was conducted. The samples were dried in a
569 forced air oven for 48 h at 55ºC. Fecal DM was determined as follows: (dried sample weight at
570 48 h - pan weight)/ (initial wet sample weight - pan weight) × 100. In phase 2 (d 10 to 24), an
571 indigestible marker, titanium dioxide (TiO_2), was added to the diet (0.5%). After fecal DM
572 estimation, samples were dried, ground, and pooled on a per-pen basis, ensuring equal
573 representation from the three pigs in each pen. Fecal samples were then analyzed for Zn and
574 TiO_2.

575 **Zinc excretion and serum concentration**

576 A blood sample was collected from 36 pigs at weaning (randomly selected), and then
577 from the middle body weight pig from each pen on d 24. Blood samples were collected via
578 jugular venipuncture using a heparinized blood collection tube (BD Vacutainer, Franklin Lakes,
579 NJ) between 12:00 to 13:00 on d 0 and between 07:00 to 8:00 on d 24. The plasma was obtained
580 through centrifugation at 1,800 × *g* for 30 min at 2°C and stored at -20°C.

581 The Zn concentration in feed and fecal samples were analyzed in duplicate using
582 inductively coupled plasma optical emission spectrometry (ICP-OES model 5,800, Agilent

583 Technologies, Santa Clara, CA) at the K-State Research and Extension Soil Testing Laboratory
584 (adapted from Sposito et al., 1982). Briefly, 0.3 g of feed and dried feces were ashed for 12 h at
585 450°C and then digested in 20 mL of 4 M nitric acid (HNO_3) at 90°C for 4 h. For the 4 M HNO_3
586 preparation, 750 mL of deionized water were mixed with 250 mL of 63% nitric acid trace
587 mineral (Fisher Chemical, Pittsburgh, PA). Before analysis, samples were diluted (1:10 or 1:100)
588 based on the expected Zn concentration to ensure that the resulting solution was within the linear
589 range of the method (0 to 1 mg/kg of Zn). All diluted samples were prepared in 0.4 M nitric acid.
590 For Plasma Zn concentration, 2 mL of plasma was submitted to the Kansas State Veterinary
591 Diagnostic Laboratory (KSVDL), where Zn concentration was obtained utilizing a KSVDL
592 method performed by an AAVLD (American Association of Veterinary Laboratory
593 Diagnosticians) accredited laboratory on an ICP- MS spectrometer (PerkinElmer, Shelton, CT).
594 Accuracy of Zn estimations was verified using a 4 M nitric acid solution with known Zn
595 concentrations (in a range from 0 to 1 mg/kg). Additionally, the coefficient of variation (**CV**)
596 between replicates of the same sample was required to be below 5%; if this threshold was
597 exceeded, the analysis was repeated.
598 The TiO_2 concentration in feed and feces was analyzed in duplicate using the
599 colorimetric method described by Short et al. (1996). The absorbance of the samples was
600 measured at 408 nm using an Epoch 2 plate reader (BioTek Instruments, Inc., Winooski, VT).
601 Accuracy of TiO_2 estimations was assessed based on the CV of the results. Absorbance for each
602 sample was measured in duplicate, and the CV between readings was required to be below 2% to
603 be considered acceptable. Additionally, the CV between replicated samples was required to be
604 below 5% for the results to be considered valid.

Total feces production (g/d) was estimated using the following equation: (Feed TiO_2, % × ADFI, g/d) / (fecal TiO_2, %). The daily excretion of Zn was estimated by multiplying the daily feces production by the fecal Zn concentration. Zinc retention was calculated as the difference between Zn intake and excretion in feces. Apparent total tract digestibility of Zn was estimated using the equation: (Zn intake, g/d – Zn excretion, g/d) / Zn intake, g/d × 100 (Almeida and Stein, 2010).

Statistical analysis

Data were analyzed as a generalized randomized block design. The lmer function was used from the lme4 package (Bates et al., 2015) in RStudio [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen serving as the experimental unit and using the Kenward–Roger approximation to determine *P*-values. For growth performance and Zn status data, the model used treatment as a fixed effect and BW block and barn as random effects. Fecal DM was analyzed at the pig level, in addition to the previous model components, the pen was used as a random effect to account for the subsampling associated with multiple individual pigs (observational unit) analyzed from each pen.

Linear and quadratic effects of increasing Zn was tested on growth performance and fecal DM within the five low ABC– 4 diets. Contrast coefficients were established based on the Zn concentration fed within a specific dietary phase or the weighted average of Zn concentration based on feeding time when considering multiple dietary phases using the contr.poly from the stat package (supplemental table 1). A specific contrast was used to compare the low- and high-ABC-4 diets at the same Zn concentration (3,000 mg/kg in phase 1 and 2,000 mg/kg in phase 2).

Least squares means were estimated using the emmeans package, and pairwise comparisons among treatments were adjusted for multiple testing using the Tukey method to

control Type I error across multiple comparations. Model assumptions of normality, homoscedasticity, and independence of residuals were visually assessed using diagnostic plots generated with the DHARMa package. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS

Growth performance

No differences in growth performance were observed between pigs fed the low- and high-ABC-4 diets formulated to the same Zn concentrations in phases 1 and 2 (3,000 and 2,000 mg/kg, respectively) for any of the periods (Table 2).

From d 0 to 10 (phase 1), ADG increased (quadratic, $P = 0.004$) as dietary Zn increased. Increasing Zn had no effect on ADFI and as a result, G:F increased (linear, $P < 0.001$) with increasing dietary Zn. Body weight on d 10 increased (linear $P = 0.006$) as dietary Zn increased in low ABC-4 diets.

From d 10 to 24 (phase 2), no response to increasing Zn was observed for ADG; however, ADFI increased (linear, $P = 0.008$) as dietary Zn increased. Gain:feed ratio tended (linear, $P = 0.089$) to decrease as dietary Zn increased. Body weight on d 24 increased (linear, $P = 0.015$) as dietary Zn increased in low ABC-4 diets.

For the experimental period (d 0 to 24), ADG and ADFI increased (linear, $P < 0.05$) as dietary Zn increased with no effect on G:F. For the common (d 24 to 46) and overall period (d 0 to 46), no response to the Zn levels previously fed from d 0 to 24 were observed for ADG, ADFI, and G:F.

No effect of dietary Zn was observed for fecal DM on d 10 or 24. When 3,000 and 2,000 mg/kg of Zn were used in phases 1 and 2, respectively, pigs fed the low ABC-4 diet had greater

651 ($P = 0.002$) d 10 fecal DM than pigs fed the high ABC-4 diet; however, no differences between
652 either formulation strategies were observed for d 24 fecal DM.

653 **Zinc digestibility and plasma Zn**

654 Zinc intake, fecal Zn excretion, and Zn absorption increased (quadratic, $P < 0.05$) as
655 dietary Zn concentration increased (DM-basis, Table 3). Apparent total tract digestibility of Zn
656 tended to increase (linear, $P = 0.074$) as Zn concentration increased. Pigs fed low ABC-4 diets
657 had greater ($P < 0.05$) Zn intake, absorption, and ATTD of Zn than pigs fed the high ABC-4 diet
658 when the same pharmacological concentrations of Zn were used. However, no difference
659 between ABC-4 formulation strategy was observed for fecal Zn excretion.

660 Baseline plasma Zn concentration on d 0 was 0.81 ± 0.10 mg/L. On d 24, plasma Zn
661 increased (quadratic, $P = 0.012$) as dietary Zn concentration increased. In pigs fed with diets
662 containing 3,000 mg/kg of Zn during phase 1 (d 0 to 10), when 2,000 mg/kg of Zn were fed
663 during phase 2 (0 to 24), a marginal response ($P = 0.094$) was observed between high and low
664 ABC-4 formulation strategies, where pigs fed the low ABC-4 diets tended to have increased d 24
665 plasma Zn.

666 # DISCUSSION

667 Growing concerns about the use of pharmacological concentrations of Zn in nursery
668 diets, due to potential negative environmental impact, have highlighted the need to develop
669 nutritional strategies to maintain growth performance without employing pharmacological
670 concentrations of Zn. Dietary acidification has been proposed as a potential strategy to enhance
671 nutrient digestibility (Blank et al., 2012) and growth performance in pigs (Landero et al., 2024).
672 However, to the best of our knowledge, this is the first study to evaluate the effect of increasing
673 levels of Zn in low ABC-4 diets, providing novel insights into the interaction between dietary

674 acidification and Zn levels on growth performance and Zn utilization. While the beneficial
675 effects of the pharmacological concentrations of Zn from ZnO in reducing the incidence of
676 PWD and improving growth performance in the swine industry are well-established (Oh et al.,
677 2021; Huang et al., 2023), the exact mode of action of pharmacological concentrations of Zn is
678 not completely known. Studies summarized by Bonetti et al. (2021) and Tang et al. (2024)
679 suggest that the effects of pharmacological concentrations of Zn from ZnO may be attributed to
680 its ability to modulate the intestinal microbiota along with improvement in nutrient absorption
681 and intestinal morphology, and hormone regulation of appetite and growth.
682 In weaned pigs, the use of pharmacological levels of Zn has been shown to elevate serum
683 ghrelin (Yin et al., 2009; Zhang and Guo, 2009; De Mille et al., 2022). Ghrelin is a peptide
684 secreted mainly by X/A-like cells in the oxyntic mucosa of the stomach (Date et al., 2000) that in
685 its acetylated form acts as an orexigenic hormone, stimulating appetite and in its des-acylated
686 form promotes and stimulates growth hormone secretion through direct action on the anterior
687 pituitary (Inui, 2001). These mechanisms has been linked to improvements in BW gain in rats
688 (Tschöp et al., 2000). In the current study, ghrelin concentration was not measured; however, the
689 linear improvement in ADFI as the dietary Zn concentration increased during the experimental
690 period (d 0 to 24) could hypothetically be associated with hormonal regulation of feed intake,
691 such as ghrelin, as previously reported in pigs (Salfen et al., 2004; Vizcarra et al., 2007).
692 The absence of changes in G:F during the experimental period (d 0 to 24) across dietary
693 treatments suggests that the improvement in ADG was primarily driven by an increase in feed
694 intake. However, this response may also be attributed to other physiological effects of
695 pharmacological concentrations of Zn. For example, pharmacological concentrations of Zn from
696 ZnO have been reported to enhance the activity of digestive enzymes in the pancreas (Hedemann

et al., 2006), potentially leading to improvement in nutrient digestibility (Oh et al., 2021). In addition, ZnO is known to influence gastrointestinal morphology, including increased villus height and villus height:crypt depth ratio, which can enhance nutrient absorption (Roselli et al., 2003; Liu et al., 2014). Although intestinal morphology was not evaluated in the present study, it is hypothesized that improvements in growth performance with increasing dietary Zn levels may also be partially explained by Zn-induced changes in intestinal structure and function. Furthermore, ZnO has been shown to modulate the composition of the gut microbiota and stabilize the mucosal barrier (Vahjen et al., 2011), which plays a critical role in protecting the epithelium from pathogenic bacteria and maintaining gut barrier integrity (Forstner, 1978; Deplancke and Gaskins, 2001). Taken together, the positive effects of ZnO on growth performance observed during the experimental period likely result from a combination of increased feed intake, enhanced nutrient digestibility, improved intestinal morphology, and stabilization of the gut microbiome and mucus layer.

Similar to the present study, the beneficial effects of increasing Zn levels during the early nursery on growth performance were no longer detected once a common low Zn diet was fed later in the nursery (Carlson et al., 1999; Stas et al., 2025). De Mille et al. (2022) observed that when pigs were transitioned from a diet containing 3,000 mg/kg in the first week to a control diet with 220 mg/kg of Zn from $ZnSO_4$, ADG and ADFI were reduced by 14.3% and 12.5%, respectively, compared to pigs fed 2,000 mg/kg of Zn over the same period, response that was associated with reductions in plasma ghrelin concentration. As previously indicated, pharmacological concentrations of Zn from ZnO modulate the gut microbiome (Yu et al., 2017; Ortiz Sanjuán et al., 2024), and these effects may be reversible. Janczyk et al. (2015) demonstrated that differences in short-chain fatty acid concentrations in the cecum observed in

pigs fed 3,100 mg/kg Zn were no longer present following transition to a diet containing 100 mg/kg Zn from ZnO. These changes could be associated with shifts in microbial populations (Pieper et al., 2011) which may ultimately influence growth performance (Gardiner et al., 2020). Further research is warranted to elucidate the exact mechanisms and develop strategies to maintain the benefits of added Zn over time.

A meta-analysis conducted by Sales (2013) used data from twenty-six studies published between 1993 and 2011 that included Zn concentrations ranging from 0 to 5,000 mg/kg. The review concluded that ADG and ADFI linearly increased as the dietary Zn concentration increased with no effect on G:F. In contrast, a more recent meta-analysis (Luise et al., 2024) concluded that diets containing 201 to 1,600 mg/kg of Zn resulted in similar ADG to diets containing 1,600 to 3,000 mg/kg of Zn. The difference in the magnitude of the response between both meta-analyses could be attributed to uncontrolled factors in the experiments, like variation between experimental stations, differences in ZnO sources, or characteristics of the basal diet.

Diet acidification has been proposed as a nutritional strategy to replace pharmacological concentrations of Zn in nursery pig diets (Landero et al., 2024; Stas et al., 2025). Low gastric pH has been associated with improved crude protein utilization in the small intestine (Partanen and Mroz, 1999) and control of enteric pathogen growth (Ravindran and Kornegay, 1993). Landero et al. (2024) and Stas et al. (2025a) observed that feeding low ABC-4 diets improved growth performance of weaned pigs when dietary Zn was set at nutritional levels; however, no response to dietary ABC-4 was observed when pharmacological concentrations of Zn (3,000 and 2,000 mg/kg in phases 1 and 2 respectively) were fed. These findings are consistent with the current study, where dietary acidification did not improve growth performance in the presence of pharmacological concentrations of Zn. Furthermore, because different ingredients were used to

743 achieve the target ABC-4 values, it remains uncertain whether the observed responses were due
744 to the ABC-4 levels themselves or to changes in ingredient composition. Stas et al. (2025b)
745 evaluated the impact of various formulation strategies to achieve low ABC-4 values, including
746 reducing dietary Ca, adding acidifiers, or altering lactose sources on growth performance. Their
747 results indicated that during the experimental period (d 0 to 24 after weaning) pigs fed high
748 ABC-4 with pharmacological concentrations of Zn diets were superior to the other formulation
749 strategies, which suggests that the performance in response is mainly driven by the
750 pharmacological concentrations of Zn rather than other dietary factors.
751 Dietary acidification has been shown to provide optimal gastrointestinal conditions for
752 the proliferation of beneficial *Lactobacillus spp.* bacteria (Li et al., 2008). *Lactobacillus sp.* can
753 inhibit the colonization of *E. coli* (the main bacteria responsible for PWD; Vagios et al., 2020)
754 by inhibiting the adhesion to the intestinal mucosal (Blomberg et al., 1993) and producing
755 hydrogen peroxide, which has antimicrobial effects (Reiter et al., 1980). Although the abundance
756 of *Lactobacillus spp.* was not evaluated in this study, it is plausible that low ABC-4 diets created
757 an ideal environment for these bacteria. The modulation of intestinal microbiota may explain the
758 observed increase in fecal DM in the low ABC-4 diets. Stas et al. (2024) observed that fecal DM
759 on d 10 and 17 post-weaning linearly decreased as dietary ABC-4 increased. However, by d 23,
760 no differences in fecal DM were observed. These findings align with the result from the present
761 study, where low ABC-4 diets improved fecal DM at d 10 but did not on d 24. This suggests that
762 the effects of dietary acidification on fecal DM may be more pronounced in the early post-
763 weaning period, potentially due to the rapid adaptation of the gastrointestinal tract to the dietary
764 changes and new environment (Modina et al., 2021).

765 Plasma Zn concentration is commonly used to determine the Zn requirement estimate in
766 pigs (Hansen et al., 2022; Blavi et al., 2024). According to a meta-analysis by Jondreville et al.,
767 (2003), weaned pigs fed optimum Zn levels, plasma Zn concentrations should have a maximum
768 of 0.9 mg/L, with most of the data used for the regression line ranging from 0.8 to 1.0 mg/L. In
769 the current study, serum Zn on d 0 (0.81 mg/L) indicated that pigs were not likely Zn deficient at
770 the beginning of the experiment. However, by d 24, pigs fed diets with only 110 mg/kg Zn or
771 500 and 333 mg/kg of Zn (phases 1 and 2, respectively) had serum Zn concentrations below 0.81
772 mg/L. This suggests that the dietary Zn levels were not enough to maintain the plasma Zn levels,
773 which also may contribute to their low growth performance during the experimental period (d 0
774 to 24). Hansen et al. (2022) evaluated increasing levels of ZnO (80% Zn) in nursery pigs. At d 21
775 post-weaning, pigs fed diets containing 153 or 493 mg/kg of Zn showed no changes in serum Zn
776 concentration, whereas pigs fed higher dietary Zn levels had increased serum Zn concentration.
777 Although dietary ABC-4 was not controlled in the study by Hansen et al. (2022), their results
778 align with the quadratic response to Zn level observed for serum Zn concentration on d 24 in the
779 current study.
780 A meta-analysis conducted by Hansen et al. (2023), using data from studies published
781 between 2002 to 2022, concluded that daily fecal Zn excretion in nursery pigs linearly increases
782 with increasing dietary Zn concentration. This contrasts with the quadratic response to the Zn
783 level observed for fecal Zn excretion in our study. Zinc excretion in pigs may involve a temporal
784 component (Brugger et al., 2014), with lower excretion expected immediately after weaning until
785 endogenous Zn pools are replenished (Rincker et al., 2005), after which Zn excretion becomes
786 more directly proportional to intake (Hansen et al., 2023). The low plasma Zn concentration
787 observed on d 24 when pigs were fed 110 or 300 mg/kg of added Zn, compared to baseline

values on d 0, suggests that pigs were still actively establishing Zn pools, which may help to explain the quadratic response observed in fecal Zn excretion. For pigs fed diets containing more than 666 mg/kg, the incremental increase in fecal Zn excretion was expected, as Zn homeostasis is mainly regulated in the brush border membrane (Maares and Haase, 2020). When dietary Zn exceeds the nutritional requirement, the expression of ZIP4 transporters (main responsible for intestinal Zn absorption) is downregulated (Dalto et al., 2023), while ZnT-type transporters are upregulated to facilitate Zn efflux from enterocytes into the intestinal lumen (Liu et al., 2023), which results in greater fecal excretion.

The ATTD of Zn is dependent on the dietary Zn concentration (Ketata et al., 2024). Brugger et al. (2014) concluded that the ATTD of Zn in nursery pigs linearly increases until the dietary Zn concentration reaches 58 ± 4 mg/kg, after which a plateau is reached. Similarly, Ketata et al. (2024) concluded that there is a quadratic relation between the dietary Zn concentration and the ATTD of Zn, further supporting the existence of a saturation point. In contrast, the present study showed a tendency for a linear increase in ATTD of Zn with increasing Zn concentration in the low ABC-4 diets. However, this trend appeared to be primarily driven by the highest dietary Zn concentration, because, when pigs were fed diets containing 110 to 1,332 mg/kg of added Zn, ATTD of Zn remained relatively constant, suggesting a plateau in Zn absorption had been reached. This flat response may be explained by the limited capacity of the intestinal Zn transport mechanisms, as Zn homeostasis is tightly regulated to avoid toxicity (Maares and Haase, 2020). The increment in Zn ATTD when pigs were fed diets containing 2,000 mg/kg of added Zn could be explained by alternative routes of Zn absorption, such as passive diffusion. Passive diffusion has been shown to occur when luminal Zn concentrations exceed the capacity of the saturable, carrier-mediated transport

systems (Cousins, 1985; Cousins et al., 2006). Therefore, at high dietary Zn concentrations, passive diffusion may contribute more significantly to total Zn absorption, partially explaining the observed increase in ATTD. Another factor that may explain the improvements in ATTD of Zn in the low ABC-4 diets may be the acidification of the diet, as this process enhances mineral solubility in the small intestine by lowering digesta pH, which increases ionization and reduces complexation of minerals with dietary antagonists. Additionally, acidifiers may slow gastric emptying and optimize digestive enzyme activity, thereby facilitating mineral release and absorption (Jongbloed et al., 2000; Van Der Aar et al., 2017). Although low ABC-4 diets improved Zn absorption and ATTD of Zn, it remains unclear why fecal Zn excretion did not differ between formulation strategies. This lack of response may be explained by differences in dietary Zn concentration. Although both diets were formulated to contain the same added Zn, analyzed values indicated that Zn concentration in the low ABC-4 diets was 3% greater than in the high ABC-4 diets (1,951 vs. 1,899 mg/kg), resulting in greater Zn intake in pigs fed the low ABC-4 diets (1,147 vs. 1,056 mg/d). Because fecal Zn excretion was similar between formulations, these results suggest that pigs fed low ABC-4 diets excreted the same amount of Zn despite an 8% increase in Zn intake.

The primary value of these findings lies in their practical application. The increase in Zn utilization observed in pigs fed the low ABC-4 diets suggests that dietary acidification may serve as a viable strategy to optimize the use of Zn in nursery diets without compromising growth performance. These results have direct implications for swine nutrition programs seeking to balance animal productivity with environmental sustainability. However, more studies in this area are needed to validate the results under different scenarios.

In conclusion, increasing dietary Zn in low ABC-4 diets linearly increased ADG and ADFI during the experimental period. However, this did not translate into overall differences in performance (d 0 to 46). Fecal DM was not affected by dietary Zn level in low ABC-4 diets, but at the same dietary Zn concentration, low ABC-4 diets increased d 10 fecal DM compared with high ABC-4 diets. Zinc intake, fecal Zn excretion, Zn absorption, and d 24 plasma Zn quadratically increased as dietary Zn concentration increased. Although performance was not different between low and high ABC-4 diets when fed the highest ZnO concentrations, low ABC-4 diets increased Zn absorption, ATTD of Zn, and plasma Zn levels, suggesting that it might be a potential strategy to improve Zn utilization in nursery pigs.

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Table 3.1 Diet composition (as-fed basis)[1]

	Phase 1		Phase 2		Phase 3
Item ABC-4	Low	High	Low	High	
Ingredient, %					
Corn[2]	52.65	48.81	56.67	54.21	68.06
Soybean meal, 47.73% CP	14.63	14.66	23.73	23.75	28.12
Crystalline lactose	15.00	15.00	7.50	7.50	---
Microbial enhanced SBM[3]	9.00	---	6.25	---	---
Enzymatically treated SMB[4]	---	13.00	---	9.00	---
Spray-dried bovine plasma	2.50	2.50	---	---	---
Corn oil	2.00	2.00	1.00	1.00	---
Limestone	0.27	0.33	0.35	0.39	0.75
Monocalcium phosphate	1.15	1.05	1.15	1.05	0.85
Salt	0.78	0.78	0.80	0.80	0.60
L-Lys-HCl	0.43	0.43	0.45	0.45	0.55
DL-Met	0.27	0.25	0.26	0.25	0.21
L-Thr	0.21	0.21	0.22	0.23	0.23
L-Trp	0.06	0.04	0.06	0.05	0.05
L-Val	0.09	0.08	0.10	0.10	0.16
Vitamin premix[5]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15	0.15	0.15
Phytase[7]	0.08	0.08	0.08	0.08	0.03
Fumaric acid	0.50	---	0.50	---	---
Zinc oxide	---	0.40	---	0.26	---
TiO_2	---	---	0.50	0.50	---
TOTAL	100	100	100	100	100.0
Calculated analysis					
Standardized ileal digestible amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.30
Ile:Lys	56	57	58	59	53
Leu:Lys	119	113	119	115	113

Met:Lys	39	38	39	39	36
Met & Cys:Lys	60	61	60	60	57
Thr:Lys	65	65	65	65	63
Trp:Lys	20	20	20	20	19
Val:Lys	70	70	70	70	70
His:Lys	37	36	37	37	35
Total Lys, %	1.51	1.51	1.50	1.50	1.44
NE, kcal/kg	2,601	2,595	2,482	2,482	2,449
SID Lys:NE, g/Mcal	5.19	5.21	5.44	5.44	5.31
Crude protein, %	20.4	21.0	21.3	21.7	20.0
Lactose, %	15.0	15.0	7.5	7.5	0.0
Ca, %	0.50	0.53	0.55	0.57	0.64
P, %	0.55	0.59	0.59	0.60	0.56
STTD P, %[8]	0.50	0.51	0.49	0.49	0.43
ABC-4, meq/kg[9]	200	441	250	430	---

[1] Phases 1, 2, and 3 were fed from d 0 to 10, 10 to 24, and 24 to 46, respectively.

[2] In the low ABC-4 diets, ZnO (72% Zn) was added at the expense of corn to form the experimental diets. For phase 1, the added ZnO levels were 0, 0.05, 0.12, 0.26, and 0.40%. For phase 2, the added ZnO levels were 0, 0.03, 0.08, 0.17, and 0.26%. In both cases, these values correspond to treatments 1 through 5, respectively. Analyzed dietary Zn concentration for treatments 1 to 6 were 111, 388, 912, 1,569, 2,825, and 2,823 mg/kg in phase 1 and 294, 342, 707, 1,251, 1,951, and 1,899 mg/kg in phase 2, respectively.

[3] ME-PRO (Prairie Aquatech, Brookings, SD).

[4] HP 300 (Hamlet protein, Findlay, OH).

[5] Provided per kilogram of feed: 4,134 IU of vitamin A; 1,653 IU of vitamin D; 44 IU of vitamin E; 3.31 mg of vitamin K3; 33 μg of vitamin B_{12}; 8 mg of riboflavin; 28 mg of pantothenic acid; 50 mg of niacin.

[6] Provided per kilogram of feed: 110 mg of Zn as zinc sulfate; 17 mg of Cu as copper sulfate; 33 mg of Mn as manganese oxide; 110 mg of Fe as ferrous sulfate; 0.30 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite.

[7] Ronozyme Hiphos 2700 (dsm-firmenich, Parsippany,NJ) provided 2,000 FYT/kg for phases 1 and 2 and 811 FYT/kg in phase 3 with an assumed release of 0.14% STTD P in phases 1 and 2 and 0.12% STTD P in phase 3.

[8] STTD P = standardized total tract digestible phosphorus.

[9] ABC-4 = Acid binding capacity at a pH of 4. Analyzed ABC-4 values for treatments 1 to 6 were 205, 230, 233, 247, 273, and 430 meq/kg in phase 1 and 259, 266, 272, 280, 306, and 409 meq/kg in phase 2, respectively.

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Table 3.2. Effect of dietary Zn and ABC-4 value on nursery pig growth performance and fecal dry matter[1]

	Dietary Zn, mg/kg[2]									
Phase 1:	110	500	1,000	2,000	3,000	3,000		*P* =		
Phase 2:	110	333	666	1,332	2,000	2,000		Zn level[4]		
ABC-4[3]:	Low					High	SEM	Linear	Quadratic	ABC-4[5]
BW, kg										
d 0	6.0	6.0	6.0	6.0	6.0	6.0	0.74	0.845	0.957	0.998
d 10	7.7	7.7	7.8	7.8	8.0	8.0	0.84	0.006	0.695	0.676
d 24	13.4	13.4	13.5	13.6	14.0	13.9	1.20	0.015	0.424	0.675
d 45	28.5	28.4	28.6	28.5	28.9	28.7	1.71	0.381	0.788	0.730
Phase 1 (d 0 to 10)										
ADG, g	170	177	181	185	202	207	17.2	0.004	0.004	0.627
ADFI, g	194	196	201	199	203	211	14.1	0.348	0.855	0.383
G:F, g/kg	872	900	898	924	994	979	33.9	< 0.001	0.339	0.623
Phase 2 (d 10 to 24)										
ADG, g	412	405	408	412	430	419	28.2	0.103	0.311	0.420
ADFI, g	541	536	563	558	588	556	36.3	0.008	0.793	0.104
G:F, g/kg	762	756	727	739	732	753	12.0	0.089	0.274	0.207
Experimental period (d 0 to 24)										
ADG, g	311	310	313	315	335	331	20.6	0.023	0.286	0.689
ADFI, g	397	394	412	405	428	412	25.4	0.016	0.686	0.254
G:F, g/kg	785	785	762	778	783	802	12.1	0.969	0.233	0.199

Common phase (d 24 to 46)[6]										
ADG, g	685	679	689	678	676	673	24.1	0.540	0.817	0.834
ADFI, g	1,013	1,021	1,027	1,027	1,018	1,004	42.3	0.856	0.471	0.555
G:F, g/kg	677	665	671	660	665	672	7.1	0.185	0.332	0.449
Overall (d 0 to 46)										
ADG, g	490	487	493	487	498	494	21.8	0.463	0.609	0.736
ADFI, g	691	694	706	700	710	695	33.2	0.284	0.842	0.383
G:F, g/kg	709	701	698	696	702	712	5.1	0.405	0.101	0.168
Fecal dry matter, %[7]										
d 10	26.67	27.11	27.65	27.73	27.64	24.78	0.628	0.263	0.389	0.002
d 24	27.11	27.22	27.6	27.09	27.37	26.35	0.601	0.895	0.878	0.222

[1] A total of 360 pigs (Line 200 × 400, DNA, Columbus, NE; initially 6.0 ± 0.74 kg) were used in a 46-d growth study with five pigs per pen and 12 pens per treatment.

[2] All dietary phases were formulated to contain 110 mg/kg of Zn from $ZnSO_4$ as a basal level. Zn oxide (72% of Zn) was used as a Zn source to reach the level of the different experimental treatments.

[3] Low ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24 in their basal level (without ZnO inclusion), respectively. However, the ABC-4 capacity of these diets increased with the dietary Zn level. The high ABC-4 diets contained 441 and 430 meq/kg from d 0 to 10 and d 10 to 24, respectively.

[4] Linear and quadratic effects of the Zn level were evaluated in the low ABC-4 diets. Contrast coefficients were established based on the Zn concentrations fed within a specific dietary phase or the weighted average of Zn concentration when considering multiple dietary phases.

[5] Compares treatments with 3,000 and 2,000 mg/kg of Zn in phases 1 and 2, respectively, under the two formulation strategies (low and high ABC-4).

[6] All experimental groups were fed with the same common diet.

[7] Same three pigs per pen were sampled on d 10 and 24.

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Table 3.3 Effect of dietary Zn and ABC-4 on Zn balance at d 24 (dry matter-basis) and plasma Zn concentration[1]

	Dietary Zn, mg/kg[2]									
Phase 1:	110	500	1,000	2,000	3,000	3,000			*P* =	
Phase 2:	110	333	666	1,332	2,000	2,000		Zn level[4]		
ABC-4[3]:			Low			High	SEM	Linear	Quadratic	ABC-4[5]
Zn intake, mg/d	159	183	398	698	1,147	1,056	40.8	< 0.001	< 0.001	< 0.001
Fecal excretion of Zn, mg/d	126	154	308	578	876	878	33.2	< 0.001	< 0.001	0.921
Absorption, mg/d[6]	33	29	90	120	272	178	20.9	< 0.001	< 0.001	< 0.001
ATTD of Zn, %	20.5	15.7	22.8	17.3	23.7	16.7	2.81	0.074	0.148	0.002
Serum Zn, mg/L										
d 0[7]				0.81 ± 0.10			---	---	---	---
d 24	0.67	0.70	0.91	1.27	2.05	1.83	0.098	< 0.001	0.012	0.094

[1] Fecal samples from 216 pigs (Line 200 × 400, DNA, Columbus, NE. initially 6.0 ± 0.74 kg) were collected with three pigs per pen and 12 pens per treatment. Fecal samples were pooled on a pen basis, ensuring equal representation from the three pigs. Feed and fecal samples were analyzed in duplicated for Zn and TiO_2.

[2] All dietary phases were formulated to contain 110 mg/kg of Zn from ZnSO4 as a basal level. Zn oxide (72% of Zn) was used as a Zn source to reach the level of the different experimental treatments. Dietary analyzed Zn values were 294, 342, 707, 1,251, 1,951, and 1,899 mg/kg for treatments 1 to 6, respectively.

[3] Low ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24 in their basal level (without ZnO inclusion), respectively. However, the ABC-4 capacity of these diets increased with the dietary Zn level. The high ABC-4 diets contained 441 and 430 meq/kg from d 0 to 10 and d 10 to 24, respectively.

[4] Linear and quadratic effects of the Zn level were evaluated in the low ABC-4 diets. Contrast coefficients were established based on the Zn concentrations fed within a specific dietary phase or the weighted average of Zn concentration when considering multiple dietary phases.

[5] Compares treatments with 3,000 and 2,000 mg/kg of Zn in phases 1 and 2, respectively under the two formulation strategies (low and high ABC-4).

[6] Absorption = Zn intake – Fecal Zn excretion.

[7] At weaning, a blood sample from 10% of the pigs was collected, and serum Zn was analyzed.

Table 3.4Supplemental Table 1. Contrast coefficients used to evaluate linear and quadratic effects of Zn level in low ABC-4 diets.[1]

	Dietary Zn, mg/kg[2]					
Phase 1:	110	500	1,000	2,000	3,000	3,000
Phase 2:	110	333	666	1,332	2,000	2,000
ABC-4[3]:	Low					High
Phase 1						
Linear	-0.5157	-0.3498	-0.1370	0.2885	0.7140	0
Quadratic	0.5169	-0.0175	-0.4603	-0.5294	0.4903	0
Phase 2						
Linear	-0.502716	-0.358659	-0.143541	0.286694	0.718222	0
Quadratic	0.497932	0.020841	-0.454498	-0.553192	0.488917	0
Experimental period, common phase, and overall period						
Linear	-0.507116	-0.355966	-0.141543	0.288475	0.716149	0
Quadratic	0.504401	0.007672	-0.457585	-0.54428	0.489792	0

[1] Contrast coefficients were established based on the Zn concentrations fed within a specific dietary phase or the weighted average of Zn concentration when considering multiple dietary phases. Using the contr.poly function from the stats package from R-Studio.

Chapter 4 - Evaluation of a novel functional insoluble fiber source on growth performance and fecal dry matter in nursery pigs using different formulation strategies

ABSTRACT

Three experiments were conducted to evaluate the effects of a functional fiber ingredient (ValoproWin; VLPW) composed primarily of insoluble, poorly fermentable fiber on growth performance and fecal dry matter (**DM**) in nursery pigs. In Exp. 1, 300 weanling pigs (initially 5.6 ± 0.47 kg) were allotted to one of six dietary treatments in a 2 × 3 factorial arrangement with main effects of dietary formulation strategy (low acid-binding capacity to a pH of 4; **ABC-4**; high vs. conventional) and VLPW feeding duration (0, 10, or 24 d) with a fixed VLPW inclusion of 2.5%. In Exp. 2, 360 weanling pigs (6.3 ± 0.26 kg) were used to evaluate five VLPW levels (0, 1.75, 2.75, 3.75 and 5%) within a low ABC-4 diet and a positive control diet with no added VLPW and the ABC-4 was not considered (conventional). In Exp. 3, 335 weanling pigs (initially 5.6 ± 0.87 kg) were used in a 2 × 2 + 1 factorial evaluating VLPW addition (2.5 or 5%) and formulation method (diluted or adjusted), with a control diet without VLPW. Across all three experiments, pigs were fed experimental diets during the first two dietary phases (approximately from weaning at 21 d of age to d 24 postweaning), followed by a common phase 3 diet. In experiments 1 and 2, no nutrient adjustments were made to the diet when VLPW was added, which resulted in nutrient dilution proportional to the VLPW level. The effects of VLPW on

growth performance were inconsistent. In Exp. 1, VLPW feeding duration did not affect growth performance, whereas in Exp. 2, increasing VLPW tended (quadratic, $P = 0.067$) to decrease overall average daily gain (**ADG**). In Exp. 3, pigs fed diets with 5% of VLPW during the experimental period tended ($P = 0.057$) to have decreased gain:feed ratio compared with those fed diets with 2.5% VLPW. Across all experiments, although low ABC-4 diets or diet dilution affected growth performance during the experimental period, no overall differences were observed. In Exp. 1, added VLPW increased fecal DM at d 10 ($P = 0.019$). In Exp. 2, fecal DM increased (linear, $P < 0.005$) on d 10 and 24 as VLPW increased. Similarly, in Exp. 3, increasing VLPW increased (linear, $P < 0.001$) fecal DM on d 10, regardless of formulation method. In conclusion, VLPW increased fecal DM in weanling pigs but had limited and inconsistent effects on growth performance across multiple dietary strategies and inclusion levels.

INTRODUCTION

Dietary fiber has traditionally been defined as a carbohydrate consisting of 10 or more monomeric units that resist hydrolysis by endogenous enzymes in the gastrointestinal tract of monogastric animals (Theander et al., 1995; Howlett et al., 2010). Consequently, fiber has historically been considered an antinutritional factor in swine nutrition, particularly in nursery diets, due to its association with reduced nutrient digestibility and growth performance in young pigs (Berrocoso et al., 2015; Jha and Berrocoso, 2015a).

Advancements in analytical techniques and an improved understanding of the nutritional role of fiber has led to more precise classifications of dietary fiber based on its physicochemical properties, particularly solubility (Bach, 2001; Montagne et al., 2003). Fiber is now commonly categorized as either soluble or insoluble, depending on its solubility in water (Lattimer and

Haub, 2010). Soluble fiber can serve as a fermentable substrate for beneficial bacteria in the gastrointestinal tract (Hu et al., 2023), while insoluble fiber helps to regulate intestinal transit and reduce the colonization of pathogenic microbes (Li et al., 2020; Dorado-Montenegro et al., 2023). Despite these benefits, the effects of dietary fiber on growth performance remain inconsistent (Grześkowiak et al., 2022). Variations in outcomes are likely influenced by fiber type, inclusion level, and interaction with other dietary components (Lv et al., 2022; Zhao et al., 2023).

ValoproWin (**VLPW**; MiXscience, Bruz, France) is a functional fiber ingredient specifically designed for nursery pig diets. It is mainly composed of a purified source of indigestible fiber, oat hulls, and yeast autolysate. According to analyses conducted using the sequential fiber fractionation method (Van Soest et al., 1991), VLPW contains 75.3% neutral detergent fiber (**NDF**), 61.3% acid detergent fiber (**ADF**), and 19.4% lignin, indicating a high proportion of structural carbohydrates. The composition suggests a predominance of insoluble, poorly fermentable fiber components, which may contribute to increased intestinal motility and enhanced gut barrier function in weaned pigs (Montagne et al., 2003; Jha and Berrocoso, 2015a). The inclusion of yeast autolysate may also provide functional compounds such as β-glucans and mannans, which have been associated with immune modulation and pathogen binding (Choi and Kim, 2023).

To the best of our knowledge, no published studies have evaluated the effect of VLPW on nursery growth performance or fecal dry matter (**DM**). Therefore, the objectives of the present studies were to determine the influence of VLPW on growth performance and fecal DM of nursery pigs. Within three experiments, sub-objectives were to determine how the response was affected by: (1) VLPW feeding duration and dietary acid binding capacity at a pH of 4 (ABC-4); (2) VLPW

inclusion level; and (3) potential interactions between VLPW inclusion level and dietary formulation strategies (nutrient dilution vs. nutrient adjustment).

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments (IACUC # 4485.54, 4943, and 4942). Experiment (**Exp.**) 1 was conducted between November and December 2023 in two independent rooms within the same barn located at the Kansas State University Swine Teaching Center in Manhattan, KS. Each pen (1.2 × 1.5 m) provided approximately 0.36 m^2 per pig and was equipped with a 4-hole dry feeder and nipple waterer. Experiment 2 was conducted between August and September 2024 in two identical barns located at the Kansas State University Segregated Early Weaning facility in Manhattan, KS. Each pen (1.2 × 1.5 m) provided approximately 0.36 m^2 per pig and was equipped with a 4-hole dry feeder and a cup drinker. Experiment 3 was conducted between June and July 2025 in a single barn located at the Kansas State University Swine Teaching Center in Manhattan, KS. Each pen (1.5 × 1.5 m) provided approximately 0.45 m^2 per pig and was equipped with a 6-hole dry feeder and nipple waterer. All facilities were mechanically ventilated. Room temperatures were set at 32.2°C during the first 10 d after weaning and then gradually decreased by 0.28°C daily until the set point reached 25°C.

Animals and diets

In Exp. 1, a total of 300 pigs (Line 241 × 600, DNA, Columbus, NE) initially weighing 5.6 ± 0.47 kg were used in a 42-d study. Pigs were weaned at approximately 19 d of age and divided into 5 body weight (BW) blocks, each containing 20% of the pigs. Pigs were then

randomly assigned to pens within BW block and pens of pigs were allotted to one of six dietary treatments. Each pen had 5 pigs, and there were 10 pens per dietary treatment. The two identical rooms had an equal representation of dietary treatments and BW blocks. Pigs were fed experimental diets for the first two phases, lasting 10 and 14 d, respectively. All pigs were then fed a common phase 3 diet from d 24 to 42. Treatments were arranged in a 2 × 3 factorial with the main effects of formulation strategy (low ABC-4 or conventional) and VLPW feeding duration (0, 10, or 24 d). Low ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24, respectively, without pharmacological levels of Zn from ZnO (Table 1). Pure lactose and specialty soy protein concentrate (AX3 Digest, Protekta, Newport Beach, CA) were used in these diets as lactose and protein concentrate sources, respectively, along with fumaric and formic acid to lower pH. Other treatment diets were formulated without considering ABC-4 values (conventional) that were 493 and 470 meq/kg and 2,990 and 1,910 ppm of Zn from d 0 to 10 and 10 to 24, respectively. Spray-dried whey and enzymatically treated soybean meal (HP300, Hamlet Protein, Findlay, OH) were used as lactose and specialty soy sources, respectively. ValoproWin was added at 2.5% of the diet, without any formulation adjustments resulting in slightly different chemical composition between diets. By design, this resulted in a dilution of approximately 2.5% in all nutrients compared to the control diet. Pigs and feeders were weighed on d 0, 10, 24, and 42 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed (**G:F**) ratio. Fecal samples were collected via rectal palpation from the same three pigs per pen on d 10 and 24 of the study to determine fecal DM.

In Exp. 2, a total of 360 barrows (Line 200 × 400, DNA, Columbus, NE) initially 6.3 ± 0.26 kg were used in a 42-d study. The pigs were weaned at approximately 21 d of age and divided into six BW categories. The pigs were then randomly assigned to pens within the BW

category and pens were allotted to one of six dietary treatments. Each pen had five pigs and there were 12 pens per treatment. The two identical barns had an equal representation of dietary treatments and BW categories. Pigs were fed experimental diets for the first two dietary phases, lasting 10 and 14 d, respectively. From d 24 to 42 of the experiment, all pigs were fed a common phase 3 diet. Dietary treatments included a low ABC-4 diet with incremental levels of VLPW (0, 1.75, 2.50, 3.75, or 5% of the diet), as well as a positive control diet without VLPW, where the ABC-4 capacity of the diet was not controlled (conventional) and pharmacological levels of Zn from ZnO were added during phases 1 and 2 (Table 2). The formulation strategy was the same as Exp. 1, differing only in the specialty soy ingredients, where specialty soybean meal (AX3 Digest; Proteka, Newport Beach, CA) was replaced with microbial enhanced protein (ME-Pro, Prairie Aquatech, Brookings, SD). For VLPW diet preparation, a large batch of basal low ABC-4 phase 1 and 2 diets were mixed and placed in separate bins. A portion of the basal diet was then blended with VLPW to create experimental diets, without adjusting the nutrient levels, resulting in a reduction in the chemical composition of the diets as VLPW increased. Pigs and feeders were weighed on d 10, 24, and 42 to determine ADG, ADFI, and G:F. Fecal samples were collected via rectal palpation from the same three pigs per pen on d 10 and 24 of the study.

In Exp. 3, a total of 335 pigs (Line 241 × 600, DNA, Columbus, NE) initially weighing 5.6 ± 0.87 kg were used in a 45-d study. The pigs were weaned at approximately 19 d of age and divided into two BW categories. Pigs were then randomly assigned to pens within the BW categories, and pens were allotted to one of five dietary treatments. Each pen had four or five pigs, and there were 14 pens per treatment. Pigs were fed experimental diets for the first two dietary phases, lasting 10 and 13 d, respectively. From d 23 to 45 of the experiment, all pigs were fed a common phase 3 diet. Dietary treatments were arranged in a 2 × 2 + 1 factorial, with the

main effects of VLPW (2.5 or 5%) and formulation method (diluted or adjusted). An additional control treatment, which contained no VLPW, was used for comparison. In the diluted diets, VLPW was added at the expense of the complete diet without further adjustments to the formulation, resulting in nutrient dilution proportional to the VLPW inclusion. In contrast, adjusted diets were reformulated to maintain a similar nutrient composition to the control diet, regardless of VLPW inclusion level (Table 3). Thus, adjusted diets contained increasing soybean meal, soy oil, and monocalcium phosphate to maintain similar amino acid, energy, and phosphorus levels across diets as VLPW increased. Pigs and feeders were weighed on d 10, 23, and 45 to determine ADG, ADFI, and G:F. Fecal samples were collected via rectal palpation from the same three pigs per pen on d 10 and 23 of the study.

For all experiments, experimental diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The first two phases were fed in pellet form, and the common phase 3 diet in meal form. Feed samples by phase (approximately 2 kg) were collected during the bagging process. Samples were collected from every other bag for phases 1 and 2 and from every fifth bag for phase 3.

Chemical analysis

Feed samples were analyzed in duplicate for crude protein (CP; N × 6.25) using the Dumas combustion method (AOAC, 2006; Method 990.03) with a LECO TruMac N analyzer (LECO Corporation., St. Joseph, MI). Neutral detergent fiber, ADF, and acid lignin were determined sequentially according to the procedures of Van Soest et al. (1991) using an Ankom Fiber Analyzer (Ankom Technology, Macedon, NY).

Fecal samples were stored at 4°C. Samples were dried at 55 °C for 48 h and loss of weight was used to determine the percentage of fecal DM.

Statistical Analysis

All experiments were analyzed using RStudio [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria], and data were analyzed as a generalized randomized block design with pen serving as the experimental unit. The performance data model utilized dietary treatment as a fixed effect and the BW block as a random effect. Barn also served as a random effect in experiments 1 and 2. In Exp. 1, contrast coefficients were set to evaluate the main effect of VLPW feeding duration (0, 10, or 24 d), formulation strategy (low ABC-4 diets or conventional with and without the inclusion of ZnO), and their interaction.

In Exp. 2, linear and quadratic effects of increasing dietary VLPW on performance and fecal dry matter were evaluated for the first five treatments. Specific contrasts compared the low-ABC-4 diets and conventional with no added VLPW.

In Exp. 3, contrast coefficients were set to evaluate the main effect of VLPW inclusion (2.5 or 5%), formulation method (diluted or adjusted), and their interaction. The linear and quadratic effects of increasing VLPW (0, 2.5, or 5%) within each formulation method on performance and fecal DM were evaluated. Contrast coefficients were established based on added VLPW level.

For all experiments, fecal dry DM was analyzed as repeated measurements, accounting for the two sampling days. In addition to the performance criteria included in each model within each experiment, pen was included as a random effect to account for subsampling associated with multiple individual pigs analyzed from each pen. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

The CP, NDF, ADF, and lignin of experimental diets were consistent with formulated values, considering analytical variation (Table 1). No interactions were observed between VLPW feeding duration and formulation strategy on growth performance and fecal DM (Table 4). For the main effect of VLPW feeding duration, there were no differences observed between treatments for any of the performance criteria or periods. However, pigs fed diets containing 2.5% VLPW had greater ($P = 0.019$) fecal DM on d 10 than those fed diets without VLPW. For d 24 fecal DM, no differences between VLPW feeding duration were observed.

For the main effect of diet formulation strategy, during phase 1 (d 0 to 10), phase 2 (d 10 to 24) and the experimental period (d 0 to 24), pigs fed conventional diets with ZnO had greater ($P \leq 0.05$) d 10 and 24 BW, ADG, and ADFI than those fed low ABC-4 diets without ZnO. However, no differences were observed for G:F between both formulation strategies. When transitioned to a common phase 3 diet (d 24 to 42), pigs previously fed diets with low ABC-4 without ZnO tended ($P = 0.059$) to have increased ADG; however, no differences were observed for ADFI and G:F. Overall (d 0 to 42), no differences were observed between the two formulation strategies for any of the performance criteria. There was no difference in fecal DM between formulation strategies on d 10; however, on d 24, pigs fed low ABC-4 diets without ZnO had greater ($P = 0.004$) fecal DM than those fed conventional diets with ZnO.

Experiment 2

The CP, NDF, and ADF of experimental diets were consistent with formulated values, considering analytical variation (Table 5).

For phase 1 (d 0 to 10), increasing VLPW decreased d 10 BW (linear, $P = 0.033$) and ADG (linear, $P = 0.019$; Table 6). There were no differences in ADFI. As a result, G:F decreased (linear, $P = 0.003$) as VLPW increased. No differences in d 10 BW, ADG, and ADFI were observed between the low ABC-4 and conventional diets. However, pigs fed low ABC-4 diets had increased ($P = 0.007$) G:F ratio.

For phase 2 (d 10 to 24), d 24 BW decreased (quadratic, $P = 0.049$) as VLPW increased with no change in BW between 0 and 1.75%, followed by a reduction at 2.50 and 3.75%, and returning to the control values with 5.0% VLPW. Average daily gain tended (quadratic, $P = 0.055$) to decrease as VLPW increased, where ADG decreased as VLPW increased from 0 to 3.75% and then returned to the control values at 5%. No response to increasing VLPW was observed for ADFI, and as a result, G:F decreased (quadratic, $P = 0.007$). The gain:feed ratio decreased between 0 and 2.5% followed by an increase between 3.75 and 5.0%. There were no differences between low ABC-4 and conventional with added ZnO diets in ADG. Pigs fed low ABC-4 diets had decreased ADFI ($P = 0.025$) compared with pigs fed conventional diets. As a result, low ABC-4 diets tended ($P = 0.076$) to increase G:F ratio.

For the experimental period (d 0 to 24), ADG decreased then increased (quadratic, $P = 0.029$) with increasing VLPW. Average daily gain decreased between 0 and 3.75% and then increased at 5%. No response to increasing VLPW was observed for ADFI. As a result, G:F decreased then increased (quadratic, $P = 0.006$) as VLPW increased, with G:F becoming poorer as VLPW increased from 0 to 2.50% followed by an improvement between 3.75 and 5.0%. There were no differences between pigs fed the low ABC-4 or conventional diets for ADG. However, pigs fed the low ABC-4 diets had decreased ($P = 0.028$) ADFI, resulting in increased ($P = 0.010$) G:F compared to those fed the conventional diets.

For the common period (d 24 to 42), no response was observed in pigs previously fed VLPW during the experimental period for ADG and ADFI. However, G:F tended (linear, P = 0.091) to increase as VLPW increased in phase 1 and 2 diets. No differences between pigs previously fed low ABC-4 or conventional diets were observed for any of the performance criteria.

Overall (d 0 to 42), increasing VLPW during the experimental period (d 0 to 24) tended to decrease then increase 42 BW (quadratic, P = 0.083), where BW decreased as VLPW increased from 0 to 3.75% in phases 1 and 2 and then returned to the control values at 5.0%. Similarly, ADG tended to decrease then increase (quadratic, P = 0.067), with the lowest ADG observed in pigs fed 2.50% and returned to the control values at 5.0%. No responses were observed for ADFI and G:F. No differences in pigs fed low ABC-4 or conventional diets were observed for any performance criteria.

On d 10 and 24, fecal DM increased (linear, P < 0.005) with increasing VLPW. Pigs fed low ABC-4 diets had increased (P = 0.039) fecal DM on d 10; however, no differences were observed between formulation strategies on d 24.

Experiment 3

The CP, NDF, ADF, and lignin of experimental diets were consistent with formulated values, considering analytical variation (Table 3). No VLPW and formulation method interactions were observed for any of the performance criteria (Table 7). For phase 1 (d 0 to 10), no VLPW or formulation method main effects were observed for any of the performance criteria. Similarly, no linear or quadratic interactions or main effects were observed for any of the performance criteria with increasing VLPW.

For phase 2 (d 10 to 23), pigs fed the diluted diets had greater ADFI ($P = 0.043$) than those fed adjusted diets. However, no differences were observed in d 23 BW and ADG between formulation method. As a result, G:F increased ($P = 0.011$) in pigs fed the adjusted diets compared with those fed diluted diets. No main effects of increasing VLPW were observed for any of the performance criteria during this period. Similarly, no linear or quadratic interactions or main effects were observed for any of the performance criteria as VLPW increased.

For the experimental period (d 0 to 23), pigs fed diluted diets tended ($P = 0.091$) to have greater ADFI than those fed adjusted diets. No differences were observed in ADG between formulation methods. Consequently, pigs fed adjusted diets tended ($P = 0.084$) to have greater G:F than those fed diluted diets. No VLPW main effects were observed for ADG and ADFI; however, pigs fed diets containing 5% VLPW tended ($P = 0.054$) to have decreased G:F compared with those fed diets containing 2.5% VLPW. Increasing the VLPW resulted in no linear or quadratic interactions or main effects for d 23 BW, ADG, and ADFI. However, a tendency for a linear interaction ($P = 0.087$) was observed for G:F, where G:F tended to linearly decrease ($P = 0.055$) as VLPW increased in diluted diets but not in the adjusted diets.

When pigs were fed a common diet during phase 3 (d 23 to 45), no main effects of formulation method were observed for any of the performance criteria. Pigs previously fed diets with 5% VLPW during the experimental period tended ($P = 0.063$) to have greater ADFI than those fed 2.5% VLPW; however, no effect of VLPW level was observed for d 45 BW, ADG, or G:F. Increasing VLPW during the experimental period resulted in no linear or quadratic interactions or main effects for ADFI and G:F during the common phase. However, despite the lack of interactions, ADG tended to increase (linear, $P = 0.097$) as VLPW increased during the experimental period in diluted diets but not in adjusted diets.

For the overall period (d 0 to 45), no main effect of formulation method was observed for any performance criteria. Pigs fed diets with 5% VLPW during the experimental period tended ($P = 0.057$) to have decreased G:F compared with those fed 2.5% VLPW; however, no effect of VLPW level was observed for ADG and ADFI. No linear or quadratic effects were observed for any of the performance criteria with increasing VLPW during the experimental period.

For fecal DM, no interactions between sampling day and dietary treatment or a significant main effect of the sampling day were observed. Similarly, no interactions or main effects of formulation method or increasing VLPW were observed for d 10 and 23 fecal DM. However, increasing VLPW increased (linear, $P < 0.001$) fecal DM on d 10, independent of formulation method. However, on d 24, fecal DM tended to increase (linear, $P = 0.098$) with increasing VLPW in adjusted diets but not in diluted diets.

DISCUSSION

The addition of fiber-rich ingredients in nursery pig diets has emerged as a nutritional approach to enhance intestinal health by stimulating gut motility, modulating microbiota composition, and improving gut barrier function (Agyekum and Nyachoti, 2017). However, these positive effects do not always result in improvements in growth performance, possibly due to the complex interactions between fiber type, physicochemical properties, formulation strategy, and dietary composition, as well as intrinsic animal factors such as age, health status, and genetics (Berrocoso et al., 2015; Agyekum and Nyachoti, 2017; Hu et al., 2023).

The inclusion of fiber-rich ingredients in nursery diets without adjusting nutrient composition has been previously reported (Batson et al., 2021b; Laskoski et al., 2021). This approach is based on the assumption that pigs regulate their voluntary feed intake based on energy intake (NRC, 2012). Therefore, reducing dietary energy density through the inclusion of

fibrous ingredients may stimulate voluntary feed intake, thereby increasing the intake of nutrients other than energy. While adding fiber is a strategy to promote feed intake and overall performance in nursery pigs, other nutritional strategies have also been proposed to optimize pig growth and gut health. Among these, acidification of the gastrointestinal tract has been proposed to optimize nutrient utilization (Partanen and Mroz, 1999) and reduce colonization by pathogenic bacteria (Ravindran and Kornegay, 1993). However, it remains unclear whether an additive effect exists when combining acidification with other nutritional strategies. Therefore, the present series of experiments evaluated the interactions between dietary acidification and fiber, using VLPW as the dietary fiber source.

Experiment 1 was designed to evaluate the effects of VLPW feeding duration and low ABC-4 compared with a more conventional formulation strategy (with pharmacological additions of Zn) on growth performance and fecal DM. The results indicated that for growth performance, there was no interaction between formulation strategy and VLPW feeding duration, and contrary to what was expected, the use of VLPW did not influence growth performance. These findings suggest that 2.5% VLPW in corn–soybean meal-based diets may not have been enough to promote feed intake and improve growth performance. Consequently, Exp. 2 was conducted to evaluate increasing levels of VLPW in low ABC-4 diets. Contrary to expectations, performance responses were negative, particularly during the experimental period, suggesting that added fiber without maintaining the main nutrient concentration (controlled amino acids, energy, and macro-minerals) may have decreased nutrient intake. Given these results, it was hypothesized that formulation method could influence the response to added fiber. Therefore, Exp. 3 was designed to evaluate whether adjusting diets to account for the dilution effect of VLPW would mitigate the negative effects previously observed.

In European-type diets, the inclusion of insoluble fiber has shown positive effects on growth performance (Gerritsen et al., 2012; Superchi et al., 2017) and gut health (Molist et al., 2011). The beneficial response in these feeding systems may be attributed to the higher supply of fermentable non-starch polysaccharides from ingredients such as wheat and barley (NRC, 2012), which, in conjunction with the insoluble sources, provide adequate substrate for beneficial intestinal bacteria (Chen et al., 2020) and the production of beneficial metabolites such as volatile fatty acids (Jha and Leterme, 2012). However, when applied to corn-soybean meal-based diets, positive responses of insoluble fiber on performance and gut health are less frequently observed. This discrepancy is likely due to differences in fiber type and fermentability, as corn or soybean meal-derived fiber is primarily insoluble and less fermentable than fiber fractions from small grains (Jha et al., 2019; Lv et al., 2022).

The lack of a positive growth response to VLPW on growth performance in the present experiments may also be attributed to the ratio of soluble to insoluble fiber and the health status of the pigs. Insoluble fiber can increase digesta passage rate and reduce nutrient digestibility when not properly balanced with fermentable fractions, potentially limiting the anticipated benefits on health and growth performance (Jha and Berrocoso, 2015; Acosta et al., 2020). Additionally, nursery pigs in high-health production systems may benefit less from fiber supplementation compared with pigs reared under health-challenging conditions (Berrocoso et al., 2015) where fiber can contribute to stabilizing gut microbiota and reducing pathogen colonization (Pluske et al., 2018). Collectively, these observations underscore the importance of optimizing the balance between soluble and insoluble fiber in nursery diets and highlight the need for further research to determine the optimal fiber composition under varying formulation strategies and farm health conditions.

Despite the absence of published information directly evaluating VLPW in nursery diets, comparisons of the current results can be made with studies assessing the effect of insoluble fiber and yeast autolysate on nursery pig performance. Similar to the results observed in Exp. 1, others have reported a lack of significant responses to increasing levels of insoluble fiber in corn-soybean meal-based diets on nursery pig performance. Batson et al. (2021a) indicated that the addition of 4% wheat middlings in corn–soybean meal-based nursery diets increased dietary NDF from 4.6 to 6.0% in phase 1 and from 6.0 to 7.2% in phase 2 but did not affect growth performance in any of the phases compared with corn–soybean meal diets formulated without pharmacological levels of Zn. Similarly, Laskoski et al. (2021) observed that the addition of 4% coarse wheat bran in corn–soybean meal-based nursery diets without pharmacological levels of Zn did not influence growth performance. Also, Batson et al. (2021b) observed no changes in performance associated with the addition of 3 fiber sources (4% coarse wheat bran, 1.85% oat hulls, or 1.55% cellulose) in corn-soybean meal-based diets. Collectively, these findings suggest that in corn-soybean meal-based diets, moderate additions of insoluble fiber, such as those evaluated in Exp. 1, may not affect nursery pig growth performance.

In growing pigs fed corn–soybean meal-based diets, Petry et al. (2020) observed a decrease in ADG and G:F with diets containing 30% corn bran without solubles, which increased the NDF content of the diet from 7.54% to 21.91%. Although the NDF levels evaluated in that experiment are not directly comparable with those tested in the present studies, the observed decrease in growth performance underscores the potential negative consequences of excessive insoluble fiber inclusion, which is mainly related to a dilution effect of dietary energy. Similarly, De Jong et al. (2014) observed that increasing NDF concentration of nursery corn–soybean meal-based diets from 8.1 to 11.6% by increasing wheat middlings resulted in a linear reduction in

ADG and ADFI, whereas no effect was observed on G:F. Although the NDF concentrations in that study were similar to those evaluated in Exp. 2 of the present project, the quadratic responses for ADG and G:F observed herein differ from the linear effects observed by De Jong et al. (2014). This discrepancy may be attributed to differences in the chemical composition of the fiber sources, as the addition of VLPW in the current experiment likely contributed additional nutritional factors beyond fiber, which supported improved performance.

The increased ADFI observed during the experimental period in Exp. 3, especially in the diluted diets, was expected, as in pigs, voluntary feed intake is largely regulated by dietary energy, meaning that when dietary energy is reduced, pigs typically increase feed intake to maintain a constant energy intake (NRC, 2012). In contrast to the present results, previous studies have reported improvements in ADG and feed efficiency when fiber ingredients are added to diets formulated to maintain the same energy density as control diets (Gasa et al., 2010; Molist et al., 2011; Zhao et al., 2018). Such improvements may be related to enhanced intestinal health, which could reduce nutrient demands for immune function, thereby allowing greater nutrient utilization available for growth.

Autolyzed yeast (*Saccharomyces cerevisiae*) is one of the components in VLPW, and has been observed to improve growth performance in nursery pigs through mechanisms such as enhanced gut health, immune modulation, and nutrient absorption. Barducci et al. (2024) observed that replacing blood plasma with autolyzed yeast, with or without immunomodulators such as β-glucans, improved feed efficiency and reduced the need for feed-grade medications. Similarly, Correia et al. (2024) observed that added autolyzed yeast increased ADG and G:F by improving intestinal barrier integrity and upregulating the expression of nutrient transporters and antioxidant enzymes in the jejunum. Namted et al. (2022) also observed positive effects of added

autolyzed yeast on both growth performance and gut health in weaned pigs, likely attributed to bioactive components such as β-glucans and mannan-oligosaccharides. Collectively, these findings suggest that autolyzed yeast is a viable nutritional strategy to enhance growth performance and intestinal health in nursery pigs. However, the lack of responses observed in the present experiments may indicate potential interactions between fiber and autolyzed yeast that attenuated the response, or that the concentration of autolyzed yeast in the VLPW product was insufficient to elicit measurable improvements in growth performance.

The consistent positive response to the VLPW on fecal DM was expected, as insoluble fibers, such as cellulose and hemicellulose, increase digesta bulk and water-holding capacity, which can lead to firmer feces and more consistent fecal output (Wilfart et al., 2007; Jha and Berrocoso, 2015). In addition, the increased bulk accelerates digesta passage rate, which may reduce the proliferation of pathogenic microbiome in the gastrointestinal tract by limiting their colonization time and substrate availability (Canibe et al., 2022). This mechanical effect is complemented by a greater microbiome diversity and stimulation of hindgut fermentation (Yong et al., 2025), which together creates optimal gastrointestinal conditions that promote intestinal health, resulting in greater fecal DM. Similar to the present study, an improvement in fecal DM associated with the use of insoluble dietary fiber (Chen et al., 2020; Batson et al., 2021b) and autolyzed yeast (Lee et al., 2021) in nursery pigs have been previously reported.

As previously described, in all experiments, VLPW was added to low ABC-4 diets without pharmacological levels of Zn. In Exp. 1 and 2, the conventional diets formulated without consideration of ABC-4 contained pharmacological levels of Zn, indicating that differences in performance between the two formulation strategies reflect the combined effects of ABC-4 and pharmacological levels of Zn, making it impossible to isolate the individual contribution of each

factor. When diets did not contain pharmacological levels of Zn, the use of low ABC-4 diets improved growth performance compared with non-controlled ABC-4 diets (Landero et al., 2024; Stas et al., 2025). However, these same authors reported that when pharmacological levels of Zn from ZnO were included with the use of low ABC-4 diet, the combination did not provide any performance advantage, which is consistent with the results from Exp. 1 and 2. The lack of response to low ABC-4 diets under these conditions may be associated with factors beyond modulation of the gastrointestinal microbiota.

Pharmacological levels of Zn have been shown to increase circulating ghrelin, a hormone that in its exogenous form stimulates feed intake and growth in pigs (De Mille et al., 2022). In addition, improvements in intestinal morphology, including greater villus height, have been reported with pharmacological Zn addition (Peng et al., 2019), which may further enhance nutrient utilization and growth performance. Despite the positive effects of conventional diets with pharmacological levels of Zn on growth performance during the experimental period, these responses were not maintained overall. This response is consistent with previous reports showing that the advantages of pharmacological levels of Zn tend to disappear once pigs transition to a common diet (Carlson et al., 1999; Stas et al., 2025). The reduction in performance may be associated with reductions in circulating ghrelin, as well as restructuring of the gastrointestinal microbiome, which together could contribute to a reduction in growth rate. Consistent with previous reports (Arroyave-Jaramillo et al., 2025; Stas et al., 2025), low ABC-4 diets improved fecal DM, possibly by creating gastrointestinal conditions favorable for beneficial bacteria such as *Lactobacillus* spp., which can inhibit *E. coli* adhesion.

In conclusion, considering the results of the three experiments collectively, the addition of VLPW had minimal effects on growth performance; however, it may serve as a nutritional

approach to increase fecal DM. Within corn–soybean meal-based diets, 5% VLPW improved fecal DM while maintaining growth performance.

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

	Phase 1				Phase 2				Phase 3
	Low ABC-4		Conventional		Low ABC-4		Conventional		
ZnO:	No	No	Yes	Yes	No	No	Yes	Yes	
Item ValoproWin:	No	Yes	No	Yes	No	Yes	No	Yes	
Ingredient, %									
Corn	50.76	49.49	48.23	47.03	57.17	55.74	55.17	53.79	68.06
Soybean meal, 47.7% CP	16.30	15.89	16.27	15.86	22.40	21.84	26.06	25.41	28.12
Crystalline lactose	15.00	14.63	----	----	7.50	7.31	----	----	----
Spray-dried whey powder	----	----	20.85	20.33	----	----	10.40	10.14	----
Spray-dried bovine plasma	2.50	2.44	2.50	2.44	----	----	----	----	----
Specialty soybean meal[2]	9.38	9.14	----	----	7.50	7.31	----	----	----
Enzymatically treated soybean meal[3]	----	----	7.75	7.56	----	----	4.00	3.90	----
VLPW[4]	----	2.50	----	2.50	----	2.50	----	2.50	----
Corn oil	2.00	1.95	2.00	1.95	1.00	0.98	1.00	0.98	----
Monocalcium phosphate, 21% P	0.93	0.90	0.20	0.20	1.00	0.98	0.60	0.59	0.85
Limestone	0.35	0.34	0.33	0.32	0.51	0.50	0.52	0.51	0.75
Salt	0.65	0.63	0.28	0.27	0.70	0.68	0.55	0.54	0.60
L-Lys-HCl	0.44	0.43	0.36	0.35	0.49	0.47	0.45	0.44	0.55
DL-Met	0.19	0.18	0.16	0.16	0.19	0.19	0.19	0.19	0.21
L-Thr	0.19	0.19	0.14	0.13	0.21	0.20	0.19	0.19	0.23
L-Trp	0.05	0.05	0.03	0.03	0.05	0.05	0.04	0.04	0.05
L-Val	0.08	0.08	0.07	0.06	0.10	0.10	0.12	0.11	0.16
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[6]	0.25	0.24	0.25	0.24	0.25	0.24	0.25	0.24	0.25
Phytase[7]	0.06	0.05	0.06	0.05	0.06	0.05	0.06	0.05	0.03
Zinc oxide	----	----	0.40	0.39	----	----	0.25	0.24	----
Fumaric acid[8]	0.38	0.37	----	----	0.38	0.37	----	----	----
Formic acid[9]	0.36	0.35	----	----	0.36	0.35	----	----	----
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis									

Standardized ileal digestible amino acids, %									
Lys	1.36	1.33	1.36	1.33	1.35	1.32	1.35	1.32	1.30
Ile:Lys	56	56	58	58	58	58	58	58	53
Leu:Lys	116	116	118	118	116	116	115	115	113
Met:Lys	34	34	32	32	35	35	35	35	36
Met & Cys:Lys	56	56	56	56	56	56	56	56	57
Thr:Lys	63	63	63	63	63	63	63	63	63
Trp:Lys	20	20	20	20	20	20	20	20	19
Val:Lys	70	70	70	70	70	70	70	70	70
His:Lys	36	36	35	35	36	36	36	36	35
Net energy, kcal/kg	2,599	2,533	2,608	2,544	2,500	2,438	2,502	2,438	2,449
SID Lys:NE	5.23	5.25	5.21	5.23	5.40	5.41	5.40	5.41	5.31
CP, %	21.1	20.5	21.0	20.5	21.3	20.8	21.2	20.7	20.0
Ca, %	0.50	0.49	0.49	0.48	0.59	0.57	0.59	0.58	0.64
P, %	0.53	0.51	0.52	0.50	0.56	0.56	0.56	0.56	0.56
STTD P, %[10]	0.46	0.45	0.46	0.45	0.46	0.45	0.46	0.45	0.43
Crude fiber, %	1.64	2.97	1.88	3.11	2.00	3.33	2.26	3.57	2.44
NDF, %[11]	5.96	7.70	6.09	6.52	7.05	8.75	7.35	9.05	8.51
ADF, %[12]	2.32	3.80	2.53	4.17	2.83	4.29	3.11	4.57	3.45
Lignin, %	0.34	2.23	0.33	2.22	0.43	2.32	0.46	2.35	0.53
Analyzed composition									
Crude protein, %	19.1	19.2	20.0	19.7	19.8	19.6	20.6	20.6	18.1
NDF, %	8.0	8.1	5.8	7.6	7.0	9.8	7.4	8.3	9.09
ADF, %	2.9	4.0	2.3	3.7	2.8	4.4	2.9	4.1	3.1
ADL, %[13]	0.08	0.63	0.10	0.64	0.08	0.62	0.08	0.62	0.04
ABC-4, meq/kg[14]	206	215	510	495	261	253	488	453	---

[1] Phases 1, 2, and 3 were fed for 10, 14, and 18 d, respectively. Diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (2012) for pigs with average body weights of 6, 9, and 18 kg for phases 1, 2, and 3, respectively.

[2] AX3 Digest (Proteka, Newport Beach, CA).

[3] HP 300 (Hamlet protein, Findlay, OH).

[4] ValoproWin (Mixscience, Bruz, France).

[5] Provided per kilogram of feed: 4,134 IU of vitamin A; 1,653 IU of vitamin D; 44 IU of vitamin E; 3.31 mg of vitamin K3; 33 µg of vitamin B_{12}; 8 mg of riboflavin; 28 mg of pantothenic acid; 50 mg of niacin.

[6] Provided per kilogram of feed: 110 mg of Zn as zinc sulfate; 17 mg of Cu as copper sulfate; 33 mg of Mn as manganese oxide; 110 mg of Fe as ferrous sulfate; 0.30 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite.

[7] Ronozyme Hiphos 2700 (dsm-firmenich, Parsippany, NJ) provided 1,486 FYT/kg for phases 1 and 2 and 811 FYT/kg in phase 3 with an assumed release of 0.14% STTD P in phases 1 and 2 and 0.12% STTD P in phase 3.

[8] Primary Products Ingredients Americas LLC, Decatur, IL.

[9] pHormasil Na (Hawkins, Roseville, MN).

[10] Standardized total tract digestible phosphorus.

[11] Neutral detergent fiber.

[12] Acid detergent fiber.

[13] Acid detergent lignin.

[14] Acid binding capacity to a pH of 4.

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

	Phase 1		Phase 2		Phase 3
Item	Low ABC-4	Conventional	Low ABC-4	Conventional	---
Ingredients, %					
Corn	50.56	48.88	55.97	55.26	68.06
Soybean meal, 47.7% CP	17.34	17.35	25.35	25.35	28.12
Crystalline lactose	15.00	--	7.50	--	--
Spray-dried whey powder	--	20.85	--	10.40	--
Spray-dried bovine plasma	3.25	3.25	--	--	--
Microbial enhanced protein[2]	7.50	--	5.50	0.00	--
Specialty soybean meal[3]	--	5.26	--	4.62	--
Corn oil	2.00	2.00	1.00	1.00	--
Monocalcium phosphate, 21% P	0.91	0.20	0.97	0.60	0.85
Limestone	0.33	0.33	0.48	0.52	0.75
Salt	0.77	0.28	0.80	0.55	0.60
L-Lys-HCl	0.44	0.36	0.49	0.45	0.55
DL-Met	0.24	0.16	0.24	0.19	0.21
L-Thr	0.18	0.14	0.21	0.19	0.23
L-Trp	0.04	0.04	0.05	0.05	0.05
L-Val	0.09	0.07	0.11	0.12	0.16
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.03
Zinc oxide	--	0.40	--	0.25	--
Fumaric acid[7]	0.50	--	0.50	--	--
Formic acid[8]	0.40	--	0.40	--	--
TOTAL	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Standardized ileal digestible amino acids, %					
Lys	1.36	1.36	1.35	1.35	1.30
Ile:Lys	56	57	58	58	53
Leu:Lys	115	118	115	115	113
Met:Lys	37	31	39	35	36
Met & Cys:Lys	56	56	56	56	57
Thr:Lys	63	63	63	63	63
Trp:Lys	20	21	21	21	19
Val:Lys	70	70	70	70	70
His:Lys	36	35	36	36	35
NE, kcal/kg	2,586	2,610	2,480	2,502	2,449
SID Lys:NE	5.26	5.21	5.44	5.40	5.31
CP, %	21.05	20.80	21.46	21.24	19.98
Lactose, %	15.00	15.00	7.50	7.50	- - -
Ca, %	0.49	0.49	0.57	0.59	0.64

P, %	0.52	0.52	0.55	0.56	0.56
STTD P, %[9]	0.47	0.46	0.46	0.46	0.43
Crude fiber, %	2.20	1.87	2.48	2.27	2.44
NDF, %	7.49	6.36	8.25	7.54	8.51
ADF, %	3.10	2.63	3.49	3.20	3.45
Lignin, %	0.42	0.35	0.51	0.46	0.53

[1] Phases 1, 2, and 3 were fed for 10, 14, and 18 d, respectively. Diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (2012) for pigs with average body weights of 6, 9, and 18 kg for phases 1, 2, and 3, respectively. ValoproWin was mixed with the low ABC-4 basal diet at 0, 1.75, 2.50, 3.75, and 5.00% of the diet with no other adjustment in the formulation made.

[2] ME-Pro (Prairie Aquatech, Brookings, SD).

[3] HP 300 (Hamlet protein, Findlay, OH).

[4] Provided per kilogram of feed: 4,134 IU of vitamin A; 1,653 IU of vitamin D; 44 IU of vitamin E; 3.31 mg of vitamin K3; 33 µg of vitamin B_{12}; 8 mg of riboflavin; 28 mg of pantothenic acid; 50 mg of niacin.

[5] Provided per kilogram of feed: 110 mg of Zn as zinc sulfate; 17 mg of Cu as copper sulfate; 33 mg of Mn as manganese oxide; 110 mg of Fe as ferrous sulfate; 0.30 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite.

[6] Ronozyme Hiphos 2700 (dsm-firmenich, Parsippany, NJ) provided 1,486FYT/kg for phases 1 and 2 and 811 FYT/kg in phase 3 with an assumed release of 0.14% STTD P in phases 1 and 2 and 0.12% STTD P in phase 3.

[7] Primary Products Ingredients Americas LLC, Decatur, IL.

[8] pHormasil Na (Hawkins, Roseville, MN).

[9] Standardized total tract digestible phosphorus.

Table 4.3 Diet composition, Exp. 3 (as-fed basis).[1]

	Phase 1					Phase 2					Phase 3
		Diluted		Adjusted			Diluted		Adjusted		---
Item ValoproWin, %:	0	2.5	5	2.5	5	0	2.5	5	2.5	5	---
Ingredient, %											
Corn	50.54	49.27	48.01	46.59	42.43	55.97	54.57	53.17	51.77	47.65	68.05
Soybean meal, 47.7% CP	17.35	16.91	16.48	17.67	18.11	25.35	24.72	24.09	25.94	26.37	28.12
Crystalline lactose	15.00	14.63	14.25	15.00	15.00	7.50	7.31	7.13	7.50	7.50	---
Microbial enhanced SBM[2]	7.50	7.31	7.13	7.50	7.50	5.50	5.36	5.23	5.50	5.50	---
Spray-dried bovine plasma	3.25	3.17	3.09	3.25	3.25	---	---	---	---	---	---
VLPW[3]	---	2.50	5.00	2.50	5.00	---	2.50	5.00	2.50	5.00	---
Soybean oil	2.00	1.95	1.90	3.09	4.28	1.00	0.98	0.95	2.15	3.28	---
Monocalcium phosphate, 21% P	0.91	0.89	0.87	0.95	0.96	0.97	0.94	0.92	0.97	1.00	0.85
Limestone	0.33	0.32	0.31	0.31	0.30	0.48	0.46	0.45	0.45	0.43	0.75
Salt	0.77	0.75	0.73	0.77	0.78	0.80	0.78	0.76	0.81	0.81	0.6
L-Lys-HCl	0.44	0.43	0.42	0.43	0.43	0.49	0.47	0.46	0.47	0.46	0.55
DL-Met	0.25	0.24	0.24	0.25	0.26	0.23	0.22	0.22	0.24	0.26	0.21
L-Thr	0.18	0.18	0.17	0.19	0.20	0.21	0.20	0.19	0.21	0.22	0.23
L-Trp	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.05
L-Val	0.09	0.08	0.08	0.10	0.10	0.11	0.10	0.10	0.11	0.12	0.16
Vitamin premix[4]	0.25	0.24	0.24	0.25	0.25	0.25	0.24	0.24	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.14	0.15	0.15	0.15	0.15	0.14	0.15	0.15	0.15

Phytase[6]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.03
Fumaric acid[7]	0.50	0.49	0.48	0.50	0.50	0.50	0.49	0.48	0.50	0.50	---
Formic acid[8]	0.40	0.39	0.38	0.40	0.40	0.40	0.39	0.38	0.40	0.40	---
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis											
Standardized ileal digestible amino acids, %											
Lys	1.36	1.33	1.30	1.36	1.36	1.35	1.32	1.29	1.35	1.35	1.30
Ile:Lys	56	56	56	56	56	58	58	58	59	59	53
Leu:Lys	115	115	115	113	112	115	115	115	114	113	113
Met:Lys	38	38	38	38	38	38	38	38	38	39	36
Met & Cys:Lys	56	56	56	56	56	55	55	55	55	56	57
Thr:Lys	63	63	63	63	64	63	63	63	63	64	63
Trp:Lys	20	21	21	21	20	20	20	20	20	21	19
Val:Lys	70	70	70	70	70	70	70	70	70	70	70
His:Lys	36	36	36	36	35	36	36	36	36	36	35
NE, kcal/kg	2,586	2,535	2,482	2,584	2,586	2,480	2,432	2,383	2,480	2,480	2,449
SID Lys:NE	5.26	5.25	5.24	5.26	5.26	5.44	5.43	5.41	5.44	5.44	5.25
CP, %	21.1	20.6	20.2	21.0	21.0	21.5	21.0	20.6	21.5	21.5	20.0
Lactose, %	15.00	14.63	14.25	15.00	15.00	7.50	7.31	7.13	7.50	7.50	---
Ca, %	0.49	0.48	0.46	0.49	0.49	0.57	0.56	0.55	0.57	0.57	0.64
P, %	0.52	0.51	0.50	0.52	0.52	0.55	0.54	0.53	0.55	0.55	0.56
STTD P, %[9]	0.47	0.46	0.45	0.47	0.47	0.46	0.45	0.45	0.46	0.47	0.43
Crude fiber, %	2.20	3.52	4.84	3.51	4.81	2.48	3.79	5.10	3.79	5.10	2.44

NDF, %[10]	7.49	9.18	10.88	9.04	10.58	8.25	9.93	11.60	9.80	11.34	8.51
ADF, %[11]	3.10	4.56	6.01	4.54	5.97	3.49	4.93	6.38	4.93	6.36	3.45
Lignin, %	0.42	0.89	1.37	0.89	1.37	0.51	0.98	1.45	0.98	1.46	0.53
Analyzed composition											
CP, %	19.7	19.4	19.0	20.1	20.2	19.9	19.8	19.2	20.8	20.2	20.1
NDF, %	6.6	7.6	9.4	9.6	14.3	6.1	8.3	10.4	8.8	9.7	8.2
ADF, %	2.2	3.4	5.0	9.7	4.9	2.2	3.8	5.5	4.1	5.4	3.2
ADL, %[12]	0.04	0.56	1.18	0.60	1.16	0.04	0.62	1.19	0.50	1.25	0.06
ABC-4, meq/kg[13]	204	195	192	203	199	256	252	258	256	260	---

[1] Phases 1, 2, and 3 were fed for 10, 13, and 22 d, respectively. Diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (2012) for pigs with average body weights of 6, 9, and 18 kg for phases 1, 2, and 3, respectively.

[2] ME-PRO (Aquatech, Brookings, SD).

[3] ValoproWin (Mixscience, Bruz, France).

[4] Provided per kilogram of feed: 4,134 IU of vitamin A; 1,653 IU of vitamin D; 44 IU of vitamin E; 3.31 mg of vitamin K3; 33 µg of vitamin B_{12}; 8 mg of riboflavin; 28 mg of pantothenic acid; 50 mg of niacin.

[5] Provided per kilogram of feed: 110 mg of Zn as zinc sulfate; 17 mg of Cu as copper sulfate; 33 mg of Mn as manganese oxide; 110 mg of Fe as ferrous sulfate; 0.30 mg of I as ethylenediamine dihydriodide; 0.30 mg of Se as sodium selenite.

[6] Ronozyme Hiphos 2400 (dsm-firmenich, Parsippany,NJ) provided 2,000 FYT/kg for phases 1 and 2 and 811 FYT/kg in phase 3 with an assumed release of 0.14% STTD P in phases 1 and 2 and 0.12% STTD P in phase 3.

[7] Primary Products Ingredients Americas LLC, Decatur, IL.

[8] Phormasil NA (Hawkins, Roseville, MN).

[9] Standardized total tract digestible phosphorus.

[10] Neutral detergent fiber.

[11] Acid detergent fiber.

[12] Acid detergent lignin.

[13] Acid binding capacity to a pH of 4.

Table 4.4. Effects of ValoproWin feeding duration and formulation strategy on nursery performance and fecal dry matter, Exp. 1.[1]

	ValoproWin feeding duration, d[2]					Formulation strategy[3]				
						ABC-4:	Low ABC-4	Conventional		
Item	0	10	24	SEM	*P* =	ZnO:	No	Yes	SEM	*P* =
BW, kg										
d 0	5.6	5.6	5.6	0.47	0.998		5.6	5.6	0.47	0.957
d 10[4]	7.0	7.1		0.53	0.267		7.0	7.1	0.53	0.030
d 24	12.4	12.3	12.4	0.86	0.901		12.0	12.8	0.85	< 0.001
d 42	23.2	23.2	23.4	1.35	0.883		23.1	23.4	1.34	0.332
Phase 1 (d 0 to 10)[4]										
ADG, g	145	153		11.8	0.276		141	155	11.4	0.033
ADFI, g	172	180		7.7	0.163		166	183	7.3	0.001
G:F, g/kg	845	839		43.9	0.784		847	840	42.1	0.821
Phase 2 (d 10 to 24)										
ADG, g	380	376	386	25.4	0.726		358	403	24.9	< 0.001
ADFI, g	497	479	503	29.8	0.268		463	523	29.1	< 0.001
G:F, g/kg	767	783	766	16.9	0.469		775	769	15.6	0.674
Experimental period (d 0 to 24)										
ADG, g	286	280	285	17.4	0.808		267	300	16.9	< 0.001
ADFI, g	365	351	365	20.1	0.316		339	381	19.7	< 0.001
G:F, g/kg	782	797	780	20.9	0.375		789	784	20.2	0.596
Common diet (d 24 to 42)										
ADG, g	598	603	608	28.8	0.795		616	591	28.0	0.059
ADFI, g	866	854	869	43.6	0.739		875	850	42.8	0.134
G:F, g/kg	692	708	701	9.8	0.262		704	697	9.0	0.365
Overall (d 0 to 42)										
ADG, g	419	418	424	21.8	0.847		417	424	21.4	0.404
ADFI, g	579	566	581	29.9	0.395		569	582	29.5	0.190

G:F, g/kg	725	739	729	12.4	0.147	733	729	12.0	0.535
Fecal dry matter, %[5]									
d 10[4]	20.97	23.15		0.733	0.019	22.29	22.56	0.597	0.753
d 24	22.45	22.32	22.21	0.592	0.952	23.28	21.37	0.505	0.004

[1] A total of 300 pigs (Line 241 × 600, DNA, Columbus, NE) were used in a 42-d growth study. No interactions between VLPW feeding duration and formulation strategy were observed for growth performance criteria or fecal dry matter in any period.

[2] ValoproWin (MiXscience, Bruz, France) is a fiber ingredient that combines a purified source of coarse indigestible fiber with oat hulls and yeast autolysate. ValoproWin was fed for 0, 10, or 24 d.

[3] Low ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24, respectively. The lactose levels were reached using pure lactose, AX3 digest as a protein concentrate source, and fumaric and formic acid as acidifiers. The conventional diets were formulated to 493 and 470 meq/kg, containing 2,990 and 1,910 mg/kg of Zn from ZnO from d 0 to 10 and 10 to 24, respectively. Spray-dried milk whey powder and HP300 were used as lactose and specialty soy sources, respectively.

[4] For d 10 BW, period 1 performance, and fecal DM on d 10 only one value is presented for 10 and 24 d VLPW feeding duration because all pigs received the same diet during this period. The *P*-value tests diets with and without VLPW.

[5] Same three pigs per pen were sampled on d 10 and 24.

Table 4.5. Analyzed composition of experimental diets, Exp. 2 (as-fed basis).[1]

	Low ABC-4[2]					Conventional
VLPW, %:[3]	0.00	1.75	2.5	3.75	5.00	- - -
Phase 1						
CP, %	20.6	19.9	19.9	19.6	19.5	19.8
NDF, %[5]	8.1	8.5	9.8	8.7	12.7	6.6
ADF, %[6]	2.2	2.6	2.9	3.6	3.2	2.6
ADL, %[7]	0.03	0.28	0.54	0.86	1.22	0.05
ABC-4, meq/kg	206	203	198	210	193	451
Phase 2						
CP, %	21.1	21.0	20.9	19.7	19.4	21.1
NDF, %	7.6	7.6	7.8	9.9	10.7	5.7
ADF, %	2.2	2.8	3.3	3.2	3.6	2.8
ADL, %	0.04	0.22	0.58	0.82	1.19	0.04
ABC-4, meq/kg	253	255	260	259	264	448

[1] The values represent the mean of two samples. Phase 1 was fed for 10 d and phase 2 for 14 d in pellet form.
[2] Acid binding capacity at a pH of 4.
[3] ValoproWin (Mixscience, Bruz, France).
[4] Crude protein
[5] Neutral detergent fiber.
[6]Acid detergent fiber.
[7] Acid detergent lignin.

Table 4.6. Effects of increasing ValoproWin (VLPW) in low ABC-4 diets on performance and fecal dry matter of nursery pigs, Exp 2.[1]

		Low ABC-4[2]					Conventional		$P =$[4]		
Item	VLPW, %[3]:	0.00	1.75	2.50	3.75	5.00	0	SEM	Linear	Quadratic	ABC-4
BW, kg											
d 0		6.3	6.3	6.3	6.3	6.3	6.3	0.26	0.933	0.960	0.893
d 10		7.9	7.9	7.6	7.7	7.7	7.8	0.32	0.033	0.123	0.513
d 24		13.6	13.4	12.9	13.0	13.3	13.6	0.44	0.160	0.049	0.948
d 42		25.5	25.4	24.6	25.0	25.4	25.2	0.65	0.535	0.083	0.494
Period 1 (d 0 to 10)											
ADG, g		159	155	133	138	142	152	8.6	0.019	0.084	0.441
ADFI, g		174	177	155	165	170	183	7.9	0.302	0.114	0.310
G:F, g/kg		914	877	852	830	835	832	20.9	0.003	0.259	0.007
Period 2 (d 10 to 24)											
ADG, g		408	397	380	378	401	423	13.2	0.379	0.055	0.348
ADFI, g		545	558	534	523	539	590	15.8	0.291	0.701	0.025
G:F, g/kg		747	712	711	725	744	717	12.6	0.855	0.007	0.076
Experimental period (d 0 to 24)											
ADG, g		304	296	277	278	293	310	9.5	0.120	0.029	0.621
ADFI, g		391	399	376	374	385	420	11.1	0.229	0.439	0.028
G:F, g/kg		778	742	735	745	761	738	10.5	0.348	0.004	0.010
Common period (d 24 to 42)											
ADG, g		660	663	646	666	667	645	13.9	0.603	0.425	0.322
ADFI, g		1,056	1,046	1,023	1,042	1,037	1,021	22.7	0.428	0.407	0.149
G:F, g/kg		626	634	633	639	645	632	7.8	0.091	0.981	0.580
Overall (d 0 to 42)											
ADG, g		457	454	435	445	454	452	10.2	0.515	0.067	0.666
ADFI, g		676	676	653	660	665	675	14.3	0.224	0.310	0.961

G:F, g/kg	676	670	667	673	683	670	6.7	0.437	0.103	0.497
Fecal dry matter, %[5]										
d 10	26.92	25.78	27.92	27.81	29.01	24.32	0.875	0.027	0.464	0.039
d 24	24.41	23.9	26.66	26.44	26.57	23.51	0.875	0.018	0.612	0.466

[1] A total of 360 pigs (Line 200 × 400, DNA, Columbus, NE) were used in a 42-d growth study with five pigs per pen and 12 pens per treatment.

[2] ABC-4 = Acid binding capacity to a pH of 4. Low ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24, respectively, and did not contain pharmacological levels of Zn. High ABC-4 diets were formulated to 493 and 470 meq/kg, containing 2,990 and 1,910 mg/kg of Zn from ZnO from d 0 to 10 and 10 to 24, respectively.

[3] VLPW = ValoproWin (MiXscience, Bruz, France), is a fiber source that contains a purified source of coarse indigestible fiber with oat hulls and yeast autolysate.

[4] Linear and quadratic *P*-values evaluated the effect of VLPW level within the low ABC-4 diets, whereas the ABC-4 *P*-value compared the low and high ABC-4 diets when VLPW was not added.

[5] The same three pigs per pen were sampled on d 10 and 24. There was a significant effect of sampling day ($P = 0.001$); however, no significant interactions ($P > 0.10$) between day and the linear or quadratic effects of VLPW inclusion were observed.

Table 4.7. Effects of ValoProWin (VLPW) and diet formulation method on growth performance and fecal dry matter in nursery pigs, Exp. 3.[1]

		Diluted[2]		Adjusted			P =[4]		
Item	VLPW, %:[3] 0	2.5	5.0	2.5	5.0	SEM	Formulation × VLPW	Formulation	VLPW
BW, kg									
d 0	5.6	5.6	5.6	5.6	5.6	0.87	0.968	0.856	0.676
d 10	6.5	6.6	6.5	6.4	6.3	1.01	0.829	0.163	0.347
d 23	12.2	12.5	12.1	12.0	12.0	1.68	0.299	0.203	0.485
d 45	26.8	27.1	27.4	26.7	26.8	2.76	0.806	0.213	0.726
Period 1 (d 0 to 10)									
ADG, g	93	100	91	88	74	16.7	0.818	0.130	0.208
ADFI, g	125	129	131	126	112	17.9	0.341	0.175	0.470
G:F, g/kg	636	756	610	686	644	77.4	0.502	0.816	0.230
Period 2 (d 10 to 23)									
ADG, g	432	447	431	425	440	51.6	0.132	0.526	0.973
ADFI, g	511	529	521	491	507	59.9	0.349	0.043	0.735
G:F, g/kg	851	846	829	866	867	11.2	0.431	0.011	0.466
Experimental period (d 0 to 23)									
ADG, g	283	293	281	279	281	35.8	0.429	0.402	0.605
ADFI, g	342	352	350	332	335	41.2	0.807	0.091	0.936
G:F, g/kg	829	834	804	838	836	9.6	0.135	0.054	0.088
Period 3 (d 24 to 45)									
ADG, g	668	667	694	671	670	49.4	0.202	0.370	0.250
ADFI, g	995	995	1,040	1,001	1,032	81.8	0.723	0.950	0.063
G:F, g/kg	671	673	670	672	652	9.0	0.326	0.243	0.168
Overall (d 0 to 45)									
ADG, g	470	474	482	471	471	42.2	0.667	0.392	0.622
ADFI, g	660	663	686	659	676	60.6	0.836	0.578	0.137

G:F, g/kg	712	717	704	714	698	7.3	0.809	0.552	0.057
Fecal dry matter, %[5]									
d 10[6]	21.95	25.10	25.97	25.39	26.81	1.382	0.753	0.516	0.192
d 24[7]	23.21	24.23	25.15	23.48	25.27		0.619	0.718	0.123

[1] A total of 335 pigs (Line 241 × 600, DNA, Columbus, NE) were used in a 45-d growth study with four or five pigs per pen and 14 replicate pens per treatment. Dietary treatments were assigned in a 2 × 2 + 1 factorial experiment, with main effects of VLPW inclusion (2.5 or 5.0%) and formulation method (diluted or adjusted), and an extra treatment was included where no VLPW was included in the formulation.

[2] In the diluted formulation method, the VLPW was included without any nutritional adjustments in the diet, which resulted in a dilution of all nutrients as VLPW increased. In the adjusted formulation method, the chemical composition of the diet was adjusted to the values of the diets without VLPW.

[3] VLPW = ValoproWin (MiXscience, Bruz, France), is a fiber source that contains a purified source of coarse indigestible fiber with oat hulls and yeast autolysate.

[4] The *P*-value associated with formulation compares the diluted and adjusted formulation method when VLPW was included at 2.5 or 5.0%. Linear and quadratic effects of increasing VLPW (0, 2.5, or 5%) within each formulation method, and their interaction, were evaluated. No linear, quadratic, or interaction effects were detected for most performance criteria. However, during the experimental period, a tendency for a linear interaction ($P = 0.087$) was observed for G:F, where G:F tended to linearly increase ($P = 0.055$) with increasing VLPW in the diluted diets but not in the adjusted diets. In period 3, ADG tended to linearly increase ($P = 0.097$) with increasing VLPW in the diluted formulation method.

[5] Fecal samples were collected from the same 3 pigs per pen on d 10 and 23 No interactions between sampling day and dietary treatment or significant main effect of sampling day were observed. Linear and quadratic effects of increasing the VLPW (0, 2.5, or 5%) within each formulation method were evaluated.

[6] On d 10 fecal DM, increased (linear, $P < 0.001$) as the VLPW increased, independent of the formulation method.

[7] On d 24, Fecal DM tended to increase (linear, $P = 0.098$) as VLPW increased in the adjusted formulation method.