Evaluation of phase-feeding strategies and compensatory growth in grow-finish pigs and supplementation of probiotics to sows and progeny

by

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M.V., Federal University of Rio Grande do Sul, 2013
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
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Abstract

The dissertation consists of 5 chapters comprising a review of literature on compensatory growth following lysine restriction in grow-finish pigs, evaluation of phase-feeding strategies for grow-finish pigs, supplementation of *Bacillus subtilis*-based probiotic to sows, nursing and nursery pigs, and addition of pharmacological levels of copper to nursery diets. Chapter 1 presents a review of the current state of knowledge on compensatory growth induced by lysine restriction in grow-finish pigs. The review discusses the basis, types, factors, and dynamics involved in compensatory growth; develops a database from peer-reviewed literature to standardize comparisons to characterize the occurrence of compensatory growth; and provides practical considerations for compensatory growth under commercial conditions. Chapter 2 describes four experiments conducted to evaluate phase-feeding strategies based on lysine specifications and number of dietary phases for 27-to 127-kg grow-finish pigs. Phase-feeding strategies provide performance advantages over feeding a single dietary phase throughout the grow-finish period. Simplification of feeding strategies from 4 to 3 or 2 dietary phases with Lys specifications at 98% to 100% of estimated requirements for growth rate does not compromise overall growth performance and carcass characteristics of grow-finish pigs from 27 to 127 kg BW. Although, using feeding programs with fewer dietary phases and Lys set slightly below the requirements can compromise growth performance if initial BW and feed intake in the grow-finish period are lower than expected. Feeding strategies with Lys specifications set at 96% of estimated requirements compromise performance of grow-finish pigs unless Lys requirements are fully met in late finishing. In chapter 3, a study evaluates the effects of daily oral dose of *Bacillus subtilis* C-3102 probiotic to nursing piglets on fecal consistency, fecal microbes, and pre-weaning performance. A daily oral dose of *Bacillus subtilis* C-3102 did not influence pre-

weaning growth performance and fecal consistency of nursing piglets and only influenced *Bacillus* sp. fecal microbial population. In chapter 4, a study evaluates the effects of providing a *Bacillus subtilis* C-3102 probiotic to sows during gestation and lactation and to progeny after weaning on performance, fecal consistency, and fecal microbes. Providing a *Bacillus subtilis* C-3102 probiotic did not elicit noteworthy improvements in performance, fecal consistency, or fecal microbial populations in gestation, lactation, or nursery periods, but there was a benefit on sow lactation feed intake. Fecal microbial analysis indicated a maternal-progeny intestinal microbiota relationship with pigs born from probiotic-fed sows displaying similar fecal microbial population as sows. However, pigs born from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery. In chapter 5, a study evaluates the effect of added copper, alone or in combination with a feed-grade antimicrobial, on growth performance of 7- to 20-kg nursery pigs. The addition of 200 mg/kg copper from copper sulfate or 440 mg/kg chlortetracycline in nursery dies for 28 days exerted growth promotional effects on weaned pigs. The lack of interaction suggests the responses of copper and chlortetracycline on growth performance of nursery pigs are as efficacious when fed alone or in combination.

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Preface

This dissertation is original work completed by the author, Mariana Boscato Menegat. The chapters were formatted according to the required standards for publication in the following peer-reviewed journals: Translational Animal Science (chapter 1), Journal of Animal Science (chapters 2, 4 and 5), and Journal of Swine Health and Production (chapter 3).

Chapter 1 - A review of compensatory growth following lysine restriction in grow-finish pigs

ABSTRACT: Compensatory growth induced by Lys restriction in grow-finish pigs is a complex physiological process affected by many factors and interactions, principally genotype, stage of growth at restriction, nature of nutritional restriction, and patterns of restriction and recovery. Scarcity of standard comparisons across the literature has hindered the characterization of important determinants of compensatory growth. Therefore, the present publication aims to review the current state of knowledge on compensatory growth induced by Lys restriction in grow-finish pigs, develop a database from peer-reviewed literature to standardize comparisons to characterize the occurrence of compensatory growth, and provide practical considerations for compensatory growth under field conditions. The literature search focused on publications directly or indirectly evaluating compensatory growth by having a period of Lys restriction followed by a recovery period of Lys sufficiency for grow-finish pigs. The database included 14 publications and 57 comparisons expressed as relative differences of restricted pigs compared to non-restricted pigs. The database analysis described compensatory growth into complete, incomplete, and no compensatory growth categories, and characterized the patterns of restriction and recovery in each category. The review of literature and database analysis support the occurrence of compensatory growth induced by Lys restriction in grow-finish pigs. The degree of Lys restriction and duration of restriction and recovery periods seem to be critical in explaining differences between complete and incomplete compensatory growth, whereas Lys level in the recovery period seems to be critical between incomplete or no compensatory growth. Compensatory growth seems to be more likely if: 1) degree of Lys restriction is between 10 to

30%; 2) Lys restriction is induced before pigs reach their maximum protein deposition (Pdmax); 3) duration of Lys restriction is short and duration of recovery period is long; and 4) Lys level in recovery is close to or above the estimated requirements. In addition, compensatory growth can occur under commercial conditions and there seems to be an opportunity to exploit compensatory growth in grow-finish pigs to reduce feed cost and improve feed efficiency under certain market conditions.

Key words: amino acid restriction, catch up growth, compensatory growth, swine

INTRODUCTION

Compensatory growth is defined as a physiological process whereby animals undergo a period of accelerated growth rate following a period of restricted growth (Hornick et al., 2000). Growth restriction is typically induced by nutritional depletion and seems to be the primary requisite for compensatory growth to occur (O'Connell et al., 2006). Lysine depletion is commonly known to have a considerable impact on growth performance of lean pigs because Lys is the first limiting amino acid in most swine diets (NRC, 2012). Compensatory growth induced by Lys restriction in grow-finish pigs has been described in the literature (Chiba et al., 2002; Fabian et al., 2004; Reynolds and O'Doherty, 2006; Suárez-Belloch et al., 2015), but the response is not consistent (Chiba et al., 1999; Fabian et al., 2002; Cloutier et al., 2016). Compensatory growth is a complex phenomenon affected by a number of factors and interactions, for instance genotype, stage of growth at restriction, nature of nutritional restriction, and patterns of restriction and recovery (Wilson and Osbourn, 1960). To date, the variation in methodology and scarcity of standard comparisons across the compensatory growth literature

have hindered the characterization of important determinants of compensatory growth in grow-finish pigs.

The interest of the swine industry in compensatory growth predominantly lies on the potential to improve swine production efficiency. Strategies to exploit compensatory growth induced by Lys restriction aim at improvement of Lys and nitrogen utilization for lean growth and, consequently, reduction of nitrogen excretion in the environment (Whang et al., 2003; Fabian et al., 2004; O'Connell et al., 2006). Moreover, the high cost of protein sources favors the exploitation of compensatory growth induced by Lys restriction to allow reductions in feed cost and improvements in feed efficiency.

Thus, the present publication aims to review the current state of knowledge on compensatory growth induced by Lys restriction in grow-finish pigs. The approach in the present review is three-fold: 1) develop a database from peer-reviewed literature to standardize comparisons across the literature to characterize the occurrence of compensatory growth; 2) review the basis, types, factors, and dynamics involved in compensatory growth; and 3) provide practical considerations for compensatory growth under commercial conditions.

DATABASE

Literature search and selection criteria

A literature search was conducted to compile published studies that directly or indirectly evaluated compensatory growth by having a period of Lys restriction followed by a recovery period of Lys sufficiency in the grow-finish phase. The search was performed via the Kansas State University Libraries under the CAB International database. The following terms were applied in the electronic-based search: ("lysine" OR "amino acid" OR "protein") AND

("restriction" OR "limitation" OR "compensatory") AND ("grow*" OR "finish*" OR "grow*-finish*") AND ("pig" OR "swine"). Results were refined by language ("English") and no restrictions were applied to year of publication.

Publications were then individually evaluated for the following selection criteria: 1) peer-reviewed; 2) conducted with pigs with a minimum initial body weight (**BW**) of 15 kg; 3) had a control group of "non-restricted pigs" not subjected to a restriction period; 4) had a group of "restricted pigs" subjected to a restriction period induced by decreasing Lys alone, Lys and other amino acids, or crude protein (**CP**) in diets; 5) had a recovery period following the restriction period induced by providing the same diet to restricted and non-restricted pigs; 6) presented growth performance data for restriction and recovery periods; 7) presented detailed diet composition; and 8) allowed ad libitum feed consumption. A total of 14 publications met all selection criteria and were included in the database.

Database development

Data collected from studies were entered in a spreadsheet template and included breed, sex, age, housing, number of pigs per pen, number of replicates, initial BW (kg), average daily gain (**ADG**, g), average daily feed intake (**ADFI**, g), and gain-to-feed ratio (**G:F**, g/kg) for restriction, recovery, and overall periods, carcass leanness (%), carcass yield (%), longissimus muscle area (cm^2), and backfat thickness (mm). For studies reporting feed efficiency as feed-to-gain ratio, the inverse proportion was calculated based on ADG and ADFI. For studies on fixed-time basis, the duration (days) of restriction and recovery periods were included. For studies on fixed-weight basis, the BW at the end of restriction and recovery periods were included. Then, data from all studies were converted to fixed-time basis to standardize comparisons between studies. To convert to fixed-time basis, the duration of restriction and recovery periods were

derived by dividing the BW at the end of each period by the ADG of the respective period. The duration of restriction and recovery periods were converted to relative duration (%) by dividing the duration of each period by the overall duration in days, and to a ratio of recovery to restriction duration by dividing the duration of recovery period by the duration of restriction period in days.

Diets from all studies were reformulated by entering the diet composition into a spreadsheet-based formulator with NRC (2012) nutrient loading values for ingredients to achieve a common basis for dietary nutrient concentrations. The dietary nutrients obtained in as-fed basis included standardized ileal digestible (**SID**) Lys to calorie ratio (g/Mcal NE), CP (%), and neutral detergent fiber (**NDF**, %). The degree of Lys restriction (%) in the restriction period was estimated by dividing the dietary Lys to calorie ratio (g/Mcal NE) of restricted pigs by the dietary Lys to calorie ratio (g/Mcal NE) of non-restricted pigs. Thus, the degree of Lys restriction (%) of restricted pigs is relative to the Lys level of non-restricted pigs and based on the assumption that non-restricted pigs were under no degree of Lys restriction in the restriction period.

Comparisons were conducted between restricted pigs and non-restricted pigs within each of the 14 publications included in the database based on the number of treatments available for comparisons within each study. A total of 60 comparisons were conducted and 3 comparisons were excluded due to insufficient restriction, as restricted pigs demonstrated similar or superior performance in the restriction period compared to non-restricted pigs. Thus, the final database included 57 comparisons for all variables listed above, except for carcass leanness (9 comparisons), carcass yield (13 comparisons), longissimus muscle area (15 comparisons), and backfat thickness (20 comparisons), which were not available in all publications. For all

variables listed above, the comparisons were performed as relative differences (%) between restricted pigs compared to non-restricted pigs. The values of restricted pigs were divided by the values of non-restricted pigs, multiplied by 100 to convert to relative values, and subtracted from 100 to indicate the relative difference from non-restricted pigs:

$$Relative\ difference\ (\%) = \left[\left(\frac{Values\ of\ restricted\ pigs}{Values\ of\ non-restricted\ pigs}\right) \times 100\right] - 100$$

Database descriptive summary

A summary of publications included in the database is presented in Table 1.1 and a descriptive summary of the database is presented in Table 1.2. The database descriptive summary is important to depict the characteristics of the data generated from the literature review and to understand the scope of inference of the present review.

On average, a degree of Lys restriction of 33% during a 39-d restriction period resulted in decrease in ADG by 12.6%, G:F by 13.7%, and BW by 6.8% in restricted pigs compared to non-restricted pigs. Following the restriction, a 55-d recovery period resulted in increase in ADG by 2.4% and G:F by 3.6% in previously restricted pigs compared to non-restricted pigs. However, on average, the improvement in growth performance in the recovery period was not sufficient to lead restricted pigs to a similar overall growth performance and final BW to non-restricted pigs, as there was approximately a 3% decrease in overall ADG, overall G:F, and final BW in restricted pigs compared to non-restricted pigs. On average, carcass characteristics indicated a leaner carcass (0.7% greater carcass leanness and 1.4% greater longissimus muscle area) with virtually no difference in backfat thickness (0.1% greater backfat) or carcass yield (0.2% greater yield) in restricted pigs compared to non-restricted pigs.

BASIS OF COMPENSATORY GROWTH ACROSS SPECIES

Early studies by Osborne and Mendel (1916) described that animals with a decrease in weight gain due to nutritional restriction exhibit a subsequent rapid weight gain above normal growth rate under adequate nutrition (Figure 1.1). The authors illustrate the physiological process as 'curves of repair' alluding to the preservation of homeostasis as its central component (Osborne and Mendel, 1916; Wilson and Osbourn, 1960). During nutritional restriction, physiological maturing seems to proceed at a slower rate to preserve homeostasis (Ragsdale, 1934), but then under adequate nutrition the growth rate of previously restricted animals seems to proceed at a faster rate proportional to the growth needed to reach maturity (Brody, 1926). The term 'compensatory growth' proposed by Bohman (1955) is broadly used in the literature across species to refer to this growth phenomenon.

The animals' growth potential is determined by genotype and influenced by environmental and nutritional limitations (Gu et al., 1992; Schinckel and de Lange, 1996). However, compensatory growth demonstrates that animals have the capacity to achieve a rate of growth above the expected growth potential for a period of time. The pertaining question is why don't all animals grow at the maximum rate throughout the growth period? Particularly in some species of animals in which adult size is important for fitness, reproduction, and survival, the acceleration of growth rate would allow animals to reach adult size at younger age. However, there are often longevity costs associated with acceleration of growth in some species, including cellular damage, developmental errors, and senescence (Metcalfe and Monaghan, 2003). The intrinsic trade-off between benefits and costs of maximal growth rate varies within species, individuals, environment, and nutrition (Metcalfe and Monaghan, 2003). In the case of compensatory growth, the costs of acceleration of growth rate is often lower than the long-term

consequences of previous nutritional restriction and impairment of adult size and weight (Metcalfe and Monaghan, 2001).

TYPES OF COMPENSATORY GROWTH

Theoretically, pigs can exhibit complete or incomplete compensatory growth. Complete compensatory growth or "catch-up growth" refers to the occurrence of faster growth rate of previously restricted pigs compared to non-restricted pigs that leads to the attainment of similar body weight at a similar age (Skiba, 2005; Hector and Nakagawa, 2012). Incomplete compensatory growth refers to the occurrence of faster growth rate of previously restricted pigs compared to non-restricted pigs, but the magnitude or duration of increase in growth rate is not sufficient to result in similar body weight at a similar age (Skiba, 2005; Hector and Nakagawa, 2012).

The occurrence of complete and incomplete compensatory growth was assessed within the database. The ADG in the recovery period was plotted against the final BW in the recovery period as a relative difference between restricted pigs compared to non-restricted pigs (Figure 1.2). The scatterplot depicts the distribution of all 57 database comparisons into four quadrants. The comparisons falling in quadrant I indicate an increase in both ADG in the recovery period and final BW, which suggests restricted pigs were able to exhibit complete compensatory growth and attain at least a similar BW to non-restricted pigs at a similar age. Quadrant II indicates a decrease in ADG in the recovery period but an increase in final BW, which means restricted pigs had an increase in ADG in the restriction period compared to non-restricted pigs and, consequently, were not restricted. Because growth restriction is a primary requisite for compensatory growth to occur (O'Connell et al., 2006), comparisons falling in quadrant II (3 out

of 60) were excluded from the database due to insufficient restriction. The comparisons falling in quadrant III indicate a decrease in both ADG in the recovery period and final BW, which suggests restricted pigs were not able to exhibit compensatory growth. The comparisons falling in quadrant IV indicate an increase in ADG in the recovery period but a decrease in final BW, which suggests restricted pigs were able to exhibit incomplete compensatory growth during the recovery period but not to attain a similar BW to non-restricted pigs at a similar age.

The distinct patterns of complete, incomplete, or no compensatory growth within the database indicate there are fundamental characteristics that place restricted pigs together in a category of compensatory growth and apart from others. This prompted the analysis of a number of factors by compensatory growth category.

FACTORS AFFECTING COMPENSATORY GROWTH IN GROW-FINISH PIGS

The factors affecting compensatory growth have been clearly defined since the early literature about the subject (Wilson and Osbourn, 1960). There are generally four important factors: genotype, stage of growth at restriction, nature of nutritional restriction, and patterns of restriction and recovery. These factors alone or in combination are responsible for determining the occurrence and extent of compensatory growth. However, the complex interactions of these factors have not been well-characterized and hinder the ability to accurately predict and control the occurrence and extent of compensatory growth in practice. An analysis of factors affecting compensatory growth within the database in the present review aims to aid in the clarification some of these complex interactions.

Genotype and stage of growth at restriction

Genotype determines the potential for growth, protein deposition, and body composition in each stage of growth in swine (Gu et al., 1992). Compensatory growth can occur in contemporary lean or formerly fat strains of pigs (Hogberg and Zimmerman, 1978; de Greef et al., 1992; Chiba et al., 2002; Fabian et al., 2002), as well as in gilts, barrows, or entire males (Robinson, 1964; Smith et al., 1999; Fabian et al., 2004; Martínez-Ramírez et al., 2008). However, the compensatory growth response may vary based on the distinct genetic potential for growth, protein deposition, and body composition between strains and genders (Martínez-Ramírez and de Lange, 2007; Ruiz-Ascacibar et al., 2019). The genetic potential is relevant because the primary genetic aspects involved in compensatory growth in grow-finish pigs are the upper limit to protein deposition (**Pdmax**) and the body composition as a ratio of body lipid to body protein (Skiba, 2005; Martínez-Ramírez and de Lange, 2007).

The curves of growth and protein deposition follow a non-linear sigmoid shape in swine (Whittemore, 1986; Schinckel and de Lange, 1996). Body weight and protein deposition increase with time until the inflexion point of the sigmoid curve and plateau thereafter. The inflexion point is determined by Pdmax. Until the inflexion point, pigs are in an energy-dependent stage of growth as energy intake likely determines the rate of growth and protein deposition (Campbell and Taverner, 1988). After the inflexion point, pigs are in a protein-dependent stage of growth as the inherit Pdmax signals the attainment of maturity and likely determines the rate of growth and protein deposition (Whittemore, 1986; Schinckel and de Lange, 1996). Early studies by Wilson and Osbourn (1960) emphasized that imposing an amino acid restriction at or after the inflexion point during the protein-dependent stage of growth results in a lasting reduction in growth. In support, recent studies established that compensatory growth primarily occurs following amino acid restriction during the energy-dependent stage of growth and the extent of compensatory

growth is dictated by Pdmax (Martínez-Ramírez et al., 2008; 2009). Thus, compensatory growth primarily occurs during the energy-dependent stage of growth before pigs reach Pdmax and, as a consequence, compensatory growth is more prone to occur in genotypes of relatively high Pdmax, which is characteristic of late maturing, high lean growth potential pigs.

During the energy-dependent stage of growth, partitioning of energy intake is predominantly directed towards protein rather than lipid deposition. During the protein-dependent stage of growth, partitioning of energy intake is reversed and the ratio of protein deposition to lipid deposition decreases (Black et al., 1986; Quiniou et al., 1995). Studies by de Greef et al. (1992) suggested that partitioning of energy could be temporarily altered depending on the influence of nutritional restriction on body composition. In agreement, recent studies established that pigs have the ability to reach the target body composition represented as the ratio of body lipid to body protein following a period of amino acid restriction (Skiba et al., 2006b; Martínez-Ramírez et al., 2008; 2009). In that sense, pigs with increased body lipid to body protein ratio induced by amino acid restriction would have preference for protein deposition over lipid deposition. This would occur for a certain period of time under adequate nutrition in order to reach the target body lipid to body protein ratio (de Greef et al., 1992; Martínez-Ramírez et al., 2008, 2009). Thus, compensatory growth seems to be driven by an inherit target body composition pigs aim to achieve.

Nature of nutritional restriction

Compensatory growth can occur by imposing Lys restriction through diet formulation or through feed intake limitation. In the former, which is the scope of the present review, diets are formulated with low levels of Lys, Lys and other amino acids, or CP, but are typically offered to pigs ad libitum. In the latter, diets are formulated with adequate levels of Lys, but offered to pigs

in limited amounts. Thus, there is a restriction in intake of Lys as well as other nutrients and energy. Depending on the nature of restriction, pigs have distinct changes in body composition, visceral organs size, as well as voluntary feed intake and feed efficiency (Table 1.3). Thus, the nature of restriction determines important and distinctive aspects of compensatory growth response in grow-finish pigs (Skiba, 2005; Martínez-Ramírez and de Lange, 2007).

The primary difference in the compensatory growth response according to the nature of restriction lies on the composition of gain following restriction (Figure 1.3; Skiba, 2005). In the case of Lys restriction, compensatory growth is driven by improvements in feed efficiency and primarily occurs by an increase in protein deposition in the carcass (de Greef et al., 1992; Chiba et al., 2002; Fabian et al., 2002; Martínez-Ramírez et al., 2008; 2009). In the case of feed intake restriction, compensatory growth is driven by increase in voluntary feed intake and occurs by an increase in lipid deposition as well as size of visceral organs like liver, kidneys, and intestines, and gut fill (Bikker et al., 1996a,b; Lovatto et al., 2006; Heyer and Lebret, 2007; Chaosap et al., 2011). A similar restriction by limiting feed intake can be induced by diets with fibrous ingredients. In the case of high fiber diets, compensatory growth is driven by an increase in voluntary feed intake and lipid deposition, but the size of visceral organs and gut fill is already enlarged due to the fibrous content of the diet (Pond and Mersmann, 1990; Raj et al., 2005; Skiba et al., 2006a,b).

The distinctive aspects of compensatory growth are related to the distinct body composition characteristics induced by nutrition in the restriction period (Figure 1.3). Pigs under a period of Lys restriction typically have higher relative body lipid composition (de Greef et al., 1992; Kamalakar et al., 2009; Martínez-Ramírez et al., 2009; Suárez-Belloch et al., 2015), whereas pigs under a period of feed intake restriction have lower relative body lipid composition

(Bikker et al., 1996a,b; Lovatto et al., 2006; Heyer and Lebret, 2007; Chaosap et al., 2011). Thus, in the recovery period and under adequate nutrition, protein and lipid deposition occur at different rates and ratios for pigs previously under a period of Lys restriction compared to feed intake restriction (de Greef et al., 1992). In order to reach a target body composition, pigs previously under Lys restriction direct resources to restore body protein reserves, whereas pigs previously under feed intake restriction direct resources to restore body lipid reserves (Skiba, 2005).

The target body composition also determines the main drivers of compensatory growth. In pigs previously under Lys restriction, feed efficiency is the primary driver and feed intake does not increase considerably due to appetite suppression mediated by body lipid stores and leptin (Chiba et al., 2002; Fabian et al., 2002; O'Connell et al., 2006; Reynolds and O'Doherty, 2006; Martínez-Ramírez et al., 2009). In pigs previously under feed intake restriction, feed intake is the primary driver to promptly increase energy intake (Bikker et al., 1996a,b; Lovatto et al., 2006; Heyer and Lebret, 2007; Chaosap et al., 2011). The database analysis agrees on drivers of compensatory growth for pigs previously under Lys restriction, as pigs exhibiting complete compensatory growth have considerable improvements in feed efficiency but virtually no increase in feed intake (Table 1.4).

Patterns of restriction and recovery

The patterns of restriction and recovery refer to both the nutrition and the duration of restriction and recovery periods. From the nutrition standpoint, both degree of Lys restriction and dietary Lys level are important. The degree of Lys restriction refers to the severity of Lys restriction in restricted pigs compared to non-restricted, whereas the dietary Lys level refers to absolute Lys content. From the duration standpoint, both individual duration of restriction and

recovery periods and ratio of recovery to restriction periods are important. A recovery to restriction ratio below 1 indicates the period of restriction is longer than the period of recovery, whereas a ratio above 1 indicates the period of recovery is longer than the period of restriction.

The patterns of restriction and recovery determine the occurrence and extent of compensatory growth. The interactions among patterns are complex and have not been completely characterized but have already been well-described (Wilson and Osbourn, 1960). While mild degrees of Lys restriction and/or short periods of restriction can cause minor effects in growth and not incite compensatory growth, severe degrees of Lys restriction and/or long periods of restriction can cause permanent stunting and prevent compensatory growth. Moreover, low Lys levels in recovery and/or short periods of recovery can prevent compensatory growth even following an ideal pattern of restriction, while high Lys levels in recovery and/or long periods of recovery cannot compensate for a severe pattern of restriction. Thus, the key to achieving compensatory growth seems to lie on finding ideal combinations and balances among all aspects involved in the patterns of restriction and recovery.

The patterns of restriction and recovery according to compensatory growth categories defined in Figure 1.2 are characterized in Table 1.4. The table summarizes differences and similarities between compensatory growth categories and aids in the identification of relevant aspects related to the occurrence of complete, incomplete, or no compensatory growth in grow-finish pigs. The differences in BW, ADG, and Lys levels in restriction and recovery periods by compensatory growth category are further illustrated in Figure 1.4. Although the database analysis performed in the present review does not reflect cause-and-effect associations or is able to predict compensatory growth responses based on patterns of restriction and recovery, it

provides important support for characterization and conceptualization of compensatory growth in pigs.

First, the BW at restriction is similar across the compensatory growth categories, as indicated by the initial BW at restriction and recovery periods. The BW at restriction is a relevant factor to observe beforehand because it determines the potential for compensatory growth to occur (Martínez-Ramírez et al., 2008, 2009). Compensatory growth primarily occurs during the energy-dependent stage of growth before pigs reach Pdmax. Pigs at lower BW at restriction are more prone to have compensatory growth because are likely in the energy-dependent stage of growth, whereas pigs at heavier BW at restriction are less prone to have compensatory growth because may be near their Pdmax and transitioning to the protein-dependent stage of growth (Möhn and de Lange, 1998).

The degree of Lys restriction across compensatory growth categories is substantial at approximately 30 to 35%. Pigs exhibiting complete compensatory growth were induced to the least degree of Lys of restriction of 30% and fed diets with higher Lys level and CP content during restriction, whereas pigs exhibiting incomplete or no compensatory growth were induced to more severe degrees of Lys restriction of 35% and 33%, respectively, and fed diets with lower Lys level and CP content during restriction. Also, pigs exhibiting complete compensatory growth were exposed to shorter restriction duration and longer recovery duration (37% and 63% of overall duration, respectively) than pigs exhibiting incomplete or no compensatory growth (44 to 45% and 55 to 56% of overall duration in restriction and recovery, respectively). However, in the recovery period, pigs exhibiting incomplete compensatory growth were fed diets with higher Lys level and CP content compared to pigs exhibiting complete or no compensatory growth.

Comparing the patterns of restriction and recovery, it is possible to identify important factors for complete, incomplete, or no compensatory growth in grow-finish pigs. The degree of Lys restriction and duration of restriction and recovery periods seem to be critical between complete and incomplete compensatory growth. If the restriction is too severe, too long, or both, and the recovery is too short, pigs seem to be more prone to exhibit incomplete over complete compensatory growth. The Lys level and CP content of diets in the recovery period seem to be critical between incomplete and no compensatory growth. If the Lys level and CP content of diets in the recovery period are too low, pigs seem to be unable to exhibit compensatory growth.

DYNAMICS OF COMPENSATORY GROWTH IN GROW-FINISH PIGS

The physiological mechanisms involved in compensatory growth in pigs have not been completely elucidated. Characterizing the dynamics of compensatory growth allows understanding when compensatory growth occurs and what are the potential underlying mechanisms of compensatory growth in pigs.

Body composition and carcass characteristics

The rates of protein deposition and lean growth are increased in pigs following a period of Lys restriction (Chiba et al., 1999; Whang et al., 2003; Martínez-Ramírez et al., 2008). Recent models in rats suggest both an increase in protein synthesis and decrease in proteolysis contribute to greater protein deposition and lean growth, but at distinct points in time (Ishida et al., 2011). The changes in rate of body lipid to body protein ratio typically occur into the early recovery period (Reynolds and O'Doherty, 2006), with a decrease in proteolysis occurring only in the first days and an increase in protein synthesis prevailing throughout the entire period of compensatory growth (Ishida et al., 2011). Once protein stores have been replenished and target body

composition is achieved, pigs return to normal protein and lipid deposition rates (O'Connell et al., 2006). Thus, the duration of compensatory protein deposition is determined by the amount of time required by the pig to achieve a target body composition (Martínez-Ramírez et al., 2008). There is consistent indication that compensatory growth induced by Lys restriction is not driven by changes in composition or size of visceral organs (Fabian et al., 2002; Martínez-Ramírez et al., 2008, 2009; Kamalakar et al., 2009) or by increases in water deposition (Martínez-Ramírez et al., 2008).

The body composition of pigs during compensatory growth is often assessed by nitrogen balance (Fabian et al., 2004; O'Connell et al., 2006; Reynolds and O'Doherty, 2006; Ishida et al., 2012). Nitrogen utilization and nitrogen retention are improved while nitrogen excretion is decreased during compensatory growth (Fabian et al., 2004; Reynolds and O'Doherty, 2006; Ishida et al., 2012). The considerable improvements in nitrogen utilization and nitrogen retention have been described in restricted pigs from the restriction to recovery period (O'Connell et al., 2006), as well as compared to non-restricted pigs (Fabian et al., 2004), which indicates an effort to replenish nitrogen reserves after restriction. Although the carry-over effect of Lys restriction on nitrogen metabolism during compensatory growth is not well-understood (Fabian et al., 2004; O'Connell et al., 2006), there seems to be a consistent improvement in efficiency of Lys utilization for gain in pigs following a period of restriction compared to non-restricted pigs (Whang et al., 2003; Fabian et al., 2004; O'Connell et al., 2006; Ishida et al., 2012; Cloutier et al., 2016). Because of higher efficiency of Lys utilization in the recovery period, some authors suggest the Lys requirements are also greater during compensatory growth (Whang et al., 2003), but this has not been confirmed experimentally.

The changes in body composition can be reflected in carcass characteristics. However, the influence of compensatory growth on carcass characteristics is variable and in many instances no effects are observed (Fabian et al., 2002; 2004; Reynolds and O'Doherty, 2006). The database analysis indicates distinct changes in carcass characteristics based on patterns of restriction and recovery and compensatory growth category (Table 1.4). Pigs exhibiting complete compensatory growth have less carcass leanness by 2.5% compared to non-restricted pigs due to an increase in backfat thickness by 3.7% despite a 2.6% increase in longissimus muscle area, whereas pigs exhibiting incomplete compensatory growth have virtually no changes in carcass characteristics compared to non-restricted pigs. The carcass composition data indicates that pigs exhibiting both complete or incomplete compensatory growth attempt to achieve a target body composition by adjusting fat and lean deposition, as indicated by changes in longissimus muscle area alongside changes in backfat thickness or vice-versa.

Metabolic activity and endocrine status

Metabolites and hormones indicators of metabolic activity and endocrine status in pigs are prone to be affected during compensatory growth (Skiba, 2005). Previous studies have focused on the description of metabolic changes during compensatory growth (Whang et al., 2003; Fabian et al., 2004; Yang et al., 2008), while more recent studies have focused on the endocrine regulation of compensatory growth (Martínez-Ramírez et al., 2009; Ishida et al., 2012).

A period of Lys restriction promotes a metabolic change in energy partitioning toward lipid deposition over protein deposition, with increases in triglycerides, cholesterol, and glucose concentrations and decreases in albumin and urea nitrogen concentrations in serum (Whang et al., 2003; Yang et al., 2008; Kamalakar et al., 2009; Suárez-Belloch et al., 2015). However, Lys

restriction does not seem to have a long-term effect on metabolism, as most serum metabolites rapidly return to normal concentrations during recovery (Fabian et al., 2004; Yang et al., 2008; Suárez-Belloch et al., 2015). The serum metabolite most often related to compensatory growth in pigs is urea nitrogen (Fabian et al., 2002; Whang et al., 2003; Yang et al., 2008). Urea nitrogen is an indicator of amino acid catabolism and efficiency of amino acid utilization (Coma et al., 1995). During compensatory growth, urea nitrogen is often low which indicates an improvement in efficiency of Lys utilization for growth (Fabian et al., 2002; Whang et al., 2003; Yang et al., 2008). However, there is no consensus about the use of urea nitrogen concentrations as an indicator of compensatory growth (Whang et al., 2003; Martínez-Ramírez et al., 2009).

The hormones involved in growth regulation and protein and lipid metabolism are the most likely to influence compensatory growth. A period of Lys restriction influences the endocrine system and promotes an increase in concentration of growth hormone (**GH**) and leptin and a decrease in insulin-like growth factor I (**IGF-I**), IGF-binding proteins (**IGFBP**), cortisol, and corticosterone (Whang et al., 2003; Martínez-Ramírez et al., 2009; Ishida et al., 2012). Insulin-like growth factor I stimulates growth and protein synthesis (Sacheck et al., 2004), while cortisol and corticosterone stimulate proteolysis (Simmons et al., 1984). Leptin is a sensor of body adiposity and regulates lipid deposition (Barb et al., 1998). Thus, the endocrine status reflects the slow growth rate, low protein deposition, and high body lipid composition of pigs under Lys restriction. However, the concentrations of IGF-I, IGFBP, cortisol, and corticosterone immediately increase in recovery and in concert with improvements in growth rate and protein deposition (Martínez-Ramírez et al., 2009; Ishida et al., 2012). Moreover, GH and leptin remain at high concentrations in the immediate recovery to regulate protein and lipid deposition, respectively, and aid in the achievement of the target body composition (Martínez-Ramírez et al.,

2009). Thus, there seems to be important endocrine components involved in compensatory growth in pigs.

The Lys level in the recovery period is also an important component of compensatory growth. However, it is often not possible to separate the influence of Lys level from endocrine components (Ishida et al., 2013). Recent *in vitro* models with myotubes have been conducted to determine the individual contribution of Lys level and endocrine components to compensatory growth (Ishida et al., 2013). Interestingly, the increase in Lys level alone or the modulation of IGF-I and glucocorticoid levels alone were not able to influence protein accumulation rate of myotubes. Thus, there seems necessary to have a combination of increased dietary Lys and modulation of endocrine status, indicated by IGF-I and glucocorticoid levels, to induce compensatory growth following a period of Lys restriction in pigs (Ishida et al., 2013). Further investigations in the area of metabolic and endocrine regulation of growth are warranted to characterize the influence and interaction of metabolites, hormones, and dietary components on compensatory growth in pigs.

PRACTICAL CONSIDERATIONS ABOUT COMPENSATORY GROWTH IN GROW-FINISH PIGS

The review of literature and database analysis provide robust evidence to support the occurrence of compensatory growth induced by Lys restriction in grow-finish pigs. However, as the database analysis in the present review mostly includes studies conducted under research conditions, the authors recognize there could be a concern about the occurrence of compensatory growth under field or commercial production conditions. Although the physiological aspects of compensatory growth are prone to occur under research or commercial conditions, there are

additional factors under commercial conditions that could influence growth and, consequently, compensatory growth responses. For example, stocking density, number of pigs per feeder, environmental conditions, health challenges, and water quality and availability (Cornelison et al., 2018; Flohr et al., 2018; Wastell et al., 2018; De Oliveira et al., 2019).

Recent studies with grow-finish pigs reared in commercial research conditions validate the database analysis and indicate compensatory growth can occur in the field (Menegat et al., 2019). The same criteria and methods used to develop the database were applied to the commercial studies. A total of 11 comparisons were conducted within 4 commercial studies between restricted pigs and non-restricted pigs based on the number of treatments available for comparisons within each study, as previously described. To assess the occurrence of complete and incomplete compensatory growth within the commercial studies, the ADG in the recovery period was plotted against the final BW in the recovery period as a relative difference between restricted pigs compared to non-restricted pigs (Figure 1.5). The distribution of the field comparisons into quadrants depicts a similar pattern to the database comparisons, indicating the occurrence of complete, incomplete, and no compensatory growth. The growth patterns and occurrence of compensatory growth throughout the grow-finish period are further illustrated in Menegat et al. (2019).

Thus, there seems to be an opportunity to exploit compensatory growth in grow-finish pigs raised in a commercial environment. In addition to recognizing the determining factors of compensatory growth, it is essential to consider the economic implications of modifications in feeding programs or diet formulation to exploit compensatory growth. In economic scenarios of expensive dietary protein sources, relying on compensatory growth might be an economical approach. However, the economic feasibility of compensatory growth must be evaluated on a

case by case basis, considering the costs of feeding programs and diet formulation, the potential improvements in feed usage and feed efficiency, and the projections in market weight under different market conditions.

CONCLUSIONS

Compensatory growth induced by Lys restriction is a measurable and repeatable response in grow-finish pigs as long as fundamental concepts are considered: 1) there are differences in types, rates, and extents of compensatory growth; 2) there are differences in physiological mechanisms of compensatory growth according to the nature of nutritional restriction, i.e., Lys restriction through diet formulation or through feed intake limitation; and 3) genotype, stage of growth at restriction, nature of nutritional restriction, and patterns of restriction and recovery notably influence compensatory growth. The present review indicates compensatory growth seems to be more likely if: 1) degree of Lys restriction is around 10 to 30%; 2) Lys restriction is induced before pigs reach their maximum protein deposition (Pdmax); 3) duration of Lys restriction is short and duration of recovery period is long; and 4) Lys level in recovery is close to or above the estimated requirements. Compensatory growth can occur under commercial conditions and there seems to be an opportunity to exploit compensatory growth in grow-finish pigs to reduce feed cost and improve feed efficiency under certain market conditions.

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Table 1.1 Summary of publications included in the database to evaluate compensatory growth following a period of lysine restriction in grow-finish pigs

Publication	Number of comparisons[1]	Breed	Sex	Number of pigs per pen	Number of pen replicates	Diet main ingredients	Average diet NDF, %	Average initial BW, kg	Average final BW, kg	Overall duration, d
Wahlstrom and Libal, 1983	15	Crossbred	Mixed	7	3-4	Corn soybean meal	8.7	26.9	101.1	98
Chiba et al., 1999	4	Crossbred	Mixed	1	4	Corn soybean meal	8.7	23.0	105.4	89
Smith et al., 1999	7	Crossbred	Gilt	2	5	Corn soybean meal	8.5	29.5	107.6	82
Fabian et al., 2002	3	Duroc	Mixed	2	4	Corn soybean meal	8.8	20.7	108.3	117
Chiba et al., 2002	4	Duroc	Mixed	2	8	Corn soybean meal	8.7	19.6	113.0	121
Fabian et al., 2004	1	Crossbred	Barrow	1	8	Corn soybean meal	8.8	21.2	107.8	102
O'Connell et al., 2006	4	Crossbred	Mixed	2	9	Barley wheat soy	12.7	34.9	95.9	68
Reynolds and O'Doherty, 2006	2	Crossbred	Mixed	11	9	Wheat barley peas soy	11.5	42.0	88.6	56
Skiba et al., 2006a	2	Crossbred	Gilt	1	6	Corn wheat barley soy	12.5	25.0	104.9	87
Yang et al., 2008	3	Crossbred	Mixed	4	4	Corn wheat soy	9.5	34.3	115.1	91
Main et al., 2008	3	Crossbred	Gilt	27	7	Corn soybean meal	8.1	32.8	116.4	103
Kamalakar et al., 2009	4	Yorkshire	Mixed	2	6	Corn soybean meal	8.8	22.7	111.0	91
Suárez-Belloch et al., 2015	3	Crossbred	Mixed	6	5	Corn wheat barley soy	11.0	26.3	124.8	115
Cloutier et al., 2016	2	Crossbred	Barrow	9	9-10	Corn wheat barley soy	10.7	26.6	103.4	85

[1] Comparisons were conducted between restricted pigs and non-restricted pigs within each publication based on the number of treatments available for comparisons in each study. A total of 57 comparisons were conducted from 14 publications, except for carcass characteristics which were not determined in all publications.

Table 1.2 Descriptive summary of the database used to evaluate compensatory growth following a period of lysine restriction in grow-finish pigs[1,2,3]

Item[4]	Mean	Median	Minimum	Maximum	SD[5]	n[6]
Restriction period						
Initial BW, kg	27.7	26.7	18.2	52.0	6.5	57
Degree of lysine restriction, %[7]	33	33	7	59	14	57
Lysine to calorie ratio, g/Mcal[8]	2.40	2.18	1.57	4.07	0.68	57
Crude protein, %	14.9	13.9	11.0	21.5	2.8	57
Duration, d	39	37	28	75	11	57
Recovery period						
Initial BW, kg	56.7	49.9	32.2	78.4	12.4	57
Lysine to calorie ratio, g/Mcal[8]	2.58	2.27	1.60	4.96	0.70	57
Crude protein, %	15.6	14.7	11.7	23.4	2.7	55
Duration, d	55	59	26	86	18	57
Recovery to restriction ratio[9]	1.5	1.7	0.4	3.1	0.7	57
Restriction period growth performance						
ADG, % difference	-12.6	-11.5	-46.1	-1.3	8.5	57
ADFI, % difference	1.6	1.6	-17.8	26.4	7.1	57
G:F, % difference	-13.7	-14.9	-34.6	2.3	8.9	57
Final BW, % difference	-6.8	-5.6	-27.3	-0.6	4.8	57
Recovery period growth performance						
ADG, % difference	2.4	3.1	-14.9	16.4	6.4	57
ADFI, % difference	0.3	0.5	-17.0	22.5	6.7	57
G:F, % difference	3.6	2.8	-16.2	36.5	9.1	57
Final BW, % difference	-2.7	-2.5	-13.2	6.2	4.0	57
Overall period growth performance						
ADG, % difference	-3.4	-2.8	-19.9	15.3	5.9	57
ADFI, % difference	0.0	0.2	-13.8	14.2	5.1	57
G:F, % difference	-3.3	-2.4	-20.7	17.6	6.0	57
Carcass characteristics						
Yield, % difference	0.2	0.2	-3.3	4.4	1.9	13
Leanness, % difference	0.7	-0.4	-5.5	9.0	4.3	9
Longissimus muscle area, % difference	1.4	0.0	-12.2	22.2	9.5	15
Backfat thickness, % difference	0.1	0.6	-23.5	20.8	8.6	20

[1] Comparisons were conducted between restricted pigs and non-restricted pigs within each publication based on the number of treatments available for comparisons in each study. A total of 57 comparisons were conducted from 14 publications, except for carcass characteristics which were not determined in all publications.

[2] For values listed as percentage difference, the comparisons were performed as relative differences between restricted pigs compared to non-restricted pigs. The values of restricted pigs were divided by the values of non-restricted pigs, multiplied by 100 to convert to relative values, and subtracted from 100 to indicate the relative difference from non-restricted pigs.

[3] Restriction period is defined as a period of lysine restriction induced by decreasing lysine alone, lysine with other amino acids, or crude protein in diets offered to restricted pigs only. Recovery period is defined as a period of lysine sufficiency following the period of lysine restriction induced by providing the same diet to restricted and non-restricted pigs.

[4] ADG = average daily gain, ADFI = average daily feed intake, G:F = gain to feed ratio, BW = body weight.

[5] SD = standard deviation.

[6] Number of comparisons conducted.

[7] Estimated by dividing the dietary lysine to calorie ratio of restricted pigs by the dietary lysine to calorie ratio of non-restricted pigs.
[8] Expressed as a ratio of standardized ileal digestible lysine to net energy in g per Mcal.
[9] Estimated by dividing the duration of recovery period by the duration of restriction period in days.

Table 1.3 Characteristic aspects of compensatory growth depending on nature of nutritional restriction[1,2]

Item	Lysine restriction	Feed intake restriction
Restriction period		
Method of imposing restriction	Diet formulation	Intake limitation
Relative body protein composition	Lower	Higher
Relative body lipid composition	Higher	Lower
Visceral organs size	Similar	Lower
Recovery period		
Voluntary feed intake	Similar/Higher	Higher
Feed efficiency	Better	Similar/Better
Body protein deposition	Higher	Similar
Body lipid deposition	Similar	Higher
Visceral organs size	Similar	Higher

[1] Description of characteristics as lower, higher, better, or similar in regard to restricted pigs compared to non-restricted pigs in restriction and recovery periods.
[2] Summarized from de Greef et al. (1992), Bikker et al. (1996a,b), Chiba et al. (2002), Fabian et al. (2002), O'Connell et al. (2006), Lovatto et al. (2006), Reynolds and O'Doherty (2006), Heyer and Lebret (2007), Kamalakar et al. (2009), Martínez-Ramírez et al. (2009), Chaosap et al. (2011), and Suárez-Belloch et al. (2015).

Table 1.4 Database analysis and characterization of compensatory growth categories in grow-finish pigs[1,2,3]

Item[4]	Complete compensatory growth	Incomplete compensatory growth	No compensatory growth
n[5]	12	28	17
Restriction period			
Initial BW, kg	27.7	29.9	24.2
Degree of lysine restriction, %[6]	30	35	33
Lysine to calorie ratio, g/Mcal[7]	2.53	2.38	2.34
Crude protein, %	15.1	14.8	14.8
Duration, % overall duration[8]	37	45	44
Recovery period			
Initial BW, kg	53.6	58.6	55.7
Lysine to calorie ratio, g/Mcal[7]	2.47	2.68	2.50
Crude protein, %	15.2	16.0	15.3
Duration, % overall duration[8]	63	55	56
Recovery to restriction ratio[9]	1.8	1.4	1.5
Restriction period growth performance			
ADG, % difference	-6.3	-15.2	-12.8
ADFI, % difference	6.9	0.3	0.0
G:F, % difference	-11.4	-15.4	-12.6
Final BW, % difference	-3.2	-7.9	-7.4
Recovery period growth performance			
ADG, % difference	8.0	4.4	-5.1
ADFI, % difference	1.2	2.5	-3.9
G:F, % difference	9.7	2.4	1.2
Final BW, % difference	2.5	-2.6	-6.5
Overall period growth performance			
ADG, % difference	3.4	-2.9	-9.1
ADFI, % difference	1.2	1.8	-3.8
G:F, % difference	2.2	-4.3	-5.3
Carcass characteristics			
Yield, % difference	2.1	-0.2	-0.7
Leanness, % difference	-2.5	0.1	4.5
Longissimus muscle area, % difference	2.6	0.3	0.7
Backfat thickness, % difference	3.7	-0.1	-3.4

[1] Compensatory growth categories were defined by the distribution of all 57 database comparisons by plotting the ADG in the recovery period against the final BW in the recovery period as a relative difference between restricted pigs compared to non-restricted pigs. Complete compensatory growth indicates an increase in both ADG and final BW in the recovery period. Incomplete compensatory growth indicates an increase in ADG in the recovery period but a decrease in final BW. No compensatory growth indicates a decrease in both ADG and final BW in the recovery period.

[2] For values listed as percentage difference, the comparisons were performed as relative differences between restricted pigs compared to non-restricted pigs. The values of restricted pigs were divided by the values of non-restricted pigs, multiplied by 100 to convert to relative values, and subtracted from 100 to indicate the relative difference from non-restricted pigs.

[3] Restriction period is defined as a period of lysine restriction induced by decreasing lysine alone, lysine with other amino acids, or crude protein in diets offered to restricted pigs only. Recovery period is defined as a period of lysine

sufficiency following the period of lysine restriction induced by providing the same diet to restricted and non-restricted pigs.
[4] ADG = average daily gain, ADFI = average daily feed intake, G:F = gain to feed ratio, BW = body weight.
[5] Number of comparisons conducted. Comparisons were conducted between restricted pigs and non-restricted pigs within 14 publications for a total of 57 comparisons, except for carcass characteristics which were not determined in all publications.
[6] Estimated by dividing the dietary lysine to calorie ratio of restricted pigs by the dietary lysine to calorie ratio of non-restricted pigs.
[7] Expressed as a ratio of standardized ileal digestible lysine to net energy in g per Mcal.
[8] Estimated by dividing the duration of each period by the overall duration in days.
[9] Estimated by dividing the duration of recovery period by the duration of restriction period in days.

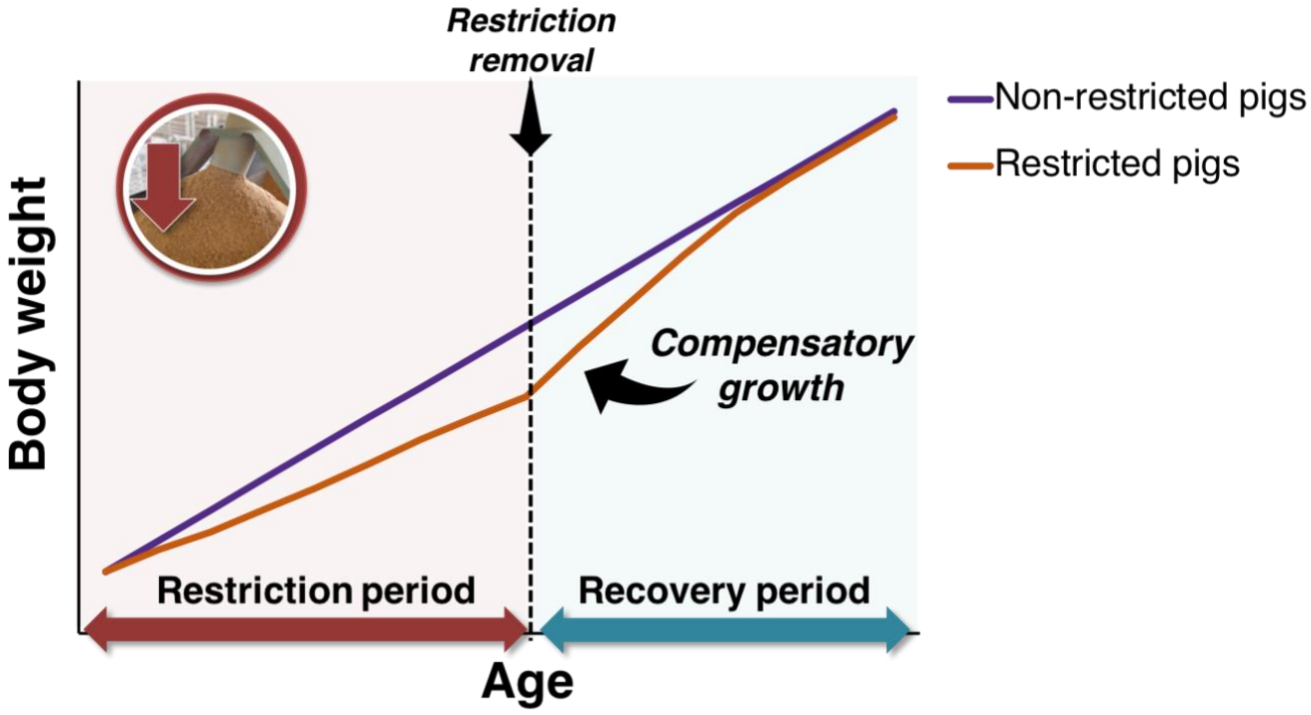

Figure 1.1 Representation of compensatory growth.

The graph depicts a period of accelerated growth rate in restricted pigs compared to non-restricted pigs following a period of growth restriction induced by nutritional deficiency.

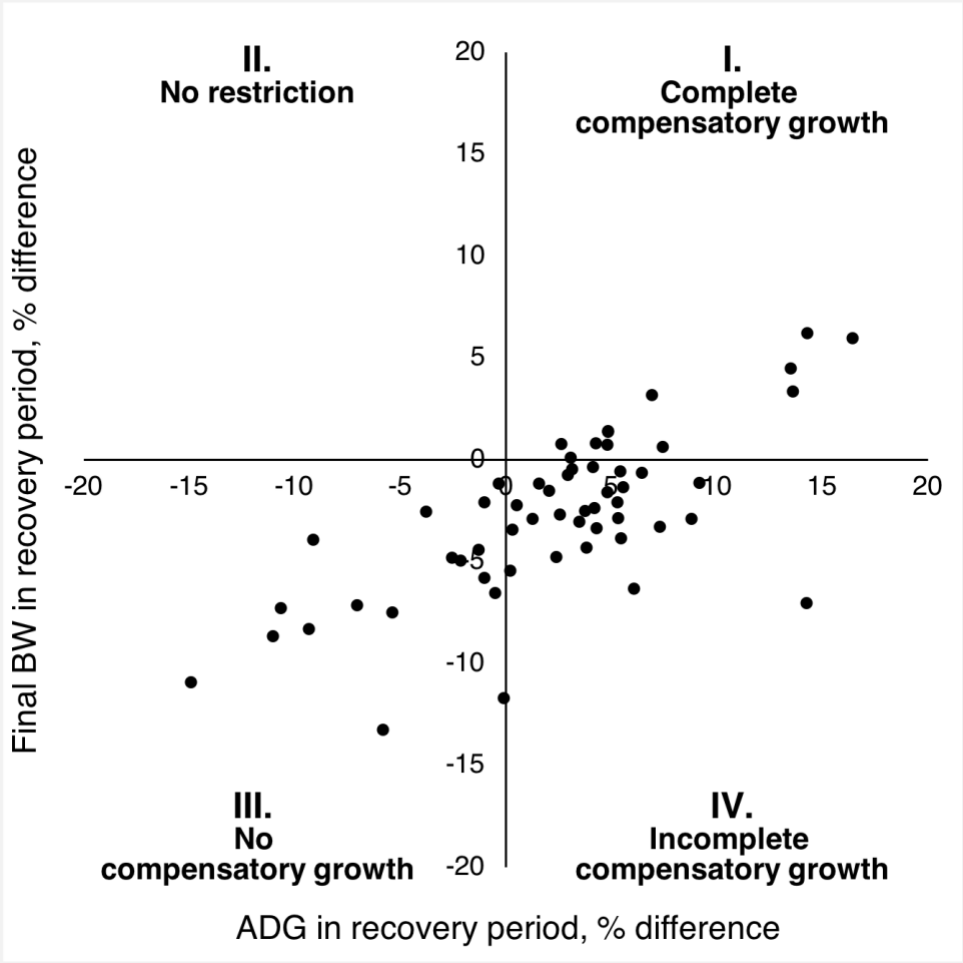

Figure 1.2 Plot of average daily gain (ADG) in the recovery period against final body weight (BW) in the recovery period as a relative difference between restricted pigs compared to non-restricted pigs.

The scatterplot depicts the distribution of all 57 database comparisons into four quadrants indicators of compensatory growth categories: I. complete compensatory growth due to an increase in ADG and final BW in restricted pigs compared to non-restricted pigs; III. no compensatory growth due to a decrease in ADG and final BW in restricted pigs compared to non-restricted pigs; and IV. incomplete compensatory growth due to increase in ADG but decrease in final BW in restricted pigs compared to non-restricted pigs. In quadrant II, there is a decrease in ADG in the recovery period but an increase in final BW in restricted pigs compared to non-restricted pigs, which means no restriction and, therefore, comparisons falling in quadrant II (3 out of 60) were excluded from the database.

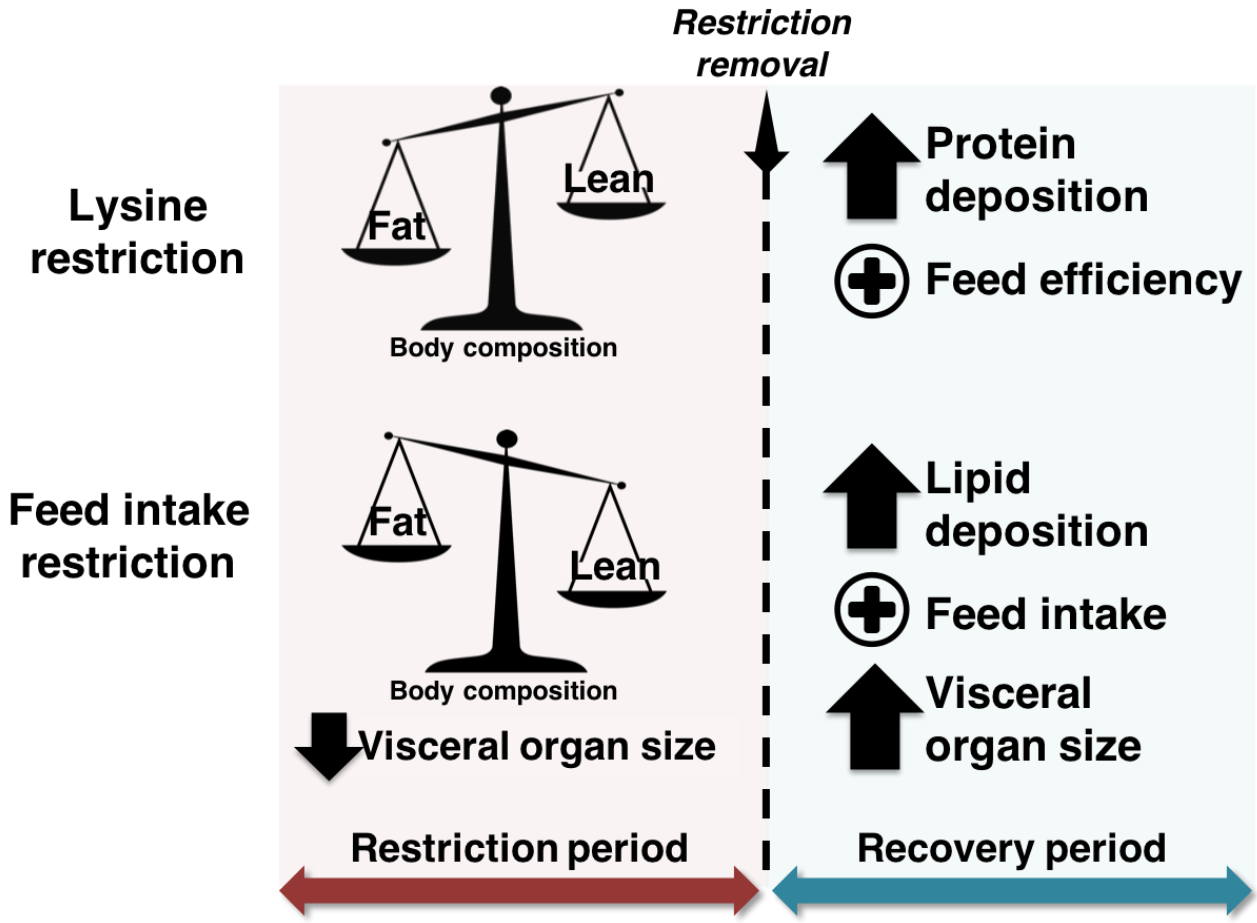

Figure 1.3 Representation of compensatory growth responses according to the nature of nutritional restriction: dietary lysine restriction or feed intake restriction.

The figure depicts the differences in relative body composition during restriction and in composition of gain during compensatory growth according to the nature of nutritional restriction.

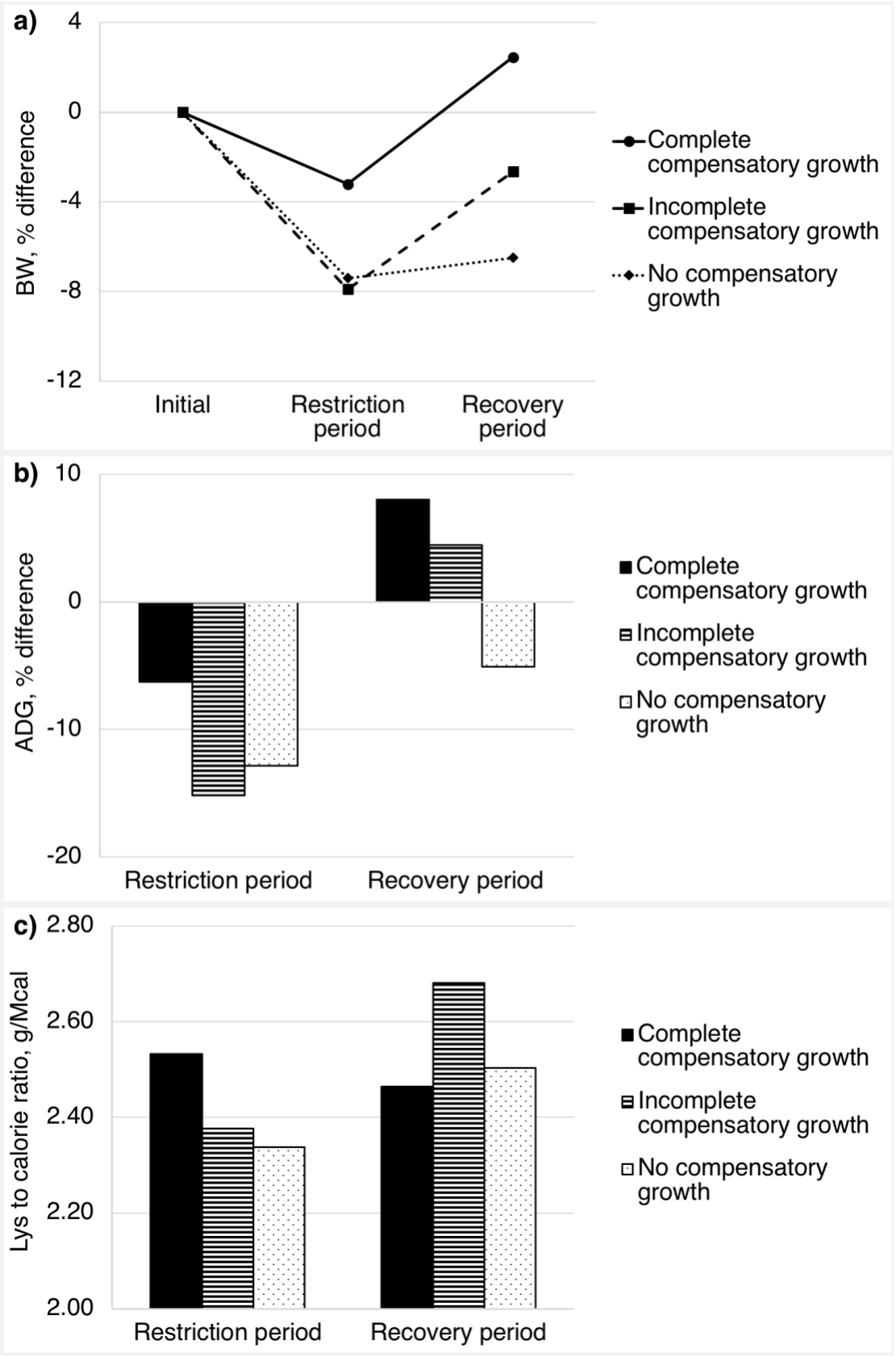
a)
4
0
-4
-8
-12
BW, % difference
Initial
Restriction period
Recovery period
Complete compensatory growth
Incomplete compensatory growth
No compensatory growth
b)
10
0
-10
-20
ADG, % difference
Restriction period
Recovery period
Complete compensatory growth
Incomplete compensatory growth
No compensatory growth
c)
2.80
2.60
2.40
2.20
2.00
Lys to calorie ratio, g/Mcal
Restriction period
Recovery period
Complete compensatory growth
Incomplete compensatory growth
No compensatory growth

Figure 1.4 Database comparisons: differences in a) body weight (BW) as a relative difference between restricted pigs compared to non-restricted pigs; b) average daily gain (ADG) as a relative difference between restricted pigs compared to non-restricted pigs; and c) lysine (Lys) to calorie ratio as a ratio of standardized ileal digestible Lys to net energy, according to category of compensatory growth in restriction and recovery periods.

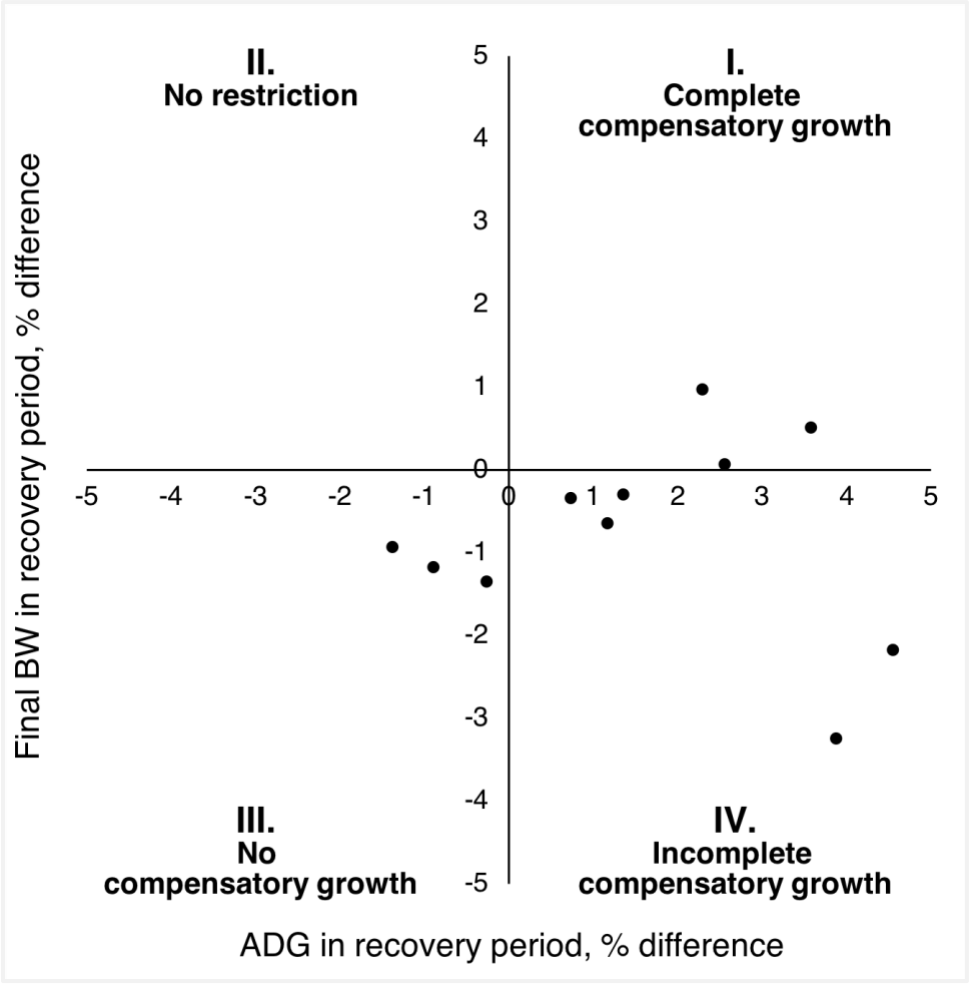

Figure 1.5 Field comparisons: plot of average daily gain (ADG) in the recovery period against final body weight (BW) in the recovery period as a relative difference between restricted pigs compared to non-restricted pigs.

The scatterplot depicts the distribution of 11 comparisons within 4 commercial studies into four quadrants indicators of compensatory growth categories: I. complete compensatory growth due to an increase in ADG and final BW in restricted pigs compared to non-restricted pigs; III. no compensatory growth due to a decrease in ADG and final BW in restricted pigs compared to non-restricted pigs; and IV. incomplete compensatory growth due to increase in ADG but decrease in final BW in restricted pigs compared to non-restricted pigs. There were no comparisons falling in quadrant II, which means no restriction.

Chapter 2 - Phase-feeding strategies based on lysine specifications for grow-finish pigs

ABSTRACT: Four experiments were conducted using 1,100 to 1,188 pigs each (PIC 359 × 1050) from approximately 27 to 127 kg body weight (**BW**) to evaluate phase-feeding strategies based on Lys specifications and number of dietary phases for grow-finish pigs. Different phase-feeding strategies were used in each experiment with treatments consisting of a combination of 3 Lys specifications at 96, 98, or 100% of estimated requirement for growth rate and 4 phase-feeding strategies with 1, 2, 3, or 4 dietary phases. A single-phase feeding strategy reduced ($P < 0.05$) overall growth performance, live BW, and hot carcass weight (**HCW**) whether Lys specifications were at 98% or 100% of estimated requirements compared to multi-phase feeding strategies. Lysine specifications at 96% of estimated requirements in a 4-phase feeding strategy reduced ($P < 0.05$) overall growth performance compared to feeding strategies with Lys at 100% of estimated requirements, unless Lys specifications were increased to 100% of estimated requirements in the late finishing phase. Lysine specifications at 98% or 100% of estimated requirements in a 2-, 3- or 4-phase feeding strategy led to similar ($P < 0.05$) overall growth rate, live BW, and HCW of grow-finish pigs. Pigs fed 1, 2, or 3-phase feeding strategies or feeding strategies with Lys below the requirements in early grow-finish had improved growth performance driven by improved feed efficiency in the period following low Lys levels, indicating the occurrence of compensatory growth. For carcass characteristics, there was no evidence ($P > 0.10$) for differences in carcass yield, backfat, loin depth, or lean percentage across feeding strategies in any of the experiments. In conclusion, phase-feeding strategies provide performance advantages over feeding a single dietary phase throughout the grow-finish period. Simplification of feeding strategies from 4 to 3 or 2 dietary phases with Lys specifications at

98% to 100% of estimated requirements for growth rate does not compromise overall growth performance and carcass characteristics of grow-finish pigs from 27 to 127 kg BW. Although, using feeding programs with fewer dietary phases and Lys set slightly below the requirements can compromise growth performance if initial BW and feed intake in the grow-finish period are lower than expected.

Key words: carcass, compensatory growth, feeding program, performance, protein, swine

INTRODUCTION

Phase-feeding strategies have been widely used to closely meet the nutrient requirements of grow-finish pigs (Han et al., 2000). The core component of developing phase-feeding strategies lies on determining dietary Lys specifications as Lys is the first limiting amino acid in most swine diets and determines optimal growth and lean deposition (Main et al., 2008). Dietary Lys concentration decreases over the grow-finish period with phase-feeding used as an attempt to meet the biological requirements of pigs as their ability to consume feed exceeds their capacity for protein deposition (NRC, 2012). However, in practice, the variation in weight, growth rate, and feed intake within a population prevents accurate estimation and delivery of optimal Lys concentration for growth on an individual basis even with meticulously designed phase-feeding strategies (Pomar and Remus, 2019).

Phase-feeding strategies with fewer dietary phases typically provide at or below Lys requirements initially and provide adequate or excess Lys levels later in the phase. Previous studies suggest simplification of phase-feeding strategies to fewer dietary phases can lead to optimal grow-finish performance and carcass characteristics and, from an economic and environmental standpoint, can minimize dietary protein input and nitrogen excretion in early grow-finish (O'Connell et al., 2005; Moore et al., 2012). Simplification of phase-feeding

strategies may be an opportunity to exploit compensatory growth. There is evidence of compensatory growth induced by Lys restriction in nursery pigs (Totafurno et al., 2017; Nemechek et al., 2018) as well as grow-finish pigs (reviewed by Menegat et al., 2019). Pigs exhibiting compensatory growth have improvements in feed efficiency and Lys utilization efficiency, which may be beneficial for overall swine production efficiency (Fabian et al., 2004; Reynolds and O'Doherty, 2006).

Therefore, the objective of this study was to evaluate whether simplification of phase-feeding strategies using different Lys specifications for growth rate is possible without compromising overall performance and carcass characteristics of grow-finish pigs.

MATERIALS AND METHODS

The Kansas State University Institutional Care and Use Committee approved the protocol used in the experiments. A series of 4 experiments were conducted to evaluate phase-feeding strategies based on Lys specifications and the number of dietary phases in the grow-finish period.

Animals and dietary treatments

Experiments were conducted at a commercial research facility in southwestern Minnesota. The barns used in the experiments were identical, naturally ventilated, and double-curtain-sided. In each experiment, a total of 1,100 to 1,188 pigs (359 × 1050, Genus PIC, Hendersonville, TN) were housed in 44 pens with 25 to 27 mixed-gender pigs per pen from approximately 27 to 127 kg body weight (**BW**) and approximately 120 days. Pens (5.5 × 3.0 m) had completely slatted floors and were equipped with a four-hole stainless steel dry self-feeder and a cup waterer. Pigs were allowed ad libitum access to feed and water. Feed additions were

accomplished and recorded by a computerized feeding system (FeedPro, Feedlogic Corp., Wilmar, MN).

In each experiment, pens of pigs were blocked by BW and randomly assigned to 1 of 4 treatments arranged in a randomized complete block design with 11 replicates per treatment. Treatments consisted of feeding strategies based on Lys specifications (96%, 98%, and 100% of estimated Lys requirements for growth rate) and the number of dietary phases (1, 2, 3, or 4 dietary phases) in the grow-finish period. The Lys specifications at 96%, 98%, and 100% of estimated requirements for growth rate were derived from the genetic supplier's Lys requirement prediction equation based on commercial experiments: SID Lys, g/Mcal NE = $0.000056 \times (\text{BW, kg} \times 2.2046)^2 - 0.02844 \times (\text{BW, kg} \times 2.2046) + 6.6391$ (Genus PIC, Hendersonville, TN; Gonçalves et al., 2017). Lysine levels were set at the midpoint of the BW range within a dietary phase. The number of dietary phases were 1, 2, 3, or 4 dietary phases, termed 1-phase, 2-phase, 3-phase, and 4-phase, respectively. An overview of weight range, duration, and Lys:calorie ratio used in each experiment are outlined in Table 2.1.

In Exp. 1, treatments consisted of: a 2-phase feeding strategy with Lys specifications at 100% of estimated requirements; a 4-phase feeding strategy with Lys specifications at 100% of estimated requirements; a 4-phase feeding strategy with Lys specifications at 96% of estimated requirements; and a 4-phase feeding strategy with Lys specifications at 96% of estimated requirements in growing (27 to 72 kg BW) and at 100% of estimated requirements in finishing (72 to 127 kg BW). In Exp. 2, treatments consisted of 1-, 2-. 3-, or 4-phase feeding strategies with Lys specifications at 100% of estimated requirements. In Exp. 3, treatments consisted of 1-, 2-. 3-, or 4-phase feeding strategies with Lys specifications at 98% of estimated requirements. In Exp. 4, treatments consisted of: a 2-phase feeding strategy with Lys specifications at 98% of estimated requirements; a 2-phase feeding strategy with Lys specifications at 98% of estimated

requirements in growing-finishing (27 to 100 kg BW) and at 100% of estimated requirements in late finishing (100 to 127 kg BW); a 3-phase feeding strategy with Lys specifications at 98% of estimated requirements; and a 4-phase feeding strategy with Lys specifications at 98% of estimated requirements.

In all experiments, diets were based on corn, distillers dried grains with solubles, and soybean meal (Tables 2.2 to 2.5). Distillers dried grains with solubles were withdrawn from the last dietary phase except for 1-phase feeding strategies and all feeding strategies in Exp. 2. The sources of corn, distillers dried grains with solubles, and soybean meal used in the experiments have been regularly sampled and tested for amino acid content. These values were used in diet formulation and standardized ileal digestible amino acid coefficients were derived from NRC (2012). Lysine levels in experimental diets were achieved by changing the amount of soybean meal. All other nutrient requirements met or exceeded the NRC (2012) recommendations in all dietary phases. Diets were manufactured at a commercial feed mill in southwestern Minnesota and offered in meal form.

Growth performance and carcass characteristics

Pens of pigs were weighed and feed disappearance measured approximately every 2 weeks to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Besides determining overall performance, intermediate performance of the feeding strategies was compared to the performance of the 4-phase feeding strategy within experiments, thus considering the 4-phase feeding strategy with Lys specifications at 100% (Exp. 1 and 2) or 98% (Exp. 3 and 4) of estimated requirements as standard. The relative difference between feeding strategies was calculated as follows:

$$Relative\ difference\ (\%) = \left[\left(\frac{ADG\ of\ feeding\ strategies}{ADG\ of\ 4-Phase\ feeding\ strategy}\right) \times 100\right] - 100$$

At the end of the experimental period, pen weights were recorded and pigs were tattooed with a pen identification number and transported to a packing plant (JBS Swift and Co., Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (**HCW**), backfat thickness, loin depth, and percentage lean. Carcass percentage lean was calculated from the packing plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average live BW.

Chemical analysis

Representative samples of complete feed were collected from multiple feeders per treatment during each dietary phase. Samples were stored at -20°C until analysis. Composite samples were ground, subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter (**DM**; method 935.29 AOAC, 1990), crude protein (**CP**; method 990.03 AOAC, 1990), ether extract (**EE**; Ankom, 2004), neutral detergent fiber (**NDF**; Ankom, 1998), Ca (method 985.01 AOAC, 1990), and P (method 985.01 AOAC, 1990). In addition, composite samples were analyzed (Ajinomoto Heartland, Inc., Chicago, IL) for total amino acids (method 994.12 for all except Trp and 994.13 for Trp, AOAC, 1990).

Statistical analysis

Data were analyzed using a linear mixed model with treatment as fixed effect, block as random effect, and pen as the experimental unit. Data were modeled as normally-distributed response variables as the residual analysis assumptions were met by evaluating studentized residuals. Hot carcass weight was used as a covariate for analyses of backfat thickness, loin depth, and percentage lean. Statistical models were fit using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Chemical analysis

The analyzed DM, CP, EE, NDF, Ca, and P content of experimental diets were consistent with formulated estimates (Tables 2.2 to 2.5). The analyzed total amino acids (not shown) and Lys (Tables 2.2 to 2.5) in experimental diets were within expectations considering expected analytical variation for complete feed analysis (AAFCO, 2018).

Growth performance and carcass characteristics

Phase-feeding is the strategy of feeding multiple diets during the grow-finish period to closely meet the continuously changing Lys requirements of pigs. Phase-feeding strategies with multiple dietary phases and frequent phase changes are more likely to closely meet the Lys requirements of grow-finish pigs and minimize the supply of Lys in deficiency or excess (Andretta et al., 2014). However, the manufacture, delivery, and storage logistics for multiple dietary phases are often not feasible in commercial swine production. Moreover, the variation in weight, growth rate, and feed intake within a population prevents accurate estimation and delivery of optimal Lys concentration for growth on an individual basis even with meticulously designed phase-feeding strategies (Pomar and Remus, 2019). Thus, simplification of phase-feeding strategies to fewer dietary phases in the grow-finish period is a topic of growing interest (Moore et al., 2012; Hong et al., 2016; Presto Åkerfeldt et al., 2019). Phase-feeding strategies with fewer dietary phases typically provide at or below Lys requirements initially and provide adequate or excess Lys levels later in the phase, and generally rely on compensatory growth to ensure optimal growth performance and carcass characteristics (O'Connell et al., 2005; Garry et al., 2007). The number of dietary phases and Lys specifications comprising phase-feeding strategies require careful consideration to ensure optimal growth performance and carcass characteristics of grow-finish pigs. Thus, the present study aimed to evaluate and provide

scientific support to the widespread practice of phase-feeding in swine production, as current peer-reviewed publications with grow-finish pigs reared in commercial conditions are needed.

Growth performance in the overall grow-finish period is presented in Table 2.6 for all experiments. The first experiment was conducted to evaluate feeding strategies with distinct Lys specifications (96% vs. 100% of estimated requirements). The use of Lys specifications at 96% of estimated requirements in a 4-phase feeding strategy reduced ($P < 0.05$) overall ADG and led to numerically ($P > 0.10$) lower final live BW by 2.5 kg and HCW by 1.5 kg, approximately, compared to feeding strategies with Lys at 100% of estimated requirements. However, there was no evidence ($P > 0.05$) that Lys specifications at 96% of estimated requirements in the growing phase compromised overall growth performance and final BW provided that Lys specifications were increased to 100% of estimated requirements in the late finishing phase. In agreement with Main et al. (2008), as long as Lys requirements are met in late finishing, using Lys below the requirements in early growing does not impact overall growth performance. Interestingly, Lys specifications at 100% of estimated requirements in either a 2- or 4-phase feeding strategy led to similar overall growth performance of grow-finish pigs. The benefits of multiple dietary phases in phase-feeding strategies seems to be less evident when using Lys at the requirements, as previously proposed by Garry et al. (2007).

The second experiment was conducted to evaluate the effects of 1, 2, 3, or 4 dietary phases with Lys specifications at 100% of estimated requirements. A single-phase feeding strategy reduced ($P < 0.05$) overall ADG, live BW, and HCW compared to multi-phase feeding strategies. In contrast, previous studies have found no evidence for impact on growth performance by having a single dietary phase during the grow-finish period (Bradford and Gous, 1991; Lee et al., 2000; O'Connell et al., 2005; Garry et al., 2007; Moore et al., 2012). The divergence from the present study could be due to differences in Lys levels and weight range of

the phase-feeding strategies used in the studies, as well as experimental conditions. Among the phase-feeding strategies, either a 2-, 3- or 4-phase feeding strategy resulted in no evidence for difference ($P > 0.10$) in overall ADG and final BW of grow-finish pigs, which is in agreement with previous studies (Bradford and Gous, 1991; Lee et al., 2000; Hong et al., 2016). Validating the findings in Exp. 1, simplification of phase-feeding strategies to fewer dietary phases in the grow-finish period with Lys set at the estimated requirements seems feasible.

The third experiment was conducted to evaluate whether simplification of phase-feeding strategies could be implemented with Lys below the requirements. Because Lys specifications at 96% of estimated requirements negatively affected growth performance in Exp. 1, Lys specifications at 98% of estimated requirements were used. Validating the findings in Exp. 2, a single-phase feeding strategy reduced ($P < 0.05$) overall ADG, G:F, live BW, and HCW compared to multi-phase feeding strategies. Among the phase-feeding strategies, either a 2-, 3- or 4-phase feeding strategy led to similar ($P > 0.05$) overall ADG and final BW of grow-finish pigs. However, a 2- or 3-phase feeding strategy reduced ($P < 0.05$) overall G:F compared to a 4-phase feeding strategy. A previous study by Lee et al. (2000) corroborates the improvement in feed efficiency in 4-phase compared to 2- or 3-phase feeding strategy. Because Lys is an important component to efficient lean growth (van Milgen and Dourmad, 2015), it seems more critical to set Lys closely to the estimated requirements in phase-feeding strategies with fewer dietary phases.

The last experiment was conducted to evaluate whether increasing Lys levels from 98% to 100% of estimated requirements in late finishing in a 2-phase feeding strategy or decreasing Lys levels earlier instead of later in a 3-phase feeding strategy with Lys at 98% of estimated requirements could lead to similar overall growth performance to a 4-phase feeding strategy with Lys at 98% of estimated requirements. Indeed, both approaches resulted in no evidence for

difference ($P > 0.05$) in overall growth performance compared to a 4-phase feeding strategy even with Lys levels below the requirements. However, in contrast to Exp. 3, the 2-phase feeding strategy with Lys at 98% of estimated requirements led to similar ($P > 0.05$) overall growth performance and final BW to a 4-phase feeding strategy with Lys at 98% of estimated requirements. Although unexpected, the discrepancy between experiments could be attributed to a difference in initial BW and overall feed intake. In Exp. 3, initial BW and overall feed intake were approximately 10% and 4% lower, respectively, compared to Exp. 4. Thus, in Exp. 3 initial Lys levels were further below the estimated requirements in the early grow-finish period and Lys intake in the entire grow-finish period was lower. In agreement with previous studies (O'Connell et al., 2005; Garry et al., 2007; Presto Åkerfeldt et al., 2019), simplification of phase-feeding strategies to fewer dietary phases in the grow-finish period with Lys set slightly below the estimated requirements seems feasible. However, in feeding programs with fewer dietary phases and Lys set slightly below the requirements, growth performance can be compromised if initial BW and feed intake in the grow-finish period are lower than expected.

For carcass characteristics, there was no evidence ($P > 0.10$) for differences in carcass yield, backfat, loin depth, or lean percentage across feeding strategies in any of the experiments (Table 2.7). Thus, the influence of phase-feeding strategies on carcass characteristics was not detected except for effects on HCW driven by differences in live BW. In agreement, previous phase-feeding studies have found no evidence for influence of number of dietary phases on carcass characteristics (O'Connell et al., 2005; Garry et al., 2007; Moore et al., 2012; Hong et al., 2016). Lee et al. (2000) observed a tendency for an increase in backfat thickness in pigs fed a single-phase compared to multi-phase feeding strategies. Lipid deposition tends to increase in a single-phase strategy due to the typically low Lys levels in the early grow-finish period which coincides with the period of greater potential for lean deposition (Lee et al., 2000). In the present

study, only a numeric increase in backfat thickness was observed in pigs under the single-phase feeding strategies compared to multi-phase feeding strategies.

Compensatory growth is a physiological process whereby animals undergo a period of accelerated growth following a period of nutritional restriction (Hornick et al., 2000, O'Connell et al., 2006). Compensatory growth induced by Lys restriction in grow-finish pigs has been previously described (Chiba et al., 2002; Reynolds and O'Doherty, 2006; Suárez-Belloch et al., 2015). Although the present study was not purposefully designed to evaluate compensatory growth, there were indications of compensatory growth induced by previous Lys restriction. The influence of Lys levels in the grow-finish period on ADG is illustrated in Fig. 2.1. The initial low Lys levels of 1- and 2-phase feeding strategies or feeding strategies with Lys below the requirements in the early grow-finish (96%/100% and 98%/100%) led to considerable decrease in ADG during the period of 27 to 50 or 27 to 72 kg BW compared to 4-phase feeding strategies. However, the following adequate Lys levels led to a substantial increase in ADG compared to 4-phase feeding strategies, even though in some instances the dietary Lys levels were the same as those provided to pigs on 4-phase feeding strategies. The improvement in growth rate in the period following Lys restriction was mainly driven by feed efficiency and indicates the occurrence of compensatory growth. Compensatory growth is a complex phenomenon affected by a number of factors and interactions. Particularly in the present study, severe Lys restriction, long restriction duration, and low Lys level following restriction seem to critically impair the ability of occurrence of complete or incomplete compensatory growth. This is evidenced by the inability of pigs fed 1-phase feeding strategies to completely compensate to achieve similar performance as pigs fed 4-phase feeding strategies, as well as by the distinct ability of pigs fed 2-phase feeding strategies across experiments to completely compensate to achieve similar performance as pigs fed 4-phase feeding strategies based on Lys specifications, initial Lys

restriction, and Lys intake. Interestingly, pigs fed 3-phase feeding strategies with low Lys levels earlier in the growing period (Exp. 4) seem to have the ability to compensate, whereas pigs fed 3-phase feeding strategies with low Lys levels later in the finishing period (Exp. 2 and 3) do not.

It appears feasible to simplify phase-feeding strategies to fewer dietary phases for grow-finish pigs without compromising growth performance and carcass characteristics. Simplification of phase-feeding strategies is an opportunity to exploit compensatory growth in grow-finish pigs as an approach to minimize the costs of diet formulation and optimize efficiency of feed utilization (Menegat et al., 2019). Moreover, simplification of phase-feeding strategies provides benefits in logistics of feed manufacture, delivery, and storage within the swine production system (Moore et al., 2012). From the feed manufacture standpoint, phase-feeding strategies with fewer diets provide an opportunity to improve feed mill efficiency, particularly with a continuous throughput pellet mill as longer larger tonnage runs improve mill efficiency. However, there is often an increase in nitrogen excretion in phase-feeding strategies with fewer dietary phases (Lee et al., 2000; Moore et al., 2012; Andretta et al., 2014). Although the nitrogen excretion in earlier phases is minimal due to Lys levels set below the requirement, the nitrogen excretion in subsequent phases increases due to Lys levels set above the requirement and, therefore, not being totally utilized for lean deposition (Han et al., 2000). Interestingly, O'Connell et al. (2005) suggest a similar nitrogen excretion is possible between single-phase and multi-phase feeding strategies when the average Lys level is the same across the grow-finish period.

In conclusion, feeding strategies with Lys specifications set at 96% of estimated requirements compromise performance of grow-finish pigs unless Lys requirements are fully met in late finishing. Phase-feeding strategies provide performance advantages over feeding a single dietary phase throughout the grow-finish period. Simplification of feeding strategies from 4 to 3 or 2 dietary phases with Lys specifications at 98% to 100% of estimated requirements for growth

rate does not compromise overall growth performance and carcass characteristics of grow-finish pigs from 27 to 127 kg BW. Although, using feeding programs with fewer dietary phases and Lys set slightly below the requirements can compromise growth performance if initial BW and feed intake in the grow-finish period are lower than expected.

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Table 2.1 Overview of experiments evaluating feeding strategies based on number of dietary phases (1, 2, 3, and 4 dietary phases) and lysine specifications (96%, 98%, and 100% of estimated requirements for growth rate) in the grow-finish period[1,2]

Approximate weight range, kg		27 to 50	50 to 72	72 to 100	100 to 127
Approximate duration, d		30	30	30	30
Dietary phases	Lysine specification	Lysine:calorie ratio[3]			
Exp. 1					
2-phase	100%	3.84	3.84	3.84	3.00
4-phase	100%	4.61	3.84	3.24	3.00
4-phase	96%	4.12	3.46	2.98	2.59
4-phase	96%/100%[4]	4.12	3.46	3.24	3.00
Exp. 2					
1-phase	100%	3.23	3.23	3.23	3.23
2-phase	100%	3.83	3.83	3.83	3.01
3-phase	100%	4.57	3.51	3.51	3.01
4-phase	100%	4.57	3.81	3.21	3.01
Exp. 3					
1-phase	98%	3.10	3.10	3.10	3.10
2-phase	98%	3.61	3.61	3.61	2.81
3-phase	98%	4.31	3.34	3.34	2.81
4-phase	98%	4.31	3.60	3.08	2.81
Exp. 4					
2-phase	98%	3.61	3.61	3.61	2.81
2-phase	98%/100%[5]	3.61	3.61	3.61	3.02
3-phase	98%	3.96	3.96	3.08	2.81
4-phase	98%	4.31	3.60	3.08	2.81

[1] A total of 4 experiments were conducted at a commercial research facility with 1,100 to 1,188 pigs (PIC 359 × 1050) per experiment in pens of 25 to 27 mixed-gender pigs per pen and 11 replicates per treatment. Experiments were conducted from approximately 27 to 127 kg body weight (BW) for approximately 120 d. Exp. 1 from 27.9 to 129.1 kg BW for 117 days, Exp. 2 from 27.4 to 125.6 kg BW for 121 days, Exp. 3 from 25.9 to 131.5 kg BW for 119 days, and Exp. 4 from 28.8 to 129.9 kg BW for 114 days.

[2] Lysine specifications at 96%, 98%, and 100% of estimated requirements for growth rate were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments: SID Lys, g/Mcal NE = $0.000056 \times (\text{BW, kg} \times 2.2046)^2 - 0.02844 \times (\text{BW, kg} \times 2.2046) + 6.6391$ (Genus PIC, Hendersonville, TN; Gonçalves et al., 2017).

[3] Lysine:calorie ratio expressed as g of standardized ileal digestible lysine per Mcal of net energy.

[4] Lysine specifications at 96% of estimated requirements were applied in growing (27 to 72 kg BW) and lysine specifications at 100% of estimated requirements were applied in finishing (72 to 127 kg BW).

[5] Lysine specifications at 98% of estimated requirements were applied in growing-finishing (27 to 100 kg BW) and lysine specifications at 100% of estimated requirements were applied in late finishing (100 to 127 kg BW).

Table 2.2 Composition of experimental diets (as-fed basis), Exp. 1[1,2]

Dietary phases	2-phase		4-phase				4-phase				4-phase			
Lysine specification	100%		100%				96%				96%/100%			
Initial weight, kg	27	100	27	50	72	100	27	50	72	100	27	50	72	100
Ingredient, % Final weight, kg	100	127	50	72	100	127	50	72	100	127	50	72	100	127
Corn	54.73	81.14	47.54	54.74	60.50	81.14	51.98	58.34	62.93	85.18	51.98	58.34	60.50	81.14
DDGS[3]	30.00	---	30.00	30.00	30.00	---	30.00	30.00	30.00	---	30.00	30.00	30.00	---
Soybean meal, 47% crude protein	12.13	16.05	19.09	12.13	6.41	16.05	14.60	8.46	3.96	11.97	14.60	8.46	6.41	16.05
Tallow	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Monocalcium phosphate, 21% P	0.05	0.25	0.20	0.05	---	0.25	0.25	0.10	---	0.30	0.25	0.10	---	0.25
Calcium carbonate	1.20	0.95	1.28	1.20	1.20	0.95	1.30	1.23	1.23	0.95	1.30	1.23	1.20	0.95
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.50	0.25	0.50	0.50	0.50	0.25	0.50	0.50	0.50	0.25	0.50	0.50	0.50	0.25
DL-Methionine	---	0.02	0.02	---	---	0.02	---	---	---	0.01	---	---	---	0.02
L-Threonine	0.09	0.07	0.09	0.08	0.08	0.07	0.08	0.08	0.07	0.06	0.08	0.08	0.08	0.07
L-Tryptophan	0.05	0.02	0.03	0.04	0.05	0.02	0.03	0.04	0.05	0.02	0.03	0.04	0.05	0.02
Vitamin-trace mineral premix[4]	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis														
SID[5] amino acids, %														
Lysine	0.96	0.77	1.13	0.96	0.82	0.77	1.02	0.87	0.76	0.67	1.02	0.87	0.82	0.77
Isoleucine:lysine	62	64	63	62	61	64	62	61	60	63	62	61	61	64
Leucine:lysine	173	154	161	173	186	154	168	180	193	163	168	180	186	154
Methionine:lysine	31	30	30	31	33	30	30	32	34	30	30	32	33	30
Methionine and cysteine:lysine	59	58	57	59	63	58	57	61	65	60	57	61	63	58
Threonine:lysine	64	65	62	63	64	65	62	63	64	65	62	63	64	65
Tryptophan:lysine	19.8	19.5	18.9	18.7	19.9	19.5	18.5	18.9	19.7	19.8	18.5	18.9	19.9	19.5
Valine:lysine	75	73	74	75	76	73	74	76	77	74	74	76	76	73
Total lysine, %	1.13	0.88	1.32	1.13	0.97	0.88	1.19	1.03	0.91	0.76	1.19	1.03	0.97	0.88
NE, kcal/kg	2,498	2,566	2,452	2,498	2,533	2,566	2,476	2,518	2,546	2,588	2,476	2,518	2,533	2,566
SID Lys:NE, g/Mcal	3.84	3.00	4.61	3.84	3.24	3.00	4.12	3.46	2.98	2.59	4.12	3.46	3.24	3.00
Crude protein, %	18.6	13.6	21.4	18.6	16.3	13.6	19.6	17.1	15.3	12.0	19.6	17.1	16.3	13.6
Calcium, %	0.53	0.45	0.60	0.53	0.50	0.45	0.60	0.53	0.50	0.45	0.60	0.53	0.50	0.45
STTD[6] phosphorus, %	0.33	0.26	0.38	0.33	0.30	0.26	0.38	0.33	0.30	0.26	0.38	0.33	0.30	0.26

Chemical analysis[7]														
Dry matter, %	88.1	87.2	87.7	87.5	88.1	87.1	87.1	88.1	88.2	87.4	87.4	87.5	88.4	87.0
Crude protein, %	18.0	13.3	19.7	20.2	16.4	13.1	18.9	18.8	15.0	12.1	18.5	17.0	16.0	13.8
Ether extract, %	4.9	3.4	5.4	4.8	5.1	3.5	5.0	4.9	5.0	3.5	4.7	5.0	5.3	3.1
Neutral detergent fiber, %	11.5	5.7	13.7	12.9	12.6	5.6	11.8	12.5	11.3	5.4	11.4	11.2	13.0	5.3
Calcium, %	0.66	0.53	0.67	0.83	0.58	0.52	0.69	0.73	0.77	0.53	0.72	0.70	0.52	0.57
Phosphorus, %	0.46	0.34	0.50	0.47	0.44	0.34	0.49	0.51	0.40	0.33	0.47	0.45	0.43	0.34
Lysine, %	1.05	0.84	1.19	1.15	0.92	0.78	1.12	1.10	0.83	0.71	1.11	0.97	0.93	0.79

[1] Experiment evaluated feeding strategies based on number of dietary phases (2 and 4 dietary phases) and lysine specifications (96% and 100% of estimated requirements for growth rate) for grow-finish pigs. Lysine specifications were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments (Genus PIC; Gonçalves et al., 2017). For 96%/100%, lysine specifications for 96% of estimated requirements were applied in growing (27 to 72 kg body weight) and lysine specifications for 100% of estimated requirements were applied in finishing (72 to 127 kg body weight).

[2] Diets were fed ad libitum in meal form from 27.9 to 129.1 kg body weight for 117 days. Lysine levels in experimental diets were achieved by changing the inclusion of soybean meal.

[3] DDGS = corn distillers dried grains with solubles.

[4] Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B_{12}; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; 0.20 g Se from sodium selenite; 500,000 FTU phytase from OptiPhos® 2000 (Huvepharma Inc., Peachtree City, GA).

[5] SID = standardized ileal digestible.

[6] STTD = standardized total tract digestible.

[7] Representative samples of complete feed were collected from multiple feeders and composite samples were ground, subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE for proximate analysis and Ajinomoto Heartland, Inc., Chicago, IL for lysine analysis).

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

Dietary phases	1-phase	2-phase		3-phase			4-phase			
Lysine specification	100%	100%		100%			100%			
Initial weight, kg	27	27	100	27	50	100	27	50	72	100
Ingredient, % Final weight, kg	127	100	127	50	100	127	50	72	100	127
Corn	68.64	62.98	69.87	56.09	66.17	69.87	56.09	63.32	69.16	69.87
DDGS[3]	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal, 47% crude protein	7.68	13.39	7.19	20.33	10.51	7.19	20.33	13.37	7.64	7.19
Tallow	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Monocalcium phosphate, 21% P	0.55	0.50	---	0.40	0.25	---	0.40	0.25	0.10	---
Calcium carbonate	1.23	1.20	1.10	1.20	1.15	1.10	1.20	1.13	1.18	1.10
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.50	0.50	0.45	0.50	0.50	0.45	0.50	0.50	0.50	0.45
DL-Methionine	---	0.03	---	0.07	0.01	---	0.07	0.03	---	---
L-Threonine	0.10	0.11	0.09	0.13	0.11	0.09	0.13	0.11	0.11	0.09
L-Tryptophan	0.05	0.04	0.05	0.04	0.04	0.05	0.04	0.04	0.05	0.05
Vitamin-trace mineral premix[4]	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis										
SID[5] amino acids, %										
Lysine	0.82	0.96	0.77	1.13	0.89	0.77	1.13	0.96	0.82	0.77
Isoleucine:lysine	57	58	59	60	58	59	60	58	57	59
Leucine:lysine	166	156	176	147	161	176	147	156	166	176
Methionine:lysine	29	30	31	32	30	31	32	30	29	31
Methionine and cysteine:lysine	57	57	60	57	57	60	57	57	57	60
Threonine:lysine	63	63	64	63	63	64	63	63	64	64
Tryptophan:lysine	18.8	18.9	19.7	19.0	18.6	19.7	19.0	18.9	19.4	19.7
Valine:lysine	70	69	73	69	70	73	69	69	70	73
Total lysine, %	0.95	1.11	0.90	1.29	1.03	0.90	1.29	1.11	0.95	0.90
NE, kcal/kg	2,540	2,509	2,562	2,471	2,535	2,562	2,471	2,518	2,555	2,562
SID Lys:NE, g/Mcal	3.23	3.83	3.01	4.57	3.51	3.01	4.57	3.81	3.21	3.01
Crude protein, %	14.7	17.0	14.5	19.9	15.8	14.5	19.9	17.0	14.7	14.5
Calcium, %	0.60	0.60	0.46	0.60	0.53	0.46	0.60	0.53	0.50	0.46
STTD[6] phosphorus, %	0.38	0.38	0.27	0.38	0.33	0.27	0.38	0.33	0.29	0.27

Chemical analysis[7]										
Dry matter, %	88.2	88.1	87.9	89.0	88.2	87.5	88.9	88.0	88.6	87.1
Crude protein, %	14.3	16.7	15.1	20.3	16.4	15.0	17.9	16.3	15.0	15.2
Ether extract, %	4.2	4.2	4.6	4.1	4.5	4.5	4.3	4.3	4.5	4.1
Neutral detergent fiber, %	10.0	10.4	10.2	10.4	9.9	10.3	10.8	9.8	10.0	9.6
Calcium, %	0.80	0.75	0.63	0.81	0.68	0.47	0.68	0.71	0.57	0.74
Phosphorus, %	0.49	0.51	0.38	0.51	0.44	0.37	0.46	0.44	0.40	0.36
Lysine, %	0.90	1.03	0.90	1.25	0.99	0.92	1.08	1.04	0.88	0.99

[1] Experiment evaluated feeding strategies based on number of dietary phases (1, 2, 3, and 4 dietary phases) with lysine specifications at 100% of estimated requirement for growth rate for grow-finish pigs. Lysine specifications were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments (Genus PIC; Gonçalves et al., 2017).

[2] Diets were fed ad libitum in meal form from 27.4 to 125.6 kg body weight for 121 days. Lysine levels in experimental diets were achieved by changing the inclusion of soybean meal.

[3] DDGS = corn distillers dried grains with solubles.

[4] Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B_{12}; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; 0.20 g Se from sodium selenite; 500,000 FTU phytase from OptiPhos® 2000 (Huvepharma Inc., Peachtree City, GA).

[5] SID = standardized ileal digestible.

[6] STTD = standardized total tract digestible.

[7] Representative samples of complete feed were collected from multiple feeders and composite samples were ground, subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE for proximate analysis and Ajinomoto Heartland, Inc., Chicago, IL for lysine analysis).

Table 2.4 Composition of experimental diets (as-fed basis), Exp. 3[1,2]

Dietary phases	1-phase	2-phase		3-phase			4-phase			
Lysine specification	98%	98%		98%			98%			
Initial weight, kg	27	27	100	27	50	100	27	50	72	100
Ingredient, % Final weight, kg	127	100	127	50	100	127	50	72	100	127
Corn	69.81	64.99	80.21	58.50	67.75	80.21	58.50	65.34	70.38	80.21
DDGS[3]	20.00	20.00	---	20.00	20.00	---	20.00	20.00	20.00	---
Soybean meal, 47% crude protein	6.46	11.35	17.21	17.88	8.88	17.21	17.88	11.33	6.42	17.21
Tallow	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Monocalcium phosphate, 21% P	0.60	0.50	0.20	0.45	0.30	0.20	0.45	0.25	0.10	0.20
Calcium carbonate	1.23	1.23	0.95	1.20	1.15	0.95	1.20	1.15	1.18	0.95
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.50	0.50	0.15	0.50	0.50	0.15	0.50	0.50	0.50	0.15
DL-Methionine	---	0.03	---	0.06	0.02	---	0.06	0.03	---	---
L-Threonine	0.10	0.11	0.02	0.12	0.11	0.02	0.12	0.11	0.11	0.02
L-Tryptophan	0.05	0.04	---	0.04	0.05	---	0.04	0.04	0.06	---
Vitamin-trace mineral premix[4]	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis										
SID[5] amino acids, %										
Lysine	0.79	0.91	0.72	1.07	0.85	0.72	1.07	0.91	0.79	0.72
Isoleucine:lysine	56	58	71	59	57	71	59	58	56	71
Leucine:lysine	168	159	169	150	164	169	150	159	169	169
Methionine:lysine	30	31	31	32	31	31	32	31	30	31
Methionine and cysteine:lysine	58	58	62	58	58	62	58	58	58	62
Threonine:lysine	63	63	64	63	63	64	63	63	64	64
Tryptophan:lysine	18.7	18.6	19.7	18.8	19.0	19.7	18.8	18.6	19.9	19.7
Valine:lysine	70	69	81	69	70	81	69	69	70	81
Total lysine, %	0.92	1.05	0.83	1.23	0.98	0.83	1.23	1.05	0.92	0.83
NE, kcal/kg	2,546	2,520	2,560	2,485	2,542	2,560	2,485	2,529	2,562	2,560
SID Lys:NE, g/Mcal	3.10	3.61	2.81	4.31	3.34	2.81	4.31	3.60	3.08	2.81
Crude protein, %	14.2	16.2	14.0	18.9	15.2	14.0	18.9	16.2	14.2	14.0
Calcium, %	0.60	0.60	0.45	0.60	0.53	0.45	0.60	0.53	0.50	0.45
STTD[6] phosphorus, %	0.38	0.38	0.26	0.38	0.33	0.26	0.38	0.33	0.29	0.26

Chemical analysis[7]										
Dry matter, %	87.8	87.7	87.0	88.6	87.3	87.7	88.2	88.8	87.0	87.3
Crude protein, %	14.4	16.4	13.6	19.2	15.6	14.5	19.0	17.5	13.6	13.6
Ether extract, %	4.1	3.7	3.3	3.6	3.4	3.3	3.7	3.9	3.3	3.4
Neutral detergent fiber, %	9.5	9.1	6.6	10.6	7.8	6.4	10.0	9.9	6.9	6.7
Calcium, %	0.74	0.64	0.56	0.77	0.55	0.42	0.77	0.73	0.59	0.56
Phosphorus, %	0.51	0.45	0.33	0.52	0.40	0.35	0.50	0.46	0.34	0.34
Lysine, %	0.94	0.98	0.88	1.21	0.88	0.80	1.19	1.02	0.79	0.88

[1] Experiment evaluated feeding strategies based on number of dietary phases (1, 2, 3, and 4 dietary phases) with lysine specifications at 98% of estimated requirements for growth rate for grow-finish pigs. Lysine specifications were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments (Genus PIC; Gonçalves et al., 2017).

[2] Diets were fed ad libitum in meal form from 25.9 to 131.5 kg body weight for 119 days. Lysine levels in experimental diets were achieved by changing the inclusion of soybean meal.

[3] DDGS = corn distillers dried grains with solubles.

[4] Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B_{12}; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; 0.20 g Se from sodium selenite; 500,000 FTU phytase from OptiPhos® 2000 (Huvepharma Inc., Peachtree City, GA).

[5] SID = standardized ileal digestible.

[6] STTD = standardized total tract digestible.

[7] Representative samples of complete feed were collected from multiple feeders and composite samples were ground, subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE for proximate analysis and Ajinomoto Heartland, Inc., Chicago, IL for lysine analysis).

Table 2.5 Composition of experimental diets (as-fed basis), Exp. 4[1,2]

Dietary phases	2-phase		2-phase		3-phase			4-phase			
Lysine specification	98%		98%/100%		98%			98%			
Initial weight, kg	27	100	27	100	27	72	100	27	50	72	100
Ingredient, % Final weight, kg	100	127	100	127	72	100	127	50	72	100	127
Corn	64.98	80.21	64.98	78.17	61.76	70.39	80.21	58.48	65.33	70.39	80.21
DDGS[3]	20.00	---	20.00	---	20.00	20.00	---	20.00	20.00	20.00	---
Soybean meal, 47% crude protein	11.35	17.21	11.35	19.25	14.61	6.42	17.21	17.88	11.33	6.42	17.21
Tallow	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Monocalcium phosphate, 21% P	0.50	0.20	0.50	0.20	0.45	0.10	0.20	0.45	0.25	0.10	0.20
Calcium carbonate	1.23	0.95	1.23	0.95	1.23	1.18	0.95	1.20	1.15	1.18	0.95
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.50	0.15	0.50	0.15	0.50	0.50	0.15	0.50	0.50	0.50	0.15
DL-Methionine	0.03	---	0.03	---	0.03	---	---	0.06	0.03	---	---
L-Threonine	0.12	0.02	0.12	0.02	0.13	0.11	0.02	0.13	0.12	0.11	0.02
L-Tryptophan	0.05	---	0.05	---	0.04	0.05	---	0.04	0.05	0.05	---
Vitamin-trace mineral premix[4]	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis											
SID[5] amino acids, %											
Lysine	0.91	0.72	0.91	0.77	0.99	0.79	0.72	1.07	0.91	0.79	0.72
Isoleucine:lysine	58	71	58	71	59	56	71	59	58	56	71
Leucine:lysine	159	169	159	164	154	169	169	149	159	169	169
Methionine:lysine	31	31	31	30	30	30	31	32	31	30	31
Methionine and cysteine:lysine	58	62	58	60	56	58	62	58	58	58	62
Threonine:lysine	64	64	64	64	64	64	64	64	64	64	64
Tryptophan:lysine	19.2	19.7	19.2	19.9	19.0	19.3	19.7	19.2	19.2	19.3	19.7
Valine:lysine	69	81	69	80	69	70	81	69	69	70	81
Total lysine, %	1.05	0.83	1.05	0.88	1.14	0.92	0.83	1.23	1.05	0.92	0.83
NE, kcal/kg	2,522	2,560	2,522	2,549	2,502	2,562	2,560	2,485	2,531	2,562	2,560
SID Lys:NE, g/Mcal	3.61	2.81	3.61	3.02	3.96	3.08	2.81	4.31	3.60	3.08	2.81
Crude protein, %	16.2	14.0	16.2	14.8	17.5	14.2	14.0	18.9	16.2	14.2	14.0
Calcium, %	0.60	0.45	0.60	0.45	0.60	0.50	0.45	0.60	0.53	0.50	0.45
STTD[6] phosphorus, %	0.38	0.26	0.38	0.26	0.38	0.29	0.26	0.38	0.33	0.29	0.26

Chemical analysis[7]											
Dry matter, %	87.8	87.9	87.3	87.9	87.7	86.6	87.0	88.6	87.4	87.7	87.9
Crude protein, %	15.8	14.2	14.8	14.1	18.7	13.7	12.8	16.8	14.0	13.7	13.1
Ether extract, %	3.8	3.3	3.4	3.3	3.8	3.4	3.8	4.2	4.0	3.6	4.1
Neutral detergent fiber, %	8.9	7.2	7.4	6.5	8.7	6.2	7.5	10.2	10.2	7.5	7.7
Calcium, %	0.70	0.55	0.62	0.67	0.67	0.62	0.64	0.65	0.67	0.57	0.72
Phosphorus, %	0.45	0.37	0.39	0.36	0.46	0.33	0.38	0.44	0.37	0.34	0.41
Lysine, %	0.93	0.81	0.89	0.80	1.20	0.82	0.71	1.02	0.87	0.82	0.73

[1] Experiment evaluated feeding strategies based on number of dietary phases (2, 3, and 4 dietary phases) and lysine specifications (98% and 100% of estimated requirements for growth rate) for grow-finish pigs. Lysine specifications were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments (Genus PIC; Gonçalves et al., 2017). For 98%/100%, lysine specifications at 98% of estimated requirements were applied in growing-finishing (27 to 100 kg body weight) and lysine specifications at 100% of estimated requirements were applied in late finishing (100 to 127 kg body weight).

[2] Diets were fed ad libitum in meal form from 28.8 to 129.9 kg body weight for 114 days. Lysine levels in experimental diets were achieved by changing the inclusion of soybean meal.

[3] DDGS = corn distillers dried grains with solubles.

[4] Provided per kg of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B_{12}; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 74 g Zn from zinc sulfate; 74 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.22 g I from calcium iodate; 0.20 g Se from sodium selenite; 500,000 FTU phytase from OptiPhos® 2000 (Huvepharma Inc., Peachtree City, GA).

[5] SID = standardized ileal digestible.

[6] STTD = standardized total tract digestible.

[7] Representative samples of complete feed were collected from multiple feeders and composite samples were ground, subsampled, and analyzed (Ward Laboratories, Inc., Kearney, NE for proximate analysis and Ajinomoto Heartland, Inc., Chicago, IL for lysine analysis).

Table 2.6 Effects of feeding strategies based on number of dietary phases (1, 2, 3, and 4 dietary phases) and lysine specifications (96%, 98%, and 100% of estimated requirements for growth rate) on growth performance of grow-finish pigs[1,2]

Item[3]	Exp. 1					
Overall, d 0 to 117	2-phase 100%	4-phase 100%	4-phase 96%	4-phase 96%/100%	SEM	Probability, *P* <
Initial BW, kg	27.9	27.9	27.9	27.9	0.79	0.998
Final BW, kg	129.9	129.8	127.2	129.4	1.09	0.110
ADG, kg	0.880[a]	0.876[ab]	0.855[b]	0.870[ab]	0.006	0.044
ADFI, kg	2.29	2.29	2.26	2.28	0.022	0.514
G:F	0.385	0.382	0.379	0.381	0.003	0.202
	Exp. 2					
Overall, d 0 to 121	1-phase 100%	2-phase 100%	3-phase 100%	4-phase 100%	SEM	Probability, *P* <
Initial BW, kg	27.4	27.4	27.4	27.4	0.66	0.951
Final BW, kg	123.9[y]	126.2[xy]	125.5[xy]	126.7[x]	1.16	0.051
ADG, kg	0.797[b]	0.816[a]	0.814[ab]	0.822[a]	0.006	0.007
ADFI, kg	2.24	2.29	2.26	2.30	0.031	0.126
G:F	0.356	0.357	0.361	0.357	0.004	0.398
	Exp. 3					
Overall, d 0 to 119	1-phase 98%	2-phase 98%	3-phase 98%	4-phase 98%	SEM	Probability, *P* <
Initial BW, kg	25.9	25.9	25.8	25.9	0.31	0.997
Final BW, kg	129.1[b]	131.6[ab]	131.8[ab]	133.4[a]	1.10	0.022
ADG, kg	0.862[b]	0.881[ab]	0.887[ab]	0.899[a]	0.008	0.009
ADFI, kg	2.29	2.29	2.31	2.29	0.024	0.953
G:F	0.376[c]	0.385[b]	0.385[b]	0.392[a]	0.002	0.001
	Exp. 4					
Overall, d 0 to 114	2-phase 98%	2-phase 98%/100%	3-phase 98%	4-phase 98%	SEM	Probability, *P* <
Initial BW, kg	28.7	28.9	28.9	28.9	0.47	0.593
Final BW, kg	128.8	130.3	130.9	129.6	1.05	0.317
ADG, kg	0.895	0.907	0.911	0.899	0.006	0.215
ADFI, kg	2.36	2.40	2.42	2.39	0.025	0.305
G:F	0.379	0.378	0.376	0.376	0.003	0.765

[1] A total of 4 experiments were conducted at a commercial research facility with 1,100 to 1,188 pigs (PIC 359 × 1050) per experiment in pens of 25 to 27 mixed-gender pigs per pen and 11 replicates per treatment. Experiments were conducted from approximately 27 to 127 kg body weight (BW) for approximately 120 d.

[2] Lysine specifications at 96%, 98%, and 100% of estimated requirements for growth rate were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments (Genus PIC; Gonçalves et al., 2017). For 96%/100%, lysine specifications at 96% of estimated requirements were applied in growing (27 to 72 kg BW) and lysine specifications at 100% of estimated requirements were applied in finishing (72 to 127 kg BW). For 98%/100%, lysine specifications at 98% of estimated requirements were applied in growing-finishing (27 to 100 kg BW) and lysine specifications at 100% of estimated requirements were applied in late finishing (100 to 127 kg BW).

[3] BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

[abc] Means with different superscripts are significantly different ($P \leq 0.05$) in the row.

[xy] Means with different superscripts have a tendency to be different ($0.05 < P \leq 0.10$) in the row.

Table 2.7 Effects of feeding strategies based on number of dietary phases (1, 2, 3, and 4 dietary phases) and lysine specifications (96%, 98%, and 100% of estimated requirements for growth rate) on carcass characteristics of grow-finish pigs[1,2]

Item[3]	Exp. 1					
	2-phase 100%	4-phase 100%	4-phase 96%	4-phase 96%/100%	SEM	Probability, $P <$
HCW, kg	94.6	94.2	92.7	94.0	0.81	0.219
Yield, %	72.8	72.6	72.9	72.6	0.15	0.338
Backfat[4], mm	17.0	16.6	17.1	17.0	0.31	0.450
Loin depth[4], mm	68.7	68.4	68.3	67.6	0.57	0.459
Lean[4], %	56.7	57.0	56.6	56.6	0.22	0.430
	Exp. 2					
	1-phase 100%	2-phase 100%	3-phase 100%	4-phase 100%	SEM	Probability, $P <$
HCW, kg	95.1[b]	97.6[a]	96.4[ab]	97.8[a]	1.05	0.014
Yield, %	76.8	77.4	76.8	77.2	0.22	0.120
Backfat[4], mm	16.8	16.3	16.5	16.3	0.24	0.365
Loin depth[4], mm	68.1	68.3	66.8	67.1	0.59	0.111
Lean[4], %	56.8	57.1	56.8	57.0	0.19	0.519
	Exp. 3					
	1-phase 98%	2-phase 98%	3-phase 98%	4-phase 98%	SEM	Probability, $P <$
HCW, kg	95.4[b]	97.0[ab]	97.6[ab]	98.6[a]	0.73	0.005
Yield, %	74.0	73.7	74.1	74.0	0.37	0.932
Backfat[4], mm	17.0	16.7	16.8	16.4	0.27	0.429
Loin depth[4], mm	67.5	68.8	67.6	68.0	0.61	0.400
Lean[4], %	56.6	56.9	56.7	57.0	0.21	0.411
	Exp. 4					
	2-phase 98%	2-phase 98%/100%	3-phase 98%	4-phase 98%	SEM	Probability, $P <$
HCW, kg	95.3	96.0	96.9	96.2	0.66	0.231
Yield, %	74.1	73.7	74.1	74.2	0.32	0.652
Backfat[4], mm	16.5	17.1	16.8	16.6	0.28	0.582
Loin depth[4], mm	68.9	68.6	68.8	69.9	0.52	0.327
Lean[4], %	57.0	56.7	56.8	57.1	0.20	0.487

[1] A total of 4 experiments were conducted at a commercial research facility with 1,100 to 1,188 pigs (PIC 359 × 1050) per experiment in pens of 25 to 27 mixed-gender pigs per pen and 11 replicates per treatment. Experiments were conducted from approximately 27 to 127 kg body weight (BW) for approximately 120 d.

[2] Lysine specifications at 96%, 98%, and 100% of estimated requirements for growth rate were derived from the genetic supplier's lysine requirement prediction equation based on commercial experiments (Genus PIC; Gonçalves et al., 2017). For 96%/100%, lysine specifications at 96% of estimated requirements were applied in growing (27 to 72 kg BW) and lysine specifications at 100% of estimated requirements were applied in finishing (72 to 127 kg BW). For 98%/100%, lysine specifications at 98% of estimated requirements were applied in growing-finishing (27 to 100 kg BW) and lysine specifications at 100% of estimated requirements were applied in late finishing (100 to 127 kg BW).

[3] HCW = hot carcass weight.

[4] Adjusted using HCW as covariate.

[abc] Means with different superscripts are significantly different ($P \leq 0.05$) in the row.

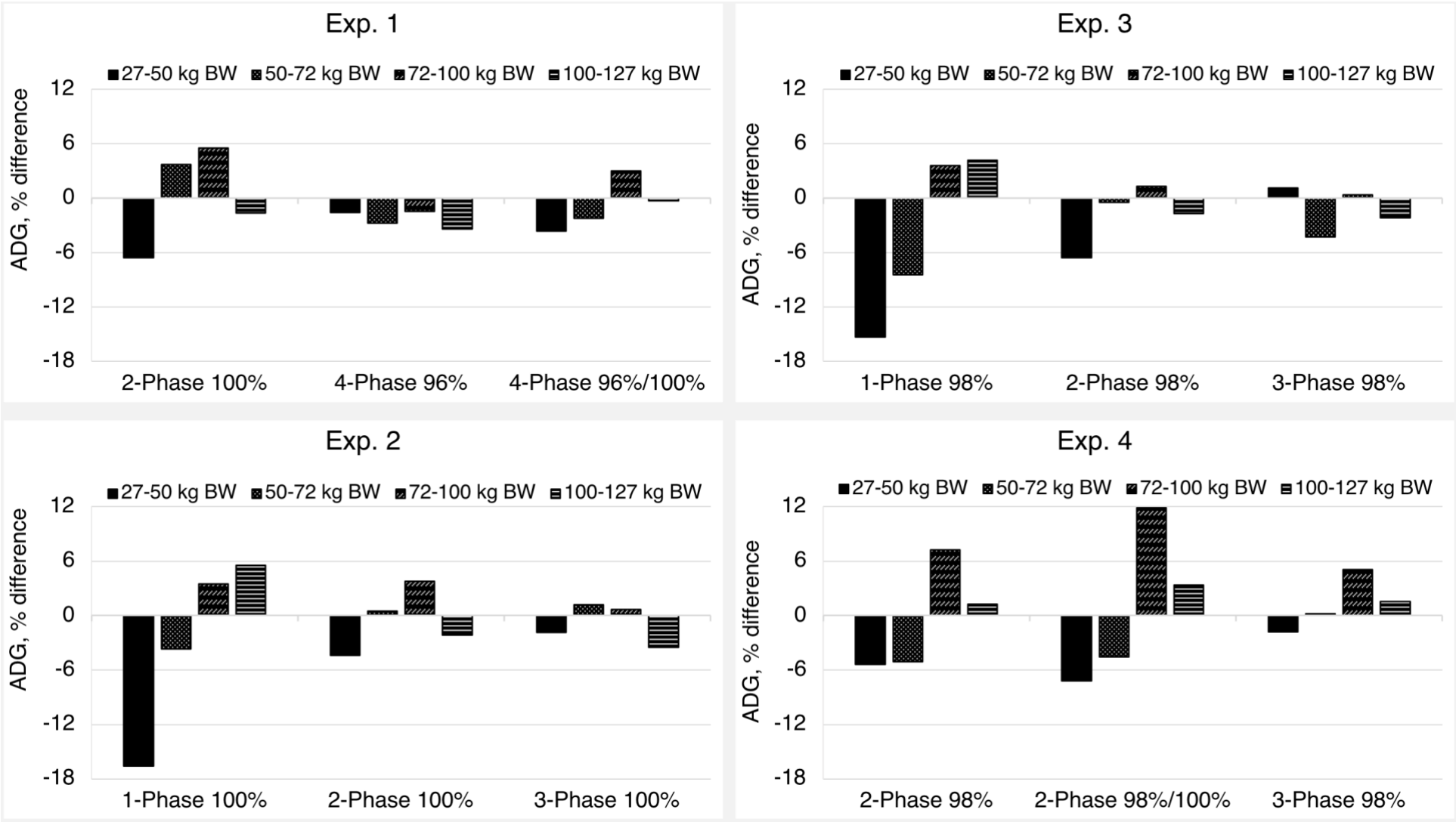

Figure 2.1 Relative intermediate performance of grow-finish pigs under feeding strategies based on number of dietary phases (1, 2, 3, and 4 dietary phases) and lysine specifications (96%, 98%, and 100% of estimated requirements for growth rate) in the grow-finish period.

Relative difference in average daily gain (ADG) determined by comparing within experiments the performance of the feeding strategies to the performance of the 4-phase 100% (Exp. 1 and 2) and 4-phase 98% (Exp. 3 and 4), which were considered as the standard feeding strategies.

Chapter 3 - Effects of oral administration of *Bacillus subtilis* C-3102 to nursing piglets on pre-weaning growth performance, fecal consistency, and fecal microbes

Summary

Objective: To evaluate the effects of daily oral dose of *Bacillus subtilis* C-3102 to nursing piglets on fecal consistency, fecal microbes, and pre-weaning performance in a controlled trial.

Materials and methods: A total of 26 litters of nursing piglets were assigned to receive a daily oral dose of placebo (n = 14 litters) or probiotic (n = 12 litters) for 18 days beginning on day 2 after birth until weaning on day 19. The probiotic treatment was based on the probiotic *B subtilis* C-3102 (Calsporin, Calpis Co. Ltd.). Treatments were applied orally once daily to individual piglets via 1 mL sugar-based gel solution alone or with Calsporin for placebo or probiotic treatments, respectively. Growth performance and litter size were measured on days 2, 9, 16, and 19. Fecal scoring and sampling were performed on days 2, 9, and 16 to categorize fecal consistency and conduct microbial analysis by isolation and enumeration method.

Results: There was no evidence for difference ($P > .10$) on growth performance, litter size, mortality, and fecal consistency in the pre-weaning period between placebo- and probiotic-treated litters. The numbers of *B subtilis* C-3102, total *Bacillus* sp., and total aerobes were increased ($P < .05$) in litters receiving probiotic compared to placebo. The numbers of *Lactobacillus* sp., *Enterococcus* sp., *Clostridium perfringens*, and Enterobacteriaceae were not influenced by treatment.

Implications: A daily oral dose of *B subtilis* C-3102 probiotic did not influence pre-weaning growth performance and fecal consistency of nursing piglets and only influenced *Bacillus* sp. fecal microbial population.

Keywords: swine, *Bacillus subtilis*, diarrhea, fecal bacterial population, suckling pigs

Introduction

Strategies to improve pig performance and preserve health while minimizing the use of antibiotics is of great interest for the swine industry. The pre-weaning period is particularly important to focus efforts on improving piglet viability and survivability as pre-weaning mortality rate typically ranges between 10 to 20% in commercial swine production.[1] Moreover, diarrhea incidence in nursing piglets contributes to poor growth rate and low survivability before weaning as well as a rise in antibiotic use.[2,3]

Porcine gastrointestinal tract bacterial colonization begins at birth and influences the gastrointestinal tract structural, functional, and immunological maturation in neonatal piglets.[4,5] Studies suggest establishing a healthy intestinal microbiota in early life might be essential for preventing pathogen colonization and immune system stimulation later in life.[6-9] Dietary strategies meant to modulate piglet intestinal microbiota during the pre-weaning period can ultimately lead to these expected health benefits.

Probiotics are non-pathogenic live microorganisms that provided in adequate amounts can improve the intestinal microbial balance and confer a health benefit to the host.[10] *Bacillus subtilis* C-3102 is a non-genetically-modified strain of a Gram-positive spore-forming bacteria used as probiotic for swine. The effects of *B subtilis* C-3102 on fecal microbiota have been associated to the increase of beneficial bacteria population on sows, particularly the

Lactobacillus sp., and the reduction on pathogenic bacteria population and diarrhea incidence in the nursing progeny.[11,12] However, to the best of the authors' knowledge, the investigation of this bacillary probiotic as directly administered to nursing piglets has not previously been conducted. The objective of this study was to evaluate the effects of a daily oral dose of a bacillary probiotic administered to piglets during the nursing phase on fecal consistency, fecal microbes, and pre-weaning performance.

Materials and methods

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment.

Facilities and health status

The experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS, for a 20-day period in December. The facility is a farrow-to-finish operation with approximately 120 sows in a 5-week batch farrowing system. Replacement gilts are routinely introduced into the herd from the genetic supplier (DNA Genetics) after a quarantine period. Sows are individually housed in environmentally-controlled and mechanically-ventilated gestation and farrowing barns. All sows are housed within a single gestation barn and a single farrowing room.

The herd is free of Porcine Reproductive and Respiratory Syndrome virus and Porcine Epidemic Diarrhea virus. Sows are routinely vaccinated on every reproductive cycle for parvovirus, leptospirosis and erysipelas (FarrowSure Gold, Zoetis Services), for enterotoxigenic *Escherichia coli* and *Clostridium perfringens* type C (LitterGuard LT-C, Zoetis Services), and with a bacterin for *Haemophilus parasuis*. Piglets are vaccinated for Porcine Circovirus type 2 and *Mycoplasma*

hyopneumoniae (Circumvent PCV-M G2, Merck Animal Health), and *Lawsonia intracellularis* (Porcilis Ileitis, Merck Animal Health). Sows and piglets are administered intramuscular antimicrobial treatment following veterinary directions in the occurrence of clinical signs of bacterial disease.

Animals, housing and management

A total of 26 lactating sows (DNA 241, DNA Genetics, Columbus, NE; 2.5 average parity) and litters (412 piglets DNA 241 × 600, DNA Genetics) were used in the study. Sows were individually housed in an environmentally-controlled and mechanically-ventilated farrowing house from day 110 of gestation to weaning on day 19 of lactation. Farrowing stalls were equipped with an individual water nipple and an electronic feeding system (Gestal Solo Feeders, Jyga Technologies). Sows were fed 2.7 kg of feed per day until farrowing and gradually transitioned to *ad libitum* feed intake after parturition. Farrowing stalls were equipped with rubber mat and heating lamp for piglet comfort. Piglets had free access to sow milk and water and no creep feed was provided during lactation. Piglets were processed and cross-fostered to equalize litter size within 24 hours of birth.

Treatments

Treatments were assigned to litters of nursing piglets in a randomized complete block design based on sow parity and farrowing date. Within a farrowing date, sows were blocked by parity and litters were randomly assigned to one of two treatment using a spreadsheet-based randomization procedure. Treatments consisted of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets for a period of 18 days beginning on day 2 after birth until weaning on day 19 of lactation. The probiotic treatment was based on a probiotic product containing *B subtilis* C-3102 (Calsporin, Calpis Co. Ltd.) provided at

approximately 20×10^6 CFU/mL per kg of body weight (BW). A daily dosage of 45.0×10^6, 77.5×10^6 and 108.3×10^6 CFU/mL was used on days 2 to 8, 9 to 15, and 16 to 19, respectively. Treatments were applied orally to individual piglets using a dosing device once daily at approximately 0700 h via 1 mL gel solution. The gel solution was composed of a sugar-based carrier (Headstart, Animal Science Products, Inc.) alone or with Calsporin for placebo or probiotic treatments, respectively. The preparation of the solution consisted of dissolving the carrier in warm water with or without Calsporin while continuously mixing the solution with a magnetic stirrer. The solution was prepared immediately before use. Both placebo and probiotic suspensions were analyzed for quantification of *B subtilis* C-3102.

Growth performance

Piglets were individually weighed and litter size recorded on days 2, 9, 16, and 19, which corresponded to weaning day. Piglet average daily gain (ADG) was calculated based on piglet BW gain on each period of days 2 to 8, 9 to 15, 16 to 19, and 2 to 19. Pre-weaning mortality was calculated based on litter size on days 2 and 19. Sow farrowing performance was recorded as number of piglets total born, born alive, stillborn, and mummified. Sows were weighed on days 2 and 19 to calculate lactation BW loss. Sow feed intake was recorded daily from days 2 to 19 to calculate overall average lactation feed intake.

Fecal score

Fecal scoring was conducted on days 2, 9, and 16 to categorize the consistency of piglets' feces per litter into the following categories: hard feces, firm formed feces, soft moist feces, soft unformed feces, and watery feces. Fecal score evaluation was conducted by a trained individual blind to treatments.

Fecal microbial analysis

Fecal samples were collected from piglets on days 2, 9, and 16 for microbial analysis. Fecal samples were freshly collected from piglets using sterile mini cotton tip swabs and pooled by litter for analysis. Fecal samples were kept at 4 °C until analysis within 24 hours of collection. Microbial analysis of fecal samples was performed by isolation and enumeration method of *B subtilis* C-3102, total *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Clostridium perfringens*, *Salmonella* spp., Enterobacteriaceae, total aerobes, and total anaerobes.

For microbial platting, approximately 1 g of feces was suspended in 9 mL of anaerobic diluent and serial 10-fold dilutions were prepared according to procedures described previously.[11] Aliquots of 0.05 mL of each dilution were inoculated into selective and non-selective media. All media were incubated at 37 °C unless otherwise noted. *Bacillus subtilis* C-3102 were enumerated on tryptic soy broth with 2% agar after incubation for 1 day.[13] Total *Bacillus* sp. were enumerated by chromogenic method using a differential medium (92325 Bacillus *ChromoSelect* Agar, Sigma-Aldrich) after incubation for 1 day and spores were quantified after incubation at 80 °C for 15 minutes.[12] *Lactobacillus* sp. were enumerated on modified lactobacilli selective agar after anaerobic incubation for 2 days.[11] *Enterococcus* sp. were enumerated on triphenyltetrazolium chloride-acridine orange- thallous sulfate aesculin crystal violet (TATAC) agar after incubation for 2 days.[11] *Clostridium perfringens* were enumerated on neomycin-brilliant green-taurocholate-nagler (NN) agar after anaerobic incubation for 3 days.[11] *Salmonella* spp. were enumerated on mannitol lysine crystal violet brilliant green (MLCB) agar after incubation for 1 day.[14] Enterobacteriaceae were enumerated on neomycin-brilliant green-taurocholate-blood (NBGT) agar after incubation for 1 day.[11] Total aerobes were enumerated on trypticase soy agar after incubation for 2 days.[11] Total anaerobes were enumerated on glucose

blood liver agar and Eggerth-Gagnon agar after anaerobe incubation for 3 days.[11] Limit of detection was 2×10^2 CFU/g. Microbial analysis was performed by the microbiology laboratory of Calpis America, Inc.

Statistical analysis

Experiment was a randomized complete block design with sow parity within farrowing date serving as the block and litter as the experimental unit. Data were analyzed using a linear mixed model with treatment included as fixed effect and block as random effect as:

$$Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij} ,$$

where μ is the overall mean, τ_i is the effect of treatment *i*, β_j is the effect of block *j*, and ϵ_{ij} is the random error.

Model assumptions were met by evaluating studentized residuals and QQ plots. All response variables were analyzed assuming a normal distribution unless otherwise noted. Pre-weaning mortality was analyzed assuming a binomial distribution and fecal score assuming a multinomial distribution. For binomial responses, the logit link function was used and for fecal score the cumulative probit link function was used. Fecal score and fecal microbial analysis were analyzed as repeated measures. Piglet initial BW (day 2) was included as a covariate for piglet BW and ADG during lactation. Statistical models were fit and pairwise comparisons were performed using the GLIMMIX procedure of SAS (SAS Institute Inc.). Results were considered significant at $P < .05$ and a tendency at $.05 \leq P \leq .10$.

Results

Quantification of Bacillus subtilis C-3102

Quantification of *B subtilis* C-3102 in the oral suspension provided daily to piglets revealed undetectable levels in the placebo, and 7.9×10^8, 10.4×10^8, and 9.8×10^8 CFU/mL in the probiotic treatment for days 2 to 8, 9 to 15, and 16 to 19, respectively.

Performance

Analysis of sow performance demonstrated no evidence for difference ($P > .05$) on farrowing and lactation performance between treatments (Table 3.1). For nursing piglet performance, no evidence for difference ($P > .10$) was observed on BW, ADG, litter size, and mortality in the pre-weaning period between treatments (Table 3.2). On day 2, there was a tendency ($P = .07$) for increased BW of placebo pigs compared to probiotic pigs. On d 9, there was a tendency ($P = .07$) for larger litter size of placebo litters compared to probiotic litters.

Fecal score

Fecal score of nursing piglets was not influenced ($P > .05$) by treatment or treatment by day interaction, as observed by the similar frequency distribution of fecal score categories on both placebo- and probiotic-treated litters within lactation day (Figure 3.1). Fecal score of nursing piglets was influenced ($P < .001$) by day of lactation, as observed by the shift in frequency distribution of fecal score categories throughout the lactation period regardless of treatment (Figure 3.1). The frequency of firm formed and hard feces increased from day 2 to 9 of lactation, suggesting hardening of feces in the first week of study. Then, from day 9 to 16, the frequency of soft moist and soft unformed feces increased, suggesting a shift to a looser fecal consistency in the second week of study.

Fecal microbial analysis

Fecal microbial analysis revealed an interaction between treatment and day of lactation ($P < .05$) on number of *B subtilis* C-3102, total *Bacillus* sp., and total anaerobes (Table 3.3). The numbers of *B subtilis* C-3102 and total *Bacillus* sp. increased ($P < .001$) in litters receiving probiotic compared to placebo on days 9 and 16 of lactation. On day 2 of lactation, the detection of *B subtilis* C-3102 also increased ($P = .02$) in probiotic litters compared to placebo litters, but total *Bacillus* sp. was similar ($P > .05$) between litter treatments. The levels of *B subtilis* C-3102 and total *Bacillus* sp. in placebo litters gradually increased ($P < .01$) throughout lactation, whereas the levels in probiotic litters considerably increased ($P < .001$) from day 2 to 9 and then remained constant ($P > .05$) until day 16. There was a tendency ($P = .09$) for increased number of total anaerobes in probiotic litters compared to placebo litters on day 9 of lactation, but there was no evidence for differences ($P > .05$) between litter treatments on days 2 and 16 of lactation. The levels of total anaerobes in placebo litters remained constant ($P > .05$) from day 2 to 9 and then decreased ($P < .001$) until day 16, whereas, in probiotic litters, there was a tendency ($P = .05$) for increased levels from day 2 to 9 and then decreased levels ($P < .001$) until day 16. The number of total aerobes was influenced ($P < .05$) by treatment and day of lactation. The number of total aerobes was increased ($P = .03$) in placebo litters compared to probiotic litters (8.79 vs. 8.64 $\log_{10}$ CFU/g, respectively; SEM = 0.046) and the levels decreased ($P < .001$) throughout lactation irrespective of treatment (9.30, 8.53, and 8.32 $\log_{10}$ CFU/g on days 2, 9, and 16, respectively; SEM = 0.066).

The numbers of *Lactobacillus* sp., *Enterococcus* sp., and Enterobacteriaceae were influenced ($P < .001$) by day of lactation (Table 3.3). The levels of *Lactobacillus* sp. increased from day 2 to 9 and then decreased until day 16 of lactation (7.94, 8.85, and 8.47 CFU/g, respectively; SEM = 0.074; $P < .001$). The levels of *Enterococcus* sp. (8.66, 7.42. and 6.06 CFU/g on days 2, 9, and

16, respectively; SEM = 0.151) and Enterobacteriaceae (9.13, 8.33, and 7.36 CFU/g on days 2, 9, and 16, respectively; SEM = 0.074; $P < .001$) decreased throughout lactation.

The number of *C perfringens* was not influenced ($P > .05$) by litter treatment and remained constant ($P > .05$) throughout lactation (Table 3.3). The fecal microbial analysis revealed non-detectable levels of *Salmonella* spp. in piglets' feces with exception of one placebo litter sample on day 2 of lactation with 2.75×10^7 CFU/g.

Discussion

Bacterial colonization of the porcine gastrointestinal tract begins at birth and mainly comes from the sow and the environment surrounding the newborn piglet. The first two weeks of life have been reported as a "developmental window" for piglets,[6] in which the gastrointestinal tract is undergoing critically important steps of development including structural, functional, and immunological maturation concomitantly with the establishment of the gut microbiota.[4,5] The establishment of the gut microbiota in early stages of life exerts a long-term influence on pigs described as "microbial imprinting",[15] particularly in terms of pathogen colonization and immune system development on the adult pig.[6-9] The evidence that gut microbiota is critically determined at early stages of life presents an opportunity to develop dietary strategies to modulate the gut microbiota of piglets and ultimately lead to an impact on lifetime performance. Because it is difficult to induce a change once the gut microbiota is established and stable,[16] early after birth represents the best opportunity to modulate gut microbiota with dietary strategies.[17] The delivery of probiotics has been recently appointed as a promising additive to piglet nutrition as studies have shown a beneficial impact on growth performance and health of nursing piglets orally supplemented with probiotics in the pre-weaning period.[18-21] However, to

the best of the authors' knowledge, this is the first published study with bacillary probiotics directly administered to nursing piglets.

The delivery of nutritional strategies to nursing piglets is often challenging, even for research purposes. Different strategies have been proposed for early administration of probiotics to piglets, including via sow milk, creep feeding, or suspension in water or milk replacers. The administration of probiotics via sow milk provides dual benefits to sows and piglets, as probiotics are fed to sows and are able to modulate milk bacterial population through the entero-mammary pathway.[22] However, the origin of milk bacterial population is complex and influenced by the bacterial population in the sow skin and the environment.[23] Moreover, from a research standpoint, it is difficult to determine a standard amount of probiotic being delivered by the milk and consumed by the piglets during lactation. The traditional approach to nutritional supplementation of nursing piglets is via creep feeding. However, studies have shown that not all piglets consume creep feed and those that consume have low intake during the nursing period.[24] Again, from a research standpoint, it is difficult to determine a standard amount of probiotic being consumed by the piglets in the creep feed during lactation. A new approach undertaken by recent studies on probiotic supplementation of nursing piglets consists of individual oral administration of the probiotic in liquid or gel suspension.[18-20] The approach is labor intensive for regular farm application, but practicable for research purposes. Most importantly, the direct oral administration to individual piglets ensures the delivery of an accurate dose of probiotics to every piglet in a litter. The consistent delivery of probiotics to nursing piglets was the main reason for choosing the oral administration approach in the present study.

Sow performance at farrowing and during lactation was similar for placebo- and probiotic-treated litters which was expected and thereby not likely to influence the litter response to

treatments. The nursing piglet performance in the pre-weaning period was not influenced by providing a daily oral dose of probiotic until weaning. In contrast, previous studies evaluating the effects of oral administration of probiotic to nursing piglets have found a growth rate improvement ranging from 7 to 15% in litters supplemented with probiotics from the first days after birth until 5 to 21 days of age.[18-21] The fecal consistency of nursing piglets was also not influenced by probiotic administration. The pre-weaning fecal consistency was mostly classified as firm formed feces and the frequency distribution of fecal score categories was similar in placebo- and probiotic-treated litters during the nursing period. In contrast to our study, a reduction in diarrhea incidence and severity has also been observed along with the improvement in growth performance in the previously-referred studies in nursing piglets receiving early administration of probiotics.[18-21]

The divergence between our study and the literature could be related to the use of different probiotic bacteria with distinct modes of action. In the referred studies,[18-21] nursing piglets received lactic acid bacteria-based probiotics, including species of *Lactobacillus* sp. and *Enterococcus* sp., whereas in the present study piglets received a bacillus-based probiotic. Lactic acid bacteria are Gram-positive, non-sporulating bacteria that produce lactic acid as the main metabolic product of carbohydrate fermentation.[25] The lactic acid produced by bacteria contributes to an acidic environment in the gastrointestinal tract to a level which influences growth of pathogenic bacteria. In addition, lactic acid bacteria colonize the intestine and inhibit pathogenic bacteria by competitive exclusion for nutrients or binding sites on the intestinal epithelium.[26] Consequently, the reduction in pathogen load can contribute to an improvement in piglet growth rate.[19]

Bacillus-based probiotics as the *B subtilis* C-3102 used in the present study are Gram-positive, spore-forming bacteria that germinate but not proliferate in the gastrointestinal tract.[25] The germination of *B subtilis* spore results in blocking of binding sites of pathogenic bacteria on the intestinal epithelium. However, the main mode of action of bacillus-based probiotics is through the production of enzymes subtilisin and catalase as metabolites.[27] The enzymes create a favorable environment for growth and colonization of beneficial bacteria in the gastrointestinal tract, particularly the *Lactobacillus* sp. However, in the present study the administration of *B subtilis* C-3102 to nursing piglets did not elicit an increase in number of *Lactobacillus* sp. in the feces. This could explain the lack of probiotic effect on pre-weaning growth performance and fecal consistency of nursing piglets in the present study. Importantly, the normal microbial population of the piglets should be taken into consideration. In the present study, the number of *Lactobacillus* sp. in fecal microbial population of nursing piglets was almost equivalent to the number of *C perfringens*. The high levels of *C perfringens* were not causing diarrhea in piglets and were considered within normal levels for the farm under study, as evaluated in other instances before and after the present study. It could be speculated that the dose of *B subtilis* C-3102 used in this study was not enough to influence the high fecal levels of *C perfringens*[11] or to elicit an effect in number of *Lactobacillus* sp. of sufficient magnitude to outnumber *C perfringens*.

The fecal microbial population of nursing piglets was moderately influenced by providing a daily oral dose of probiotic until weaning. The number of total *Bacillus* sp. increased in fecal microbial population of piglets from probiotic-treated litters compared to placebo-treated litters. The increase in total *Bacillus* sp. was mainly driven by *B subtilis* C-3102, which was expected to be found in increased number in fecal microbial population of litters receiving the probiotic. The

presence of substantial levels of *B subtilis* C-3102 in fecal microbial population of probiotic-treated litters also substantiates our decision to orally dose piglets individually in this study as a means of ensuring the ingestion of the expected dose of probiotic by all piglets in the litters assigned to the probiotic treatment. The number of total aerobes was decreased in fecal microbial population of piglets receiving probiotic compared to the ones receiving placebo. Total aerobe count is commonly used as an indicator of general bacterial population on fecal samples.[25] The decrease in number of total aerobes indicates the probiotic contributes to maintaining a low bacterial load in the feces of nursing piglets and, consequently, in the environment.[28,29] The number of total anaerobes was mostly similar in placebo- or probiotic-treated litters, except for day 9 in lactation when the number of total anaerobes was inexplicably increased in litters receiving the probiotic but soon returned to a similar level to placebo litters on day 16 in lactation. Total anaerobe count is commonly used as an indicator of anaerobic populations in the posterior portion of the gastrointestinal tract, which includes *Lactobacillus* sp., *Bacteroides* sp., *Streptococcus* sp., among others.[25] In the present study, approximately 90% of the total anaerobes in both placebo- or probiotic-treated litters consisted primarily of *Lactobacillus* sp., which is in agreement with previous studies with young piglets.[30]

The number of *Lactobacillus* sp., *Enterococcus* sp., *C perfringens*, and Enterobacteriaceae in fecal microbial population were not influenced by providing probiotics to nursing piglets. However, earlier studies have indicated the potential to increase *Lactobacillus* sp. and decrease Enterobacteriaceae in fecal microbial population of sows in a before-and-after study with *B subtilis* C-3102.[11] Recently, a study demonstrated a decrease in *Clostridium* sp. in the fecal microflora of one-week-old progeny of sows fed *B subtilis* C-3102 probiotic following two sequential reproductive cycles.[12] The lack of influence of *B subtilis* C-3102 on fecal populations

of *Lactobacillus* sp., *Enterococcus* sp., *C perfringens*, and Enterobacteriaceae in nursing piglets in the present study could be due to the same hypothesized reason for the lack of effect on growth performance and fecal consistency: the dose of *B subtilis* C-3102 was not enough to influence the fecal levels of *Enterococcus* sp., *C perfringens*, and Enterobacteriaceae or to elicit an increase in *Lactobacillus* sp. Furthermore, the fact that the fecal population of these bacteria remained unaffected by the probiotic treatment could be responsible for the lack of effect on pre-weaning growth performance and fecal consistency of nursing piglets during lactation. Finally, it is noteworthy that a variation in probiotic effect could be attributed to a multitude of factors, including environmental conditions and health status. In this regard, it has been suggested that growth-promoting effects of probiotics are more evident under conditions of environmental stress or health challenge,[31] which were not expressive in the current study. The effects of *B subtilis* C-3102 probiotic on pre-weaning performance should be evaluated under typical environmental stress and health challenge of commercial swine production in further studies.

Implications

Under the conditions of this study, providing a daily oral dose of *Bacillus subtilis* C-3102 probiotic to nursing piglets until weaning:

- Did not influence pre-weaning growth performance and fecal consistency.
- Influenced only total *Bacillus* sp. fecal microbial populations.

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Table 3.1 Analysis of sow performance according to litter treatment*

	Placebo	Probiotic	SEM	Probability, *P*†
Parity	2.6	2.5	0.23	.30
Total born, n	17.7	17.5	0.90	.85
Born alive, n	16.5	16.3	0.64	.80
Stillborn, n	0.6	0.8	0.26	.59
Mummified, n	0.6	0.4	0.25	.33
Lactation feed intake, kg	6.60	6.64	0.182	.57
Lactation body weight loss, kg	6.25	6.24	2.659	.99

* A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was based on a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd.).

† Level of significance is $P < .05$ and tendency at $.05 \leq P \leq .10$ using linear mixed models.

SEM = standard error of the mean

Table 3.2 Effects of providing a daily oral dose of probiotics to nursing piglets during lactation on pre-weaning piglet performance*

	Placebo	Probiotic	SEM	Probability, *P*†
Body weight, kg				
d 2‡	1.63	1.53	0.042	.07
d 9	2.95	3.04	0.054	.30
d 16	4.76	4.81	0.107	.78
d 19	5.47	5.55	0.136	.67
ADG, g				
d 2 to 9	196	208	7.75	.30
d 9 to 16	259	252	9.51	.63
d 16 to 19	226	247	21.38	.40
d 2 to 19	205	209	7.15	.67
Litter size, n				
d 2	16.0	15.7	0.23	.31
d 9	15.7	15.1	0.23	.07
d 16	14.9	14.8	0.23	.80
d 19	14.8	14.7	0.26	.92
Mortality, %				
d 2 to 19	7.5	5.8	0.02	.51

* A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was based on a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd.).

† Level of significance is $P < .05$ and tendency at $.05 \leq P \leq .10$ using linear mixed models.

‡ Piglet initial body weight included as a covariate for piglet body weight and ADG during lactation in the statistical analysis.

SEM = standard error of the mean; ADG = average daily gain

Table 3.3 Effects of providing a daily oral dose of probiotics to nursing piglets during lactation on fecal microbes*

Microbe, log_{10} CFU/g	Placebo			Probiotic			Probability, *P*†		
	d 2	d 9	d 16	d 2	d 9	d 16	Treatment × Day	Treatment	Day
Bacillus subtilis C-3102	2.02^{xb}	2.36^{yb}	3.20^{zb}	2.24^{xa}	5.55^{ya}	5.74^{ya}	< .001	< .001	< .001
SEM	0.06	0.10	0.08	0.06	0.11	0.08			
Detected/sampled, n	2/14	7/14	14/14	7/12	12/12	12/12			
Total *Bacillus* sp.	2.44^{x}	3.32^{yb}	3.75^{zb}	2.67^{x}	5.55^{ya}	5.75^{ya}	< .001	< .001	< .001
SEM	0.13	0.10	0.12	0.13	0.11	0.12			
Detected/sampled, n	10/14	14/14	14/14	11/12	12/12	12/12			
Lactobacillus sp.	7.84	8.85	8.48	8.04	8.84	8.45	.72	.62	< .001
SEM	0.16	0.06	0.10	0.19	0.06	0.11			
Detected/sampled, n	14/14	14/14	14/14	11/11	12/12	12/12			
Enterococcus sp.	8.58	7.59	5.41	8.74	7.25	6.70	.10	.18	< .001
SEM	0.11	0.19	0.52	0.11	0.21	0.56			
Detected/sampled, n	13/13	14/14	12/14	10/10	12/12	12/12			
Clostridium perfringens	8.74	8.79	8.59	8.72	8.84	8.89	.40	.33	.66
SEM	0.02	0.13	0.15	0.02	0.14	0.17			
Detected/sampled, n	14/14	14/14	14/14	12/12	12/12	12/12			
Enterobacteriaceae	9.20	8.33	6.97	9.05	8.34	7.75	.13	.16	< .001
SEM	0.10	0.09	0.27	0.11	0.10	0.29			
Detected/sampled, n	14/14	14/14	14/14	12/12	12/12	11/12			
Total aerobes	9.32	8.64	8.41	9.28	8.42	8.24	.66	.03	< .001
SEM	0.09	0.09	0.09	0.10	0.10	0.10			
Detected/sampled, n	14/14	14/14	14/14	12/12	12/12	12/12			
Total anaerobes	9.68^{x}	9.61^{xB}	9.27^{y}	9.61^{Y}	9.76^{XA}	9.18^{Z}	.03	.99	< .001
SEM	0.08	0.06	0.07	0.08	0.07	0.08			
Detected/sampled, n	14/14	14/14	14/14	12/12	12/12	12/12			

* A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was based on a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd.). Microbial analysis of fecal samples was performed by isolation and enumeration method.

† Interactive and main effects of treatment and day. Level of significance is $P < .05$ and tendency at $.05 \leq P \leq .10$ using linear mixed models.

[a,b] Indicate significant difference ($P < .05$) between treatments within each day.

[A,B] Indicate a tendency ($.05 \leq P \leq .10$) for difference between treatments within each day.

[x,y,z] Indicate significant difference ($P < .05$) between days within each treatment.

[X,Y,Z] Indicate a tendency ($.05 \leq P \leq .10$) for difference between days within each treatment.

SEM = standard error of the mean

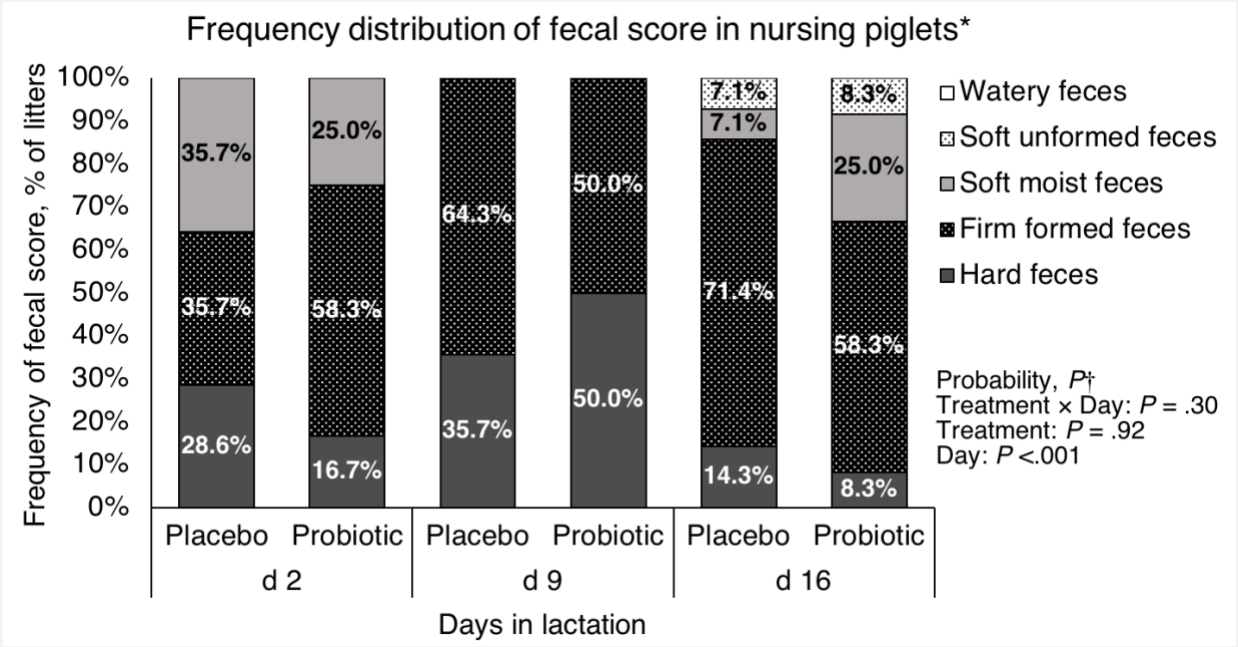

Figure 3.1 Effects of providing a daily oral dose of probiotics to nursing piglets during lactation on frequency distribution of fecal consistency assessed by litter fecal score

*A total of 26 lactating sows (DNA 241, DNA genetics) and litters were used with litter treatments consisting of providing a daily oral dose of a placebo (n = 14 litters) or a probiotic (n = 12 litters) to nursing piglets from day 2 after birth until weaning on day 19. The probiotic treatment was based on a direct-fed microbial containing *Bacillus subtilis* C-3102 (Calsporin, Calpis Co. Ltd.). Fecal score evaluation was conducted by a trained individual blind to treatments to categorize the consistency of piglets' feces per litter.

†Interactive and main effects of treatment and day. Level of significance is $P < .05$ and tendency at $.05 \leq P \leq .10$ using linear mixed models.

Chapter 4 - Effects of *Bacillus subtilis* C-3102 on sow and progeny performance, fecal consistency, and fecal microbes during gestation, lactation, and nursery periods

ABSTRACT: This study evaluated the effects of providing a dietary probiotic, *Bacillus subtilis* C-3102, to sows during gestation and lactation and to progeny after weaning on performance, fecal consistency, and fecal microbes. For the sow portion of the study, 29 sows and litters were used from d 30 of gestation until weaning. Sow treatments consisted of control diet or probiotic diet with *B. subtilis* C-3102 at 500,000 CFU/g of gestation feed and 1,000,000 CFU/g of lactation feed. For the nursery portion of the study, 358 weaned pigs, progeny of sows on study, were used in a 42-d nursery study. Nursery treatments consisted of control diet or probiotic diet with *B. subtilis* C-3102 and prebiotics at 500,000 CFU/g of nursery feed. Treatments were arranged in a split-plot design with sow treatment (control or probiotic diet) as main plot and nursery treatment (control or probiotic diet) as subplot. Performance, fecal consistency by fecal score method, and fecal microbes by isolation and enumeration method were assessed. In lactation, probiotic-fed sows tended ($P = 0.057$) to have increased feed intake, but it did not improve ($P > 0.05$) sow or litter performance in lactation. In the nursery, there were no ($P > 0.10$) interactions or main effects of sow or nursery treatments on overall growth performance. However, pigs born from control-fed sows had greater ($P < 0.05$) average daily gain, average daily feed intake, and body weight in late nursery than pigs born from probiotic-fed sows. Fecal score evaluation of nursing and nursery pigs indicated no influence ($P > 0.05$) of sow or nursery treatments on fecal consistency. Fecal microbial analysis revealed a modest modification in fecal microbial population by increasing ($P < 0.05$) the number of total *Bacillus* sp. in probiotic-fed

sows and nursery pigs. Nursing piglets born from probiotic-fed sows carried over ($P < 0.05$) this modification in fecal microbial population pre-weaning. In conclusion, providing a probiotic based on *B. subtilis* C-3102 to sows during gestation and lactation and to progeny after weaning did not elicit noteworthy improvements in performance or fecal consistency, but there was a benefit on sow lactation feed intake. Fecal microbial analysis indicated a maternal-progeny intestinal microbiota relationship with pigs born from probiotic-fed sows displaying similar fecal microbial population as sows. However, pigs born from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery.

Key words: direct-fed microbial, feed additive, microbiota, probiotic, swine

INTRODUCTION

Probiotics have been explored as feed additives in swine diets to improve performance and preserve intestinal health while minimizing the use of antibiotics (Liao and Nyachoti, 2017). The use of probiotics in sow diets is proposed to have a dual purpose benefiting sows and their progeny. The intimate maternal contact is an important determinant of gastrointestinal tract bacterial colonization of newborn piglets (Everaert et al., 2017). Moreover, sows are able to exert a diet-driven modulation of milk bacterial population and influence the progeny intestinal microbiota during lactation (Rodriguez, 2014; Chen et al., 2018). The establishment of a healthy intestinal microbiota in early life may be essential to promote growth, immunity, and health later in life (Schmidt et al., 2011; Merrifield et al., 2015; Dou et al., 2017). Thus, dietary strategies to modulate the intestinal microbiota of sows and piglets have been investigated. Studies have demonstrated that provision of probiotics to sows can modify the sow fecal microbial population and carry over to progeny in pre- and post-weaning stages (Silva et al., 2010; Baker et al., 2013; Starke et al., 2013).

Bacillus subtilis C-3102 is a non-genetically-modified strain of a Gram-positive, spore-forming bacteria used as probiotic for pigs. The effects of *B. subtilis* C-3102 are proposed to promote beneficial bacteria proliferation in sows and reduce pathogenic bacteria in their progeny (Maruta et al., 1996b; Kritas et al., 2015). This has been reflected as reduction in diarrhea incidence (Maruta et al., 1996b), improvement in growth performance (Marubashi et al., 2012), and attenuation of intestinal lesions under health challenge (Canning et al., 2017) in nursery pigs. However, to the best of the authors' knowledge, studies designed to evaluate the long-term influence of providing *B. subtilis* C-3102 to sows in gestation and lactation on the progeny through the nursery have not been previously conducted.

Therefore, the objective of this study was to evaluate the effects of providing a probiotic based on viable spores of *B. subtilis* C-3102 to sows during gestation and lactation and to progeny after weaning on performance, fecal consistency, and fecal microbes.

MATERIALS AND METHODS

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Sows and progeny were used in this study divided in sow portion, from d 30 of gestation to weaning, and nursery portion, from weaning to d 42.

Sow portion

A total of 29 crossbred sows (DNA 241, DNA Genetics, Columbus, NE; 1.9 average parity) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used for the sow portion of the study. Sows were individually housed in environmentally-controlled and mechanically-ventilated barns during gestation and lactation. Farrowing stalls were equipped

with an individual nipple waterer and an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada). Farrowing stalls were equipped with a rubber mat and heat lamp for piglet comfort. Piglets were processed and cross-fostered within sow treatment group to equalize litter size within 24 hours of birth. Piglets had free access to water and no creep feeding was provided during lactation.

Dietary treatments were assigned to sows with confirmed pregnancy on d 30 of gestation in a randomized complete block design based on sow parity and initial body weight (**BW**). Sow dietary treatments consisted of providing a control diet (n = 14 sows) or a probiotic diet (n = 15 sows) to sows during gestation and lactation. The probiotic diet was supplemented with a probiotic product based on viable spores of *B. subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan). The active ingredient in Calsporin® is dried *B. subtilis* C-3102 fermentation product in a calcium carbonate carrier. Diets were based on corn and soybean meal and fed in meal form (Table 4.1). Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Gestation diets were fed from d 30 of gestation until farrowing. Daily feed allowance was 2, 2.5, or 3 kg once per day according to body condition from d 30 to 112 of gestation, and 2.7 kg/d from d 112 of gestation until farrowing. Dietary treatments were top dressed in a common gestation diet. In the control diet, the top dress contained ground corn. In the probiotic diet, the top dress contained ground corn and Calsporin® to achieve 500,000 CFU/g of *B. subtilis* C-3102 in gestation feed at the expense of corn.

Lactation diets were fed from farrowing to weaning at approximately d 19 of lactation. Sows were allowed *ad libitum* feed intake during lactation with daily feed delivery and recording by an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, St-Lambert-de-

Lauzon, Quebec, Canada). Dietary treatments were incorporated into lactation diet formulation. In the probiotic diet, Calsporin® was included to achieve 1,000,000 CFU/g of *B. subtilis* C-3102 in lactation feed at the expense of corn.

Sow BW was measured on d 30 and 112 of gestation, post-farrow, and at weaning. Sow feed intake was recorded on a daily basis. Fecal samples were collected from sows on d 30 of gestation (baseline), d 112 of gestation (pre-farrowing), and d 18 of lactation (pre-weaning) for microbial analysis. Farrowing and litter performance were assessed by recording number of total born piglets, born alive piglets, stillborn, and mummies; individual piglet BW at birth, d 2 and 12 of lactation, and at weaning; and litter size on d 2 and 12 of lactation, and at weaning. Pre-weaning mortality was estimated considering the number of dead piglets from birth to weaning in relation to the number of piglets born alive. Fecal score was conducted to characterize piglet fecal consistency on d 2 (postnatal) and d 18 (pre-weaning). Fecal samples were collected from piglets on d 2 (postnatal) and d 18 (pre-weaning) for microbial analysis.

Nursery portion

A total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of the study. Only nine weaned pigs (five from control litters and four from probiotic litters) were not included in the nursery portion of the study due to health issues. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d period into the nursery beginning at weaning. Weaned pigs were housed in an environmentally-controlled and mechanically-ventilated nursery barn with 1.5 × 1.5 m pens equipped with a four-hole, dry, self-feeder and one cup waterer. Pigs were placed in mixed-gender pens with 4 or 5 pigs per pen.

Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot. There were 18 or 19 replicates per treatment. Nursery dietary treatments consisted of providing a control diet or a probiotic diet with supplementation of viable spores of *B. subtilis* C-3102 and prebiotics (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) to nursery pigs. In the probiotic diet, BacPack ABF™ was included at 0.05% of complete feed to achieve 500,000 CFU/g of *B. subtilis* C-3102 and proprietary amounts of yeast cell wall derivatives. The active ingredients in BacPack ABF™ are dried *B. subtilis* C-3102 fermentation product and mannan oligosaccharides in a calcium carbonate carrier.

Diets were based on corn and soybean meal and fed in three dietary phases: phase 1, fed from d 0 to 7 in pellet form; phase 2, fed from d 7 to 21 in meal form; and phase 3, fed from d 21 to 42 in meal form (Table 4.2). Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Pigs and feeders were weighed on d 0, 7, 14, 21, 28, 35, and 42 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Fecal score was conducted to characterize piglet fecal consistency on d 0, 7, 14, 21, 28, 35, and 42. Fecal samples were collected from pigs on d 21 and 42 for microbial analysis.

Fecal score

Fecal score was conducted to categorize the piglet fecal consistency using the following categories: hard feces, firm formed feces, soft moist feces, soft unformed feces, and watery feces. Fecal scoring was assigned to litters in the sow portion of the study and to pens in the nursery portion of the study by visually assessing feces in farrowing stalls or nursery pens. Fecal

score evaluation was performed by three trained individuals and the concordant score was considered as the definite score.

Fecal microbial analysis

Fecal samples were freshly collected from sows by rectal grab, from nursing piglets with sterile mini cotton tip swabs, and from nursery pigs with sterile cotton tip swabs. In the sow portion of the study, fecal samples were collected from individual sows for analysis (n = 29) and from all nursing piglets pooled by litter for analysis (n = 27). Fecal samples from one litter of each sow treatment group were not collected for microbial analysis due to sows farrowing later compared to the group. In the nursery portion of the study, fecal samples were collected from two pigs per pen and three pens of the same treatment were pooled for analysis (n = 24). Fecal samples were kept at 4 °C until analysis within 24 hours of collection.

Microbial analysis of fecal samples was performed by isolation and enumeration method of *B. subtilis* C-3102, total *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Clostridium perfringens*, *Salmonella* spp., Enterobacteriaceae, total aerobes, and total anaerobes.
For microbial platting, approximately 1 g of feces was suspended in 9 mL of anaerobic diluent and serial 10-fold dilutions were prepared according to procedures described previously (Maruta et al., 1996b). Aliquots of 0.05 mL of each dilution were inoculated into selective and non-selective media. All media were incubated at 37 °C unless otherwise noted. *Bacillus subtilis* C-3102 were enumerated on tryptic soy broth with 2% agar after incubation for 1 day (Marubashi et al., 2012). Total *Bacillus* sp. were enumerated by chromogenic method using a differential medium (92325 Bacillus *ChromoSelect* Agar, Sigma-Aldrich, Saint Louis, MO) after incubation for 1 day and spores were quantified after incubation at 80 °C for 15 minutes (Kritas et al., 2015). *Lactobacillus* sp. were enumerated on modified lactobacilli selective agar after anaerobic

incubation for 2 days (Maruta et al., 1996b). *Enterococcus* sp. were enumerated on triphenyltetrazolium chloride-acridine orange- thallous sulfate aesculin crystal violet (TATAC) agar after incubation for 2 days (Maruta et al., 1996b). *Clostridium perfringens* were enumerated on neomycin-brilliant green-taurocholate-nagler (NN) agar after anaerobic incubation for 3 days (Maruta et al., 1996b). *Salmonella* spp. were enumerated on mannitol lysine crystal violet brilliant green (MLCB) agar after incubation for 1 day (Maruta et al., 1996a). Enterobacteriaceae were enumerated on neomycin-brilliant green-taurocholate-blood (NBGT) agar after incubation for 1 day (Maruta et al., 1996b). Total aerobes were enumerated on trypticase soy agar after incubation for 2 days (Maruta et al., 1996b). Total anaerobes were enumerated on glucose blood liver agar and Eggerth-Gagnon agar after anaerobe incubation for 3 days (Maruta et al., 1996b). Limit of detection was 2×10^2 CFU/g. Microbial analysis was performed by the microbiology laboratory of Calpis America, Inc. (Peachtree City, GA).

Chemical analysis

Feed samples were collected during the manufacturing process. Approximately 1 kg of feed was collected from each treatment for each batch of feed. Composite samples were stored at -20 °C and grinded before submission to analysis. Feed samples were analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter (method 935.29 AOAC, 1990), crude protein (method 990.03 AOAC, 1990), acid detergent fiber (Ankom, 1998), neutral detergent fiber (Ankom, 1998), ether extract (Ankom, 2004), Ca (method 985.01 AOAC, 1990), and P (method 985.01 AOAC, 1990). Feed samples were also analyzed for quantification of *B. subtilis* C-3102 (Calpis America, Inc., Peachtree City, GA).

Statistical analysis

Data were analyzed using a linear mixed model. Dietary treatment was included as fixed effect. Block was included as random effect in the sow portion analysis of the study. The

experimental units were sow or litter for the sow portion of the study and pen for the nursery portion of the study.

Response variables were fit assuming a normal distribution unless otherwise noted. Piglets born alive, stillborn, and mummies were analyzed assuming a binomial distribution as a proportion of total born piglets. Pre-weaning mortality was analyzed assuming a binomial distribution as a proportion of number of dead piglets from birth to weaning in relation to the number of piglets born alive. Fecal score was analyzed assuming a multinomial distribution and considering the frequency distribution of experimental units within each fecal score category. For normally-distributed response variables, the residual assumptions were met by evaluating studentized residuals.

In the nursery portion of this study, preplanned contrast statements were built to evaluate the main effects and interactions of sow dietary treatment and nursery dietary treatment. Repeated measures analysis was applied to fecal score and fecal microbial analysis considering the multiple measures taken on the same experimental unit over a time period.

Statistical models were fit using the GLIMMIX procedure of SAS® version 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

RESULTS

Chemical analysis

Proximate analysis, Ca, P, and *B. subtilis* C-3102 content of experimental diets (Tables 4.3 and 4.4) were consistent with formulated estimates. The presence of *B. subtilis* C-3102 in control diets is associated to the ubiquitous nature of the species and was as expected. The levels

in control diets were within expectations and in accordance to the literature (Marubashi et al., 2012), i.e., at least 1 $\log_{10}$ lower CFU/g compared to probiotic diets.

Sow portion

There was no evidence for differences ($P > 0.10$) in sow parity and BW on d 30 of gestation between dietary treatments (Table 4.5), validating the randomization process. No evidence for differences ($P > 0.10$) was observed on sow BW at the end of gestation, post-farrow, or at weaning, consequently no evidence for differences ($P > 0.10$) was observed on sow BW change from farrow to weaning between control- and probiotic-fed sows. In gestation, ADFI was similar ($P > 0.10$) for control- and probiotic-fed sows. In lactation, probiotic-fed sows had a tendency ($P = 0.057$) for increased ADFI, consuming on average 0.5 kg more feed per day in lactation than control-fed sows. There was no evidence for differences ($P > 0.10$) in number of piglets total born, born alive, stillborn, and mummies; piglet BW from birth to weaning; litter weight from birth to weaning; piglet ADG during lactation; and pre-weaning mortality between control- and probiotic-fed sows. Probiotic-fed sows had a tendency ($P = 0.060$) for larger litter size on d 2 after birth, with on average 0.5 more piglet per litter than control-fed sows. There was no evidence for differences ($P > 0.10$) in litter size on d 12 of lactation and at weaning between control- and probiotic-fed sows.

Nursery portion

Only a tendency ($P < 0.10$) for interaction of sow dietary treatment and nursery dietary treatment was observed on growth performance of nursery pigs (Table 4.6). Therefore, the main effects of sow dietary treatment and nursery dietary treatment on growth performance of nursery pigs were further explored (Table 4.7).

Initial BW in the nursery was influenced ($P < 0.01$) by sow dietary treatment, where pigs born from probiotic-fed sows were 0.1 kg heavier than pigs born from control-fed sows. The

difference in initial nursery BW was expected from the same difference in piglet weaning weight and as a consequence of split plot design used in this study. The significance was captured in the nursery due to the greater number of replicates in the nursery portion of the study (n = 36 or 38 pens per treatment) compared to the sow portion of the study (n = 14 or 15 litters per treatment), in addition to the considerably lower variation around pig BW in the nursery portion of the study (initial BW SEM = 0.01) compared to the sow portion of the study (weaning BW SEM = 0.21).

In phase 1, from d 0 to 7 of nursery, there was a tendency ($P = 0.088$) for interaction of sow dietary treatment and nursery dietary treatment on G:F, where pigs born from control-fed sows had improved G:F when fed the probiotic diet compared to the control diet, but pigs born from probiotic-fed sows had similar G:F when fed the probiotic diet or the control diet. There was no evidence ($P > 0.10$) for effect of sow dietary treatment on growth performance. There was a tendency ($P = 0.084$) for effect of nursery dietary treatment on ADG, where pigs fed the probiotic diet in the nursery had increased ADG compared to pigs fed the control diet. However, no evidence ($P > 0.10$) for effect of nursery dietary treatment was observed on ADFI. Body weight of pigs on d 7 of nursery was not influenced ($P > 0.10$) by sow or nursery dietary treatments. In phase 2, from d 7 to 21 of nursery, there was no evidence ($P > 0.10$) for effect of sow or nursery dietary treatments on growth performance. Body weight of pigs on d 21 of nursery was not influenced ($P > 0.10$) by sow or nursery dietary treatments. In phase 3, from d 21 to 42 of nursery, there was an effect ($P < 0.01$) of sow dietary treatment on ADG and ADFI, where pigs born from control-fed sows had increased ADG and ADFI compared to pigs born from probiotic-fed sows. However, no evidence ($P > 0.10$) for effect of sow dietary treatment was observed for G:F. There was a tendency ($P = 0.084$) for effect of nursery dietary treatment for G:F, where pigs fed the control diet in the nursery had increased G:F compared to pigs fed

the probiotic diet. However, no evidence ($P > 0.10$) for effect of nursery dietary treatment was observed on ADG and ADFI.

Overall, from d 0 to 42 of nursery, there was no evidence ($P > 0.10$) for effect of sow or nursery dietary treatments on growth performance. There was an effect ($P = 0.042$) of sow dietary treatment on final nursery BW, where pigs born from control-fed sows were heavier than pigs born from probiotic-fed sows on d 42 of nursery. There was no evidence ($P > 0.10$) for effect of nursery dietary treatment on final nursery BW.

Fecal score

Fecal score of nursing and nursery pigs is presented as the frequency distribution of litters and pens, respectively, within each fecal score category. Fecal score of nursing piglets was not influenced ($P > 0.10$) by interaction of sow dietary treatment by day or main effect of sow dietary treatment (Fig. 4.1). Fecal consistency was mostly classified as hard feces or firm formed feces in litters from both control- or probiotic-fed sows. There was a tendency ($P = 0.070$) for an effect of day in lactation in fecal score of nursing piglets. On d 2 of lactation, fecal consistency was mostly classified as firm formed feces or soft moist feces, but on d 18 of lactation fecal consistency mostly shifted to hard feces or firm formed feces.

Fecal score of nursery pigs was not influenced ($P > 0.10$) by interactions or main effects of sow dietary treatment and nursery dietary treatment (Fig. 4.2). Fecal consistency was mostly classified as soft moist feces or soft unformed feces across dietary treatments. There was no interactions of sow dietary treatment and nursery dietary treatment by day in nursery ($P > 0.10$). There was a tendency ($P = 0.077$) for main effect of day in fecal score of nursery pigs (Fig. 4.3). During the 42-d nursery period, fecal consistency gradually shifted to a looser pattern, with a decrease in frequency distribution of pens with firm formed feces, an increase of pens with soft unformed feces, and presence of pens with watery feces on d 42 of nursery.

Fecal microbial analysis

Sow fecal microbial analysis revealed an interaction ($P < 0.01$) between sow dietary treatment and day in lactation on number of *B. subtilis* C-3102 and total *Bacillus* sp. (Table 4.8). In probiotic-fed sows, the numbers of *B. subtilis* C-3102 and total *Bacillus* sp. increased ($P < 0.05$) from d 30 to 113 of gestation and remained at a constant level in lactation until a day prior to weaning; whereas in control-fed sows, the level of *B. subtilis* C-3102 and total *Bacillus* sp. either decreased or remained at a constant level during gestation and lactation. The numbers of *B. subtilis* C-3102 and total *Bacillus* sp. were increased ($P < 0.05$) in probiotic-fed sows compared to control-fed sows at any stage of gestation and lactation.

The numbers of *Lactobacillus* sp., *C. perfringens*, Enterobacteriaceae, and total anaerobes were influenced ($P < 0.01$) by day in lactation. The number of *Lactobacillus* sp. remained constant during gestation (7.13 and 6.84 $\log_{10}$ CFU/g on d 30 and 113, respectively), but increased in lactation (8.45 $\log_{10}$ CFU/g on d 18; $P < 0.05$). The number of *C. perfringens* decreased during gestation and lactation (8.03, 7.74, and 6.08 $\log_{10}$ CFU/g on d 30 of gestation, d 113 of gestation, and d 18 of lactation, respectively; $P < 0.05$). The number of Enterobacteriaceae remained at a constant level during gestation (7.48 and 7.36 $\log_{10}$ CFU/g on d 30 and 113, respectively), but decreased in lactation (6.57 $\log_{10}$ CFU/g on d 18; $P < 0.05$). The number of total anaerobes slightly reduced during gestation (9.15 and 9.00 $\log_{10}$ CFU/g on d 30 and 113, respectively; $P < 0.05$), but increased in lactation (9.30 $\log_{10}$ CFU/g on d 18; $P < 0.05$). For number of total aerobes, there were no evidence ($P > 0.10$) for interactions or main effects of sow dietary treatment or day in lactation. *Salmonella* spp. was detected on d 113 of gestation in two out of 14 fecal samples from control-fed sows (average 5.49 $\log_{10}$ CFU/g) and in one out of 15 fecal samples from probiotic-fed sows (4.34 $\log_{10}$ CFU/g), but it was not detectable in fecal samples on d 30 of gestation and d 18 of lactation.

Nursing piglet fecal microbial analysis revealed an interaction ($P < 0.05$) between sow dietary treatment and day in lactation on number of *B. subtilis* C-3102, total *Bacillus* sp., and *Lactobacillus* sp. (Table 4.9). The number of *B. subtilis* C-3102 increased ($P < 0.05$) from d 2 to 18 of lactation in litters from probiotic-fed sows, whereas remained at a constant level in lactation in litters from control-fed sows. The number of total *Bacillus* sp. decreased ($P < 0.05$) from d 2 to 18 of lactation in litters from both sow dietary treatments, but the magnitude of decrease was greater in litters from control-fed sows. The numbers of *B. subtilis* C-3102 and total *Bacillus* sp. were increased ($P < 0.05$) in litters from probiotic-fed sows compared to control-fed sows on d 18 of lactation. The number of *Lactobacillus* sp. increased ($P < 0.05$) from d 2 to 18 of lactation in litters from control-fed sows, whereas remained at a constant level in lactation in litters from probiotic-fed sows. The number of *Lactobacillus* sp. was similar in litters from both sow dietary treatments on d 18 of lactation.

The numbers of *C. perfringens*, Enterobacteriaceae, total aerobes, and total anaerobes were influenced ($P < 0.10$) by day in lactation. The number of *C. perfringens* (8.93 to 8.57 $\log_{10}$ CFU/g), Enterobacteriaceae (9.30 to 8.38 $\log_{10}$ CFU/g), total aerobes (8.23 to 6.70 $\log_{10}$ CFU/g), and total anaerobes (9.43 to 8.60 $\log_{10}$ CFU/g) decreased ($P < 0.10$) from d 2 to 18 of lactation. For number of *Enterococcus* sp., there was no evidence ($P > 0.10$) for interactions or main effects of sow dietary treatment or day in lactation. *Salmonella* spp. was detected on d 2 of lactation in one out of 13 fecal samples from litters from control-fed sows (7.33 $\log_{10}$ CFU/g), but it was not detectable in fecal samples on d 18 of lactation.

Nursery pig fecal microbial analysis revealed an interaction ($P < 0.01$) between sow dietary treatment, nursery dietary treatment, and day in nursery on number of *B. subtilis* C-3102 (Tables 4.10 and 4.11). Nursery pigs from control-fed sows and also fed a control diet in the nursery maintained lower levels of *B. subtilis* C-3102 during nursery, whereas pigs from control-

fed sows but fed a probiotic diet in the nursery rapidly increased and maintained higher levels of *B. subtilis* C-3102 during the nursery. Nursery pigs from probiotic-fed sows and also fed a probiotic diet in the nursery maintained higher levels of *B. subtilis* C-3102 during the nursery, whereas pigs born from probiotic-fed sows but fed a control diet in the nursery gradually decreased and maintained lower levels of *B. subtilis* C-3102 during the nursery. The number of total *Bacillus* sp. was influenced ($P < 0.01$) by nursery dietary treatment, but there was no evidence ($P > 0.10$) for interactions or main effects of sow dietary treatment or day in nursery. Nursery pigs fed a probiotic diet in the nursery had increased number of total *Bacillus* sp. compared to pigs fed a control diet in the nursery (5.69 vs. 4.09 $\log_{10}$ CFU/g, respectively; $P < 0.01$).

The number of total aerobes was influenced ($P < 0.05$) by an interaction between nursery dietary treatment and day in nursery and a main effect of sow dietary treatment. Nursery pigs fed a control diet in the nursery slightly increased the number of total aerobes during nursery (9.52 to 9.70 $\log_{10}$ CFU/g from d 21 to 42; $P < 0.05$), whereas pigs fed the probiotic diet maintained a constant number of total aerobes during nursery (9.58 to 9.57 $\log_{10}$ CFU/g from d 21 to 42; $P > 0.10$). Nursery pigs from control-fed sows had slightly increased number of total aerobes compared to pigs from probiotic-fed sows (9.65 vs. 9.54 $\log_{10}$ CFU/g, respectively; $P < 0.05$).

The number of total anaerobes was influenced ($P < 0.05$) by an interaction between sow dietary treatment and nursery dietary treatment and a main effect of day in nursery. Nursery pigs from control-fed sows and also fed the control diet in the nursery had slightly higher ($P < 0.05$) number of total anaerobes (10.23 $\log_{10}$ CFU/g) compared to pigs that were either fed the probiotic diet in the nursery (10.11 $\log_{10}$ CFU/g) or from probiotic-fed sows (10.10 $\log_{10}$ CFU/g), whereas the number of total anaerobes in pigs born from probiotic-fed sows and also fed the

probiotic diet in the nursery was intermediate (10.17 $\log_{10}$ CFU/g). The number of total anaerobes slightly decreased from d 21 to 42 of nursery (10.19 to 10.12 $\log_{10}$ CFU/g; $P < 0.05$).

For *Lactobacillus* sp., *Enterococcus* sp., and Enterobacteriaceae, there were only tendencies ($P < 0.10$) for interactions or main effects of sow dietary treatment, nursery dietary treatment, and day in nursery. The practical and biological significance of these tendencies were not considered relevant to the study. *Clostridium perfringens* and *Salmonella* spp. were not detectable in fecal samples in the nursery.

DISCUSSION

Pre-weaning piglet development is intrinsically reliant on the sow. The intimate contact of newborn piglets with the sow is an important determinant of early bacterial colonization of the porcine gastrointestinal tract (Everaert et al., 2017) and exerts a long-term influence on pigs described as "microbial imprinting" (Thompson et al., 2008; Mach et al., 2015). Maternal-to-progeny transfer of bacteria originates from the reproductive tract during parturition, and from the milk, skin, and fecal-oral contact during lactation (Buddington et al., 2009). However, the balance between beneficial and pathogenic bacteria can be altered during critical periods of the sow reproductive cycle, particularly from farrowing through weaning (Liu et al., 2019). Dietary strategies meant to modulate the bacterial population and re-establish the bacterial balance of sows can confer health benefits to sows and, indirectly, to the progeny (Baker et al., 2013). Probiotics have been appointed as promising additives to modulate the intestinal microbiota via sow nutrition because, by definition, probiotics are non-pathogenic live microorganisms that can improve the intestinal microbial balance and confer health benefits once provided in adequate amounts (Fuller, 1989). Probiotics have been found to influence the developing intestinal microbiota of nursing piglets though supplementation of sows (Baker et al., 2013; Starke et al.,

2013). Interestingly, the probiotic influence on intestinal microbiota of the progeny in early life seems to be extended to post-weaning stages later in life (Alexopoulos et al., 2001; Silva et al., 2010). In light of the available literature, the present study focused on the further comprehension of the maternal-progeny intestinal microbiota relationship and the long-term impact of providing a probiotic, *B. subtilis* C-3102, to sows on progeny through the nursery in regard to performance, fecal consistency, and fecal microbes.

The findings of the sow portion of the study indicate a benefit of providing *B. subtilis* C-3102 during gestation and lactation on sow lactation feed intake. Previous studies providing *Bacillus* sp. or *Enterococcus* sp. species to sows during late gestation and lactation support an improvement in lactation feed intake with probiotics (Alexopoulos et al., 2004; Böhmer et al., 2006). Feed consumption during lactation is important to achieve the milk production potential to support large and fast-growing litters with minimal mobilization of sow body reserves (Strathe et al., 2017). However, while probiotic-fed sows consumed on average 0.5 kg more feed per day than control-fed sows, the improvement in feed intake did not affect litter performance or sow body weight loss during lactation as previously reported (Alexopoulos et al., 2001, 2004; Stamati et al., 2006; Baker et al., 2013).

The influence of sow probiotic supplementation on litter performance until weaning is not consistent in the literature. While some studies report improvements in weaning weight, number of weaned piglets, fecal consistency, and pre-weaning mortality driven by sow supplementation with bacillus-based probiotics (Alexopoulos et al., 2001, 2004; Stamati et al., 2006), others including the present study fail to find evidence for improvements in pre-weaning performance (Böhmer et al., 2006; Baker et al., 2013). Improvements in pre-weaning performance have been attributed to beneficial effects of probiotics on milk composition and microbial balance (Alexopoulos et al., 2001, 2004; Stamati et al., 2006; Starke et al., 2013), but

only the latter has been assessed in the present study. In the present study, only litter size after cross-fostering was improved in probiotic-fed sows by an average of 0.5 piglet per litter compared to control-fed sows, but the litter size advantage was not maintained until weaning. The improvement in litter size after cross-fostering is a consequence of the numerically larger number of piglets born alive in probiotic-fed sows, with an average of 0.4 more piglet born alive per litter compared to control-fed sows. The variation in number of piglets born alive among sows likely limited the ability to find significant differences in litter size at birth, whereas the consistency in litter size after equalization allowed for a significant effect. Nevertheless, it cannot be assumed that litter size at birth is a primary effect of sow probiotic supplementation, particularly starting on d 30 of gestation as in the present study, because litter size is determined in earlier stages of pregnancy and subject to a multitude of unrelated factors (Böhmer et al., 2006; Østrup et al., 2011).

Providing a bacillus-based probiotic to sows during gestation and lactation induced a sow fecal microbial population modification by increasing the number of total *Bacillus* sp. as a consequence of increasing *B. subtilis* C-3102. Most importantly, probiotic supplementation to sows influenced the developing fecal microbial population of the progeny. Piglets born and nursed by probiotic-fed sows displayed a similar fecal microbial population with increasing number of *B. subtilis* C-3102 and total *Bacillus* sp. in the pre-weaning period. Previous studies support the influence of probiotics on sow fecal microbial population as well as the maternal transfer of probiotic strains to the progeny and the mirrored fecal microbial population in nursing piglets (Taras et al., 2005, 2006). The conditions in the gastrointestinal tract of newborn piglets are permissive for bacterial colonization (Buddington et al., 2009). The modulation of sow fecal microbiota is an effective strategy to reduce pathogen load and establish beneficial bacteria more rapidly in the gastrointestinal tract of piglets in the early postnatal period (Baker et al., 2013).

Studies providing *Bacillus* sp. or *Enterococcus* sp. species to sows during late gestation and lactation describe improvements in the population of beneficial bacteria, primarily *Lactobacillus* sp., and reductions in the population of potentially harmful bacteria, including *C. perfringens* and *Escherichia coli* (Baker et al., 2013; Starke et al., 2013). However, similar probiotic-driven modifications of fecal microbial population were not found in the present study. The potential of *B. subtilis* C-3102 to increase *Lactobacillus* sp. and decrease Enterobacteriaceae in fecal microbial population of sows and decrease *Clostridium* sp. in the fecal microbial population of the progeny has been demonstrated in previous studies (Maruta et al., 1996b; Kritas et al., 2015). However, the normal microbial population of sows and piglets should be taken into consideration. In the present study, the number of *C. perfringens* in fecal microbial population of sows and piglets was equivalent to or greater than the number of *Lactobacillus* sp. The levels of *C. perfringens* were not causing clinical signs in sows or piglets and were considered within normal levels for the farm under study, as evaluated in other instances before and after the present study. However, it could be speculated that the dose of *B. subtilis* C-3102 used in this study was not enough to displace *C. perfringens* (Maruta et al., 1996b) or to elicit an effect in number of *Lactobacillus* sp. of sufficient magnitude to outnumber *C. perfringens*. This could also explain the lack of probiotic effect on fecal consistency in the pre-weaning period. Moreover, the intestinal microbiota of sows seems to inherently control the impact of probiotics and, once established and stable, a fundamental change on bacterial population as consequence of probiotic supplementation becomes less likely (Savage et al., 1978).

The findings of the nursery portion of the study indicate a similar growth performance and fecal consistency in the overall nursery period in spite of providing *B. subtilis* C-3102 to sows and/or nursery pigs. Only few studies were designed to evaluate the long-term influence of providing bacillus-based or lactic acid bacteria-based probiotics to sows in late gestation and

lactation on the progeny through the nursery (Alexopoulos et al., 2001; Silva et al., 2010). The studies suggest the beneficial effects of probiotic seem to be additive, as growth rate and weight gain in the nursery are further improved in nursery pigs born from sows fed probiotic diets and also fed probiotic diets in the nursery (Alexopoulos et al., 2001; Silva et al., 2010). In contrast, no additive effects of probiotics were found in the nursery portion of the present study, as implied by the lack of interactions between sow and nursery dietary treatments on nursery performance. The studies also suggest the effects of probiotics on performance of nursery pigs can be indirect, when pigs are born from sows fed probiotic diets in gestation and lactation, or direct, when pigs are fed probiotic diets in the nursery (Alexopoulos et al., 2001). In the present study, the indirect effect of probiotics in nursery performance was observed in late nursery. Contrarily to expected, nursery pigs born from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery. Although the underlying cause remains unclear, the fact that growth performance impairment only occurred in late nursery suggests that a modification of intestinal microbial population driven by dietary change could be speculated. In the last phase of nursery, pigs were switched to a considerably simpler diet in comparison to the diet composition of previous nursery phases by the removal of lactose sources, specialty protein sources, and pharmacological levels of zinc oxide. Dietary changes have been associated with significant shifts on fecal microbiota of nursery pigs, including structural and functional transitions in the attempt to face a challenge (Tilocca et al., 2017). Although the fecal microbial analysis performed in the present study did not identify differences in fecal microbes between nursery pigs born from control- or probiotic-fed sows, it is plausible to speculate that there could have been more complex differences in microbiota composition not able to be identified by isolation and enumeration of a limited number of bacteria. A difference in basal microbiota composition between nursery pigs born from control- or probiotic-fed sows could have led to

distinct microbiota adaptation processes following a change in diet composition and reflected on growth performance (Tilocca et al., 2017). However, the theory presented here warrants further investigations.

The direct effect of probiotics in nursery performance was modest in the present study. In contrast, previous studies with weaned pigs fed diets with *B. subtilis* C-3102 demonstrate the potential to improve growth rate and feed efficiency by 5 to 6% in the nursery with probiotics (Marubashi et al., 2012). However, the inconsistency of growth performance effects to probiotics is common-place in the probiotic scientific literature (Zimmermann et al., 2016). The variation within the use of the same bacteria strain could be attributed to a multitude of factors, including dietary composition, environmental conditions, and health status. In this regard, it has been suggested that growth-promoting effects of probiotics are more evident under conditions of dietary, environmental, or health challenges (Madec et al., 1998). In the present study, the health status and sanitation of nursery facilities, as well as the formulation of diets at the nutrient requirements for nursery pigs (NRC, 2012) and the inclusion of pharmacological levels of zinc oxide, might have contributed to the lack of growth-promoting effect of probiotics in diets for nursery pigs. This could also explain the lack of probiotic effect on fecal consistency in the post-weaning period.

Providing a bacillus-based probiotic to nursery pigs induced a modest modification in fecal microbial population by increasing the number of total *Bacillus* sp. as a consequence of increasing *B. subtilis* C-3102 irrespective of sow diet in gestation and lactation. Although providing *B. subtilis* C-3102 to sows in gestation and lactation is able to increase total *Bacillus* sp. in fecal microbial population of nursing piglets as discussed previously, the levels of total *Bacillus* sp. are not sustained during nursery without providing probiotic to nursery pigs. This agrees with the characteristic of bacillus-bases probiotics, which spores germinate but not

proliferate in the gastrointestinal tract (Buchanan et al., 1974). Moreover, providing *B. subtilis* C-3102 to nursery pigs only seems to elicit the same increase in total *Bacillus* sp. in fecal microbial population as the supplementation of both sows and nursery pigs. However, providing *B. subtilis* C-3102 to both sows and nursery pigs seems to control the number of total aerobes in fecal microbial population of nursery pigs. Total aerobes count is a common indicator of general bacterial population on fecal samples (Buchanan et al., 1974), which indicates the probiotic contributes to maintaining a low bacterial load in the feces of nursery pigs and, consequently, in the environment (Siggers et al., 2008; Luyckx et al., 2016). However, the number of *Lactobacillus* sp., *Enterococcus* sp., and Enterobacteriaceae in fecal microbial population of nursery pigs were not influenced by providing probiotics to sows and/or nursery pigs. Bacillus-based probiotics as the *B subtilis* C-3102 used in the present study are Gram-positive, spore-forming bacteria which the main mode of action is through the production of enzymes subtilisin and catalase to create a favorable environment for growth and colonization of beneficial bacteria in the gastrointestinal tract, particularly the *Lactobacillus* sp. (Buchanan et al., 1974; Hosoi et al., 2000). However, the probiotic did not elicit an increase in number of *Lactobacillus* sp. in the feces of nursery pigs in the present study, which might have contributed to the lack of probiotic effect on growth performance and fecal consistency of nursery pigs in the present study.

In conclusion, providing a probiotic based on viable spores of *Bacillus subtilis* C-3102 to sows during gestation and lactation and to progeny during nursery did not elicit noteworthy improvements in performance or fecal consistency. The most notable benefit was seen as an improvement of sow lactation feed intake. Interestingly, fecal microbial analysis indicated the establishment of maternal-progeny intestinal microbiota relationship and its modulation by providing the probiotic to sows. In the pre-weaning period, pigs born from probiotic-fed sows displayed a similar fecal microbial population as sows, but no influence on performance. In the

post-weaning period, however, pigs from probiotic-fed sows demonstrated reduced growth rate and feed consumption in late nursery. Therefore, there seems to be a long-term influence of sow probiotic supplementation on progeny through the nursery that warrants further investigations.

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Table 4.1 Compositions of gestation and lactation diets (as-fed basis)[1]

Item	Gestation	Lactation
Ingredient, %		
Corn	80.7	63.4
Soybean meal, 47% crude protein	15.6	30.6
Choice white grease	---	2.50
Calcium carbonate	1.15	0.90
Monocalcium phosphate, 21.5% phosphorus	1.40	1.05
Sodium chloride	0.50	0.50
L-Lysine HCl	---	0.20
DL-Methionine	---	0.05
L-Threonine	0.03	0.10
L-Valine	---	0.05
Vitamin premix[2]	0.50	0.50
Trace mineral premix[3]	0.15	0.15
Phytase[4]	0.02	0.02
Probiotic[5]	+/-	+/-
Total	100.0	100.0
Calculated analysis		
SID[6] amino acids, %		
Lysine	0.56	1.08
Isoleucine:lysine	86	67
Leucine: lysine	209	139
Methionine:lysine	38	30
Methionine and cysteine:lysine	76	56
Threonine:lysine	79	67
Tryptophan:lysine	24	20
Valine:lysine	99	78
Net energy, kcal/kg	2,476	2,524
Crude protein, %	14.1	20.1
Calcium, %	0.85	0.75
STTD[7] phosphorus, %	0.48	0.44

[1] Gestation diets were fed from d 30 of gestation until farrowing and lactation diets were fed from farrowing until weaning on d 19 of lactation. Diets were fed in meal form.

[2] Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 22,455 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin B_{12}; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 88 mg biotin; 661 mg folic acid; 1,984 mg pyridoxine; 220,460 mg choline; 19,841 mg carnitine.

[3] Provided per kg of premix: 73 g Zn from zinc sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite; 0.08 g chromium picolinate.

[4] Ronozyme® HiPhos (DSM Nutritional Products, Inc., Parsippany, NJ) provided 405 FTU/kg of feed.

[5] Calsporin® 1.0B (Calpis Co. Ltd., Tokyo, Japan) provided viable spores of *Bacillus subtilis* C-3102 at 1×10^9 CFU/g of product. In gestation, it was top dressed in probiotic diets to achieve 500,000 CFU/g of feed. In lactation, it was included in probiotic diets to achieve 1,000,000 CFU/g of feed (0.10% inclusion rate). Calsporin® 1.0B was included in the diets at the expense of corn.

+/- Indicates inclusion in probiotic diets and absence of inclusion in control diets.

[6] SID = standardized ileal digestible.

[7] STTD = standardized total tract digestible.

Table 4.2 Compositions of nursery diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn	43.0	55.2	60.8
Soybean meal, 47% crude protein	18.8	24.8	34.6
Whey powder, 11.5% crude protein	25.0	10.0	---
Fish meal, 63% crude protein	4.50	---	---
Enzyme-treated soybean meal[2]	2.50	5.00	---
Choice white grease	3.00	1.00	1.00
Calcium carbonate	0.40	0.73	0.85
Monocalcium phosphate, 21.5% phosphorus	0.60	1.10	1.00
Sodium chloride	0.30	0.55	0.60
L-Lysine-HCl	0.45	0.45	0.35
DL-Methionine	0.22	0.22	0.15
L-Threonine	0.20	0.19	0.14
L-Tryptophan	0.05	0.03	0.01
L-Valine	0.15	0.10	0.04
Vitamin premix[3]	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15
Vitamin E, 20,000 IU	0.05	---	---
Choline chloride 60%	0.04	---	---
Phytase[5]	0.02	0.02	0.02
Zinc oxide	0.39	0.25	---
Probiotic[6]	+/-	+/-	+/-
Total	100.0	100.0	100.0
Calculated analysis			
SID[7] amino acids, %			
Lysine	1.40	1.35	1.30
Isoleucine:lysine	55	58	61
Leucine: lysine	107	115	124
Methionine:lysine	37	37	34
Methionine and cystine:lysine	56	58	57
Threonine:lysine	63	63	63
Tryptophan:lysine	19.3	19.1	19.0
Valine:lysine	69	69	69
Histidine:lysine	31	36	40
Net energy, kcal/kg	2,632	2,485	2,443
Crude protein, %	20.5	21.1	22.1
Calcium, %	0.75	0.70	0.70
STTD[8] phosphorus, %	0.49	0.43	0.36

[1] Nursery diets were fed in three dietary phases: phase 1, from d 0 to 7 after weaning in pellet form; phase 2, from d 7 to 21 in meal form; and phase 3, from d 21 to 42 in meal form.

[2] HP 300 (Hamlet Protein, Inc., Findlay, OH), 56% crude protein.

[3] Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin B_{12}; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[4] Provided per kg of premix: 73 g Zn from zinc sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[5] Ronozyme® HiPhos (DSM Nutritional Products, Inc., Parsippany, NJ) provided 405 FTU/kg of feed.

[6] BacPack ABF™ (Quality Technology International, Inc., Elgin, IL) provided viable spores of *Bacillus subtilis* C-3102 at 1×10^9 CFU/g of product and proprietary amounts of yeast cell wall derivatives. BacPack ABF™ was included in the diets to achieve 500,000 CFU/g of feed (0.05% inclusion rate) at the expense of corn.

+/- Indicates inclusion in probiotic diets and absence of inclusion in control diets.

[7] SID = standardized ileal digestible.

[8] STTD = standardized total tract digestible.

Table 4.3 Chemical analysis of sow diets (as-fed basis)[1]

		Lactation	
Item	Gestation	Control	Probiotic
Proximate analysis, %			
Dry matter	88.1	88.9	88.7
Crude protein	13.1	20.2	20.2
Acid detergent fiber	2.7	3.0	2.8
Neutral detergent fiber	8.2	7.6	7.4
Ether extract	2.3	5.0	5.1
Calcium	1.30	1.05	1.12
Phosphorus	0.64	0.63	0.64
Bacillus subtilis C-3102, CFU/g	*	3.0×10^3	1.1×10^6

[1] Diet samples were collected at manufacturing and composite samples were submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and quantification of *Bacillus subtilis* C-3102 (Calpis America, Inc., Peachtree City, GA).

* Top dress analysis contained *Bacillus subtilis* C-3102 at 5.1×10^3 CFU/g of control top dress and 2.2×10^7 CFU/g of probiotic top dress.

Table 4.4 Chemical analysis of nursery diets (as-fed basis)[1]

	Phase 1		Phase 2		Phase 3	
Item	Control	Probiotic	Control	Probiotic	Control	Probiotic
Proximate analysis, %						
Dry matter	91.3	91.1	89.7	89.6	88.4	88.1
Crude protein	19.6	19.9	20.6	20.9	21.7	20.9
Acid detergent fiber	2.1	2.4	2.9	2.6	4.1	3.8
Neutral detergent fiber	5.1	5.5	6.5	6.4	7.4	9.3
Ether extract	4.8	4.9	3.1	3.0	3.3	2.8
Calcium	1.11	1.05	0.90	0.92	0.92	0.95
Phosphorus	0.69	0.73	0.69	0.68	0.60	0.60
Bacillus subtilis C-3102, CFU/g	1.3×10^4	4.0×10^5	3.4×10^4	5.0×10^5	5.2×10^4	5.4×10^5

[1] Diet samples were collected at manufacturing and composite samples were submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE) and quantification of *Bacillus subtilis* C-3102 (Calpis America, Inc., Peachtree City, GA).

Table 4.5 Effect of providing *Bacillus subtilis* C-3102 during gestation and lactation on sow and piglet performance until weaning[1]

Item[2]	Control	Probiotic[3]	SEM	Probability, *P* <
Count, n	14	15	---	---
Parity	1.9	2.0	0.26	0.319
Gestation length, d	115.3	115.2	0.23	0.787
Lactation length, d	19.4	19.4	0.29	0.973
Sow BW, kg				
d 30 gestation	200.7	200.2	6.94	0.803
d 112 gestation	243.1	236.6	8.76	0.145
Post-farrow	223.6	218.9	7.74	0.218
Wean	220.2	217.0	7.69	0.366
Change, farrow to wean	-4.3	-1.9	1.87	0.377
Sow ADFI, kg				
Gestation	2.4	2.4	0.09	0.944
Lactation	5.7	6.2	0.24	0.057
Total born, n	15.5	16.8	0.95	0.201
Born alive, n	14.1	14.5	0.72	0.624
Stillborn and mummy, n	1.4	2.3	0.59	0.228
Born alive, %	90.9	86.1	2.18	0.135
Stillborn, %	8.2	10.3	1.92	0.450
Piglet BW, kg				
Birth	1.41	1.38	0.05	0.664
d 2	1.65	1.56	0.06	0.276
d 12	3.88	3.93	0.14	0.755
Wean	5.74	5.85	0.21	0.601
Piglet ADG, g	222	231	9.79	0.316
Litter weight, kg				
Birth	20.1	19.7	1.16	0.722
d 2	22.1	21.5	0.91	0.626
d 12	49.2	50.3	2.17	0.730
Wean	72.6	73.8	3.08	0.755
Litter size, n				
d 2[4]	13.3	13.8	0.24	0.060
d 12	12.6	12.8	0.31	0.719
Wean	12.7	12.7	0.32	0.916
Pre-wean mortality, %	10.5	12.4	2.24	0.557

[1] A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used in the sow portion of this study. Dietary treatments were fed to sows from d 30 of gestation until weaning at approximately d 19 of lactation.

[2] BW = body weight; ADFI = average daily feed intake; ADG = average daily gain.

[3] Probiotic diet was supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed and 1,000,000 CFU/g of lactation feed.

[4] Piglets were cross-fostered within sow treatment group to equalize litter size within 24 hours of birth.

Table 4.6 Interactive effects of sow and nursery dietary treatments on growth performance of nursery pigs[1,2]

Sow treatment[3]	Control		Probiotic			Probability, $P <$		
Nursery treatment[4]	Control	Probiotic	Control	Probiotic	SEM	Sow treatment × nursery treatment	Sow treatment	Nursery treatment
Item[5]								
BW, kg								
d 0	5.8	5.8	5.9	5.9	0.01	0.995	0.001	0.547
d 7	6.3	6.4	6.3	6.4	0.05	0.350	0.940	0.114
d 21	10.8	10.9	10.9	10.7	0.17	0.441	0.677	0.795
d 42	24.0	23.9	23.3	23.1	0.34	0.841	0.042	0.707
Phase 1 (d 0 to 7)								
ADG, g	62	82	63	69	7.49	0.333	0.418	0.084
ADFI, g	113	117	117	119	7.42	0.853	0.704	0.681
G:F, g/kg	506	691	552	550	54.35	0.088	0.383	0.093
Phase 2 (d 7 to 21)								
ADG, g	314	317	323	308	10.18	0.359	0.986	0.560
ADFI, g	435	445	451	427	11.85	0.151	0.959	0.549
G:F, g/kg	720	712	716	722	11.49	0.562	0.764	0.905
Phase 3 (d 21 to 42)								
ADG, g	627	612	594	588	9.89	0.628	0.005	0.293
ADFI, g	931	924	886	880	16.46	0.980	0.008	0.702
G:F, g/kg	674	662	672	669	4.24	0.293	0.679	0.084
Overall (d 0 to 42)								
ADG, g	424	422	414	407	8.30	0.755	0.135	0.535
ADFI, g	623	624	612	600	12.32	0.595	0.146	0.661
G:F, g/kg	681	675	678	678	3.83	0.491	0.998	0.478

[1] A total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of this study. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment.

[2] Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[3] Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 of lactation).

[4] Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 CFU/g of nursery feed.

[5] BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

Table 4.7 Main effects of sow and nursery dietary treatment on growth performance of nursery pigs[1,2]

	Sow treatment[3]			Probability,	Nursery treatment[4]			Probability,
Item[5]	Control	Probiotic	SEM	*P* <	Control	Probiotic	SEM	*P* <
BW, kg								
d 0	5.8	5.9	0.01	0.001	5.9	5.9	0.01	0.547
d 7	6.3	6.3	0.04	0.940	6.3	6.4	0.04	0.114
d 21	10.8	10.8	0.12	0.677	10.8	10.8	0.12	0.795
d 42	23.9	23.2	0.24	0.042	23.7	23.5	0.24	0.707
Phase 1 (d 0 to 7)								
ADG, g	72	66	5.30	0.418	62	75	5.23	0.084
ADFI, g	115	118	5.25	0.704	115	118	5.18	0.681
G:F, g/kg	598	551	38.43	0.383	529	621	37.92	0.093
Phase 2 (d 7 to 21)								
ADG, g	316	315	7.20	0.986	318	313	7.10	0.560
ADFI, g	440	439	8.38	0.959	443	436	8.27	0.549
G:F, g/kg	716	719	8.13	0.764	718	717	8.02	0.905
Phase 3 (d 21 to 42)								
ADG, g	619	591	7.00	0.005	610	600	6.90	0.293
ADFI, g	928	883	11.64	0.008	908	902	11.48	0.702
G:F, g/kg	668	670	3.00	0.679	673	665	2.96	0.084
Overall (d 0 to 42)								
ADG, g	423	410	5.87	0.135	419	414	5.79	0.535
ADFI, g	624	606	8.71	0.146	617	612	8.59	0.661
G:F, g/kg	678	678	2.71	0.998	679	677	2.67	0.478

[1] A total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of this study. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment.

[2] Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[3] Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 of lactation).

[4] Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 CFU/g of nursery feed.

[5] BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

Table 4.8 Effects of providing *Bacillus subtilis* C-3102 during gestation and lactation on sow fecal microbes[1, 2]

	d 30 Gestation		d 113 Gestation		d 18 Lactation		Probability, $P <$		
Item[3]	Control	Probiotic[4]	Control	Probiotic[4]	Control	Probiotic[4]	Treatment × day	Treatment	Day
Bacillus subtilis C-3102, log_{10} CFU/g	3.13[c]	4.69[b]	1.76[d]	6.14[a]	2.69[c]	6.20[a]	0.003	0.001	0.031
SEM	0.39	0.37	0.17	0.16	0.19	0.18			
Detected/sampled, n	8/10	10/10	2/14	15/15	9/14	15/15			
Total *Bacillus* sp., log_{10} CFU/g	4.86[c]	5.32[b]	4.86[c]	6.16[a]	4.25[d]	6.22[a]	0.001	0.001	0.001
SEM	0.11	0.10	0.05	0.05	0.05	0.05			
Detected/sampled, n	10/10	10/10	14/14	15/15	14/14	15/15			
Lactobacillus sp., log_{10} CFU/g	7.09	7.17	7.38	6.30	8.52	8.37	0.109	0.184	0.001
SEM	0.22	0.21	0.41	0.40	0.17	0.17			
Detected/sampled, n	10/10	10/10	14/14	13/15	14/14	15/15			
Clostridium perfringens, log_{10} CFU/g	8.06	8.01	7.93	7.55	6.14	6.02	0.351	0.196	0.001
SEM	0.08	0.07	0.13	0.13	0.24	0.23			
Detected/sampled, n	10/10	10/10	14/14	15/15	14/14	15/15			
Enterobacteriaceae, log_{10} CFU/g	7.41	7.56	7.30	7.43	6.69	6.45	0.411	0.951	0.001
SEM	0.19	0.18	0.16	0.16	0.25	0.24			
Detected/sampled, n	10/10	10/10	14/14	15/15	14/14	15/15			
Total aerobes, log_{10} CFU/g	8.23	8.60	8.32	8.32	8.69	8.38	0.117	0.869	0.368
SEM	0.17	0.16	0.16	0.16	0.14	0.13			
Detected/sampled, n	10/10	10/10	14/14	15/15	14/14	15/15			
Total anaerobes, log_{10} CFU/g	9.11	9.20	9.07	8.92	9.35	9.25	0.250	0.437	0.001
SEM	0.09	0.08	0.08	0.08	0.07	0.06			
Detected/sampled, n	10/10	10/10	14/14	15/15	14/14	15/15			

[1] A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used in the sow portion of this study. Dietary treatments were fed to sows from d 30 of gestation until weaning at approximately d 19 of lactation.

[2] Fecal samples were freshly collected from sows by rectal grab on d 30 of gestation (baseline), d 112 of gestation (pre-farrowing), and d 18 of lactation (pre-weaning). Microbial analysis was performed by isolation and enumeration method.

[3] Limit of detection was 2×10^2 CFU/g.

[4] Probiotic diet was supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 lactation).

[abcd] Indicate significant difference ($P < 0.05$) in the row.

Table 4.9 Effects of providing *Bacillus subtilis* C-3102 during gestation and lactation on nursing piglet fecal microbes[1,2]

	d 2 Lactation		d 18 Lactation		Probability, $P <$		
Item[3]	Control	Probiotic[4]	Control	Probiotic[4]	Treatment × day	Treatment	Day
Bacillus subtilis C-3102, $\log_{10}$ CFU/g	2.44[b]	2.95[b]	2.51[b]	5.39[a]	0.001	0.001	0.001
SEM	0.36	0.35	0.22	0.21			
Detected/sampled, n	5/13	9/14	7/13	14/14			
Total *Bacillus* sp., $\log_{10}$ CFU/g	5.83[ab]	6.28[a]	3.39[c]	5.41[b]	0.007	0.001	0.001
SEM	0.30	0.29	0.20	0.19			
Detected/sampled, n	13/13	14/14	11/13	14/14			
Lactobacillus sp., $\log_{10}$ CFU/g	6.91[b]	7.84[ab]	8.38[a]	8.06[a]	0.030	0.342	0.005
SEM	0.41	0.40	0.12	0.12			
Detected/sampled, n	12/13	14/14	13/13	14/14			
Enterococcus sp., $\log_{10}$ CFU/g	9.70	9.92	9.74	9.64	0.156	0.583	0.267
SEM	0.13	0.13	0.08	0.08			
Detected/sampled, n	13/13	14/14	13/13	14/14			
Clostridium perfringens, $\log_{10}$ CFU/g	8.83	9.02	8.53	8.60	0.750	0.484	0.063
SEM	0.18	0.17	0.20	0.19			
Detected/sampled, n	13/13	14/14	13/13	14/14			
Enterobacteriaceae, $\log_{10}$ CFU/g	9.33	9.28	8.35	8.40	0.623	0.983	0.001
SEM	0.11	0.10	0.14	0.13			
Detected/sampled, n	13/13	14/14	13/13	14/14			
Total aerobes, $\log_{10}$ CFU/g	8.24	8.23	6.77	6.64	0.849	0.810	0.001
SEM	0.15	0.14	0.43	0.41			
Detected/sampled, n	13/13	14/14	13/13	14/14			
Total anaerobes, $\log_{10}$ CFU/g	9.42	9.44	8.64	8.57	0.691	0.803	0.001
SEM	0.11	0.10	0.12	0.12			
Detected/sampled, n	13/13	14/14	13/13	14/14			

[1] A total of 29 sows (DNA 241, DNA Genetics, Columbus, NE) and litters (367 piglets DNA 241 × 600, DNA Genetics, Columbus, NE) were used in the sow portion of this study. Dietary treatments were fed to sows from d 30 of gestation until weaning at approximately d 19 of lactation.

[2] Fecal samples were freshly collected from piglets with sterile mini cotton tip swabs on d 2 (postnatal) and 18 of lactation (pre-weaning). Microbial analysis was performed by isolation and enumeration method.

[3] Limit of detection was 2×10^2 CFU/g.

[4] Probiotic diet was supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 lactation).

[abc] Indicate significant difference ($P < 0.05$) in the row.

Table 4.10 Effects of sow and nursery dietary treatment on nursery pigs fecal microbes[1,2]

	d 21 Nursery				d 42 Nursery			
Sow treatment[3]	Control		Probiotic		Control		Probiotic	
Nursery treatment[4]	Control	Probiotic	Control	Probiotic	Control	Probiotic	Control	Probiotic
Item[5]								
Bacillus subtilis C-3102, log_{10} CFU/g	2.67	5.57	3.38	5.52	3.54	5.81	2.45	5.75
SEM	0.20	0.20	0.20	0.20	0.25	0.25	0.25	0.25
Detected/sampled, n	4/6	6/6	6/6	6/6	6/6	6/6	3/6	6/6
Total *Bacillus* sp., log_{10} CFU/g	3.96	5.60	4.09	5.55	4.11	5.85	4.18	5.78
SEM	0.16	0.16	0.16	0.16	0.09	0.09	0.09	0.09
Detected/sampled, n	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Lactobacillus sp., log_{10} CFU/g	9.14	9.05	8.90	9.12	8.94	8.69	8.96	8.85
SEM	0.09	0.09	0.09	0.09	0.11	0.11	0.11	0.11
Detected/sampled, n	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Enterococcus sp., log_{10} CFU/g	3.97	4.23	4.05	4.45	4.47	4.76	4.94	5.13
SEM	0.53	0.53	0.53	0.53	0.52	0.52	0.52	0.52
Detected/sampled, n	6/6	5/6	6/6	5/6	6/6	5/6	6/6	6/6
Enterobacteriaceae, log_{10} CFU/g	7.58	6.71	7.22	7.57	7.49	7.44	7.26	7.43
SEM	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.23
Detected/sampled, n	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Total aerobes, log_{10} CFU/g	9.62	9.64	9.42	9.53	9.76	9.59	9.65	9.55
SEM	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Detected/sampled, n	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Total anaerobes, log_{10} CFU/g	10.25	10.13	10.14	10.22	10.21	10.09	10.05	10.13
SEM	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05
Detected/sampled, n	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6

[1] A total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of this study. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment. Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[2] Fecal samples were freshly collected from piglets with sterile cotton tip swabs on d 21 and 42 of nursery. Microbial analysis was performed by isolation and enumeration method.

[3] Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 of lactation).

[4] Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 CFU/g of nursery feed.

[5] Limit of detection was 2×10^2 CFU/g.

Table 4.11 Probability of interactions and main effects of sow dietary treatment, nursery dietary treatment, and day in nursery on nursery pigs fecal microbes[1,2,3]

Item	Sow treatment × nursery treatment × day	Sow treatment × nursery treatment	Sow treatment × day	Nursery treatment × day	Sow treatment	Nursery treatment	Day
Bacillus subtilis C-3102	0.009	0.695	0.009	0.399	0.460	0.001	0.509
Total *Bacillus* sp.	0.912	0.337	0.832	0.525	0.824	0.001	0.082
Lactobacillus sp.	0.538	0.146	0.223	0.090	0.974	0.443	0.012
Enterococcus sp.	0.862	0.979	0.689	0.897	0.486	0.487	0.068
Enterobacteriaceae	0.122	0.057	0.230	0.300	0.716	0.578	0.379
Total aerobes	0.931	0.419	0.372	0.036	0.022	0.407	0.071
Total anaerobes	0.868	0.012	0.383	0.999	0.364	0.568	0.039

[1] A total of 358 weaned pigs (DNA 241 × 600, DNA Genetics, Columbus, NE), progeny of the sows on study, were used for the nursery portion of this study. Weaned pigs were approximately 19 d of age, on average 5.9 kg initial BW, and were used in a 42-d nursery study beginning at weaning with 4 or 5 pigs per pen and 18 or 19 replicates per treatment. Pigs were assigned to pens and pens were assigned to dietary treatments in a split-plot design. Sow dietary treatment (control diet or probiotic diet) served as main plot and nursery dietary treatment (control diet or probiotic diet) as subplot.

[2] Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 of lactation).

[3] Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 CFU/g of nursery feed.

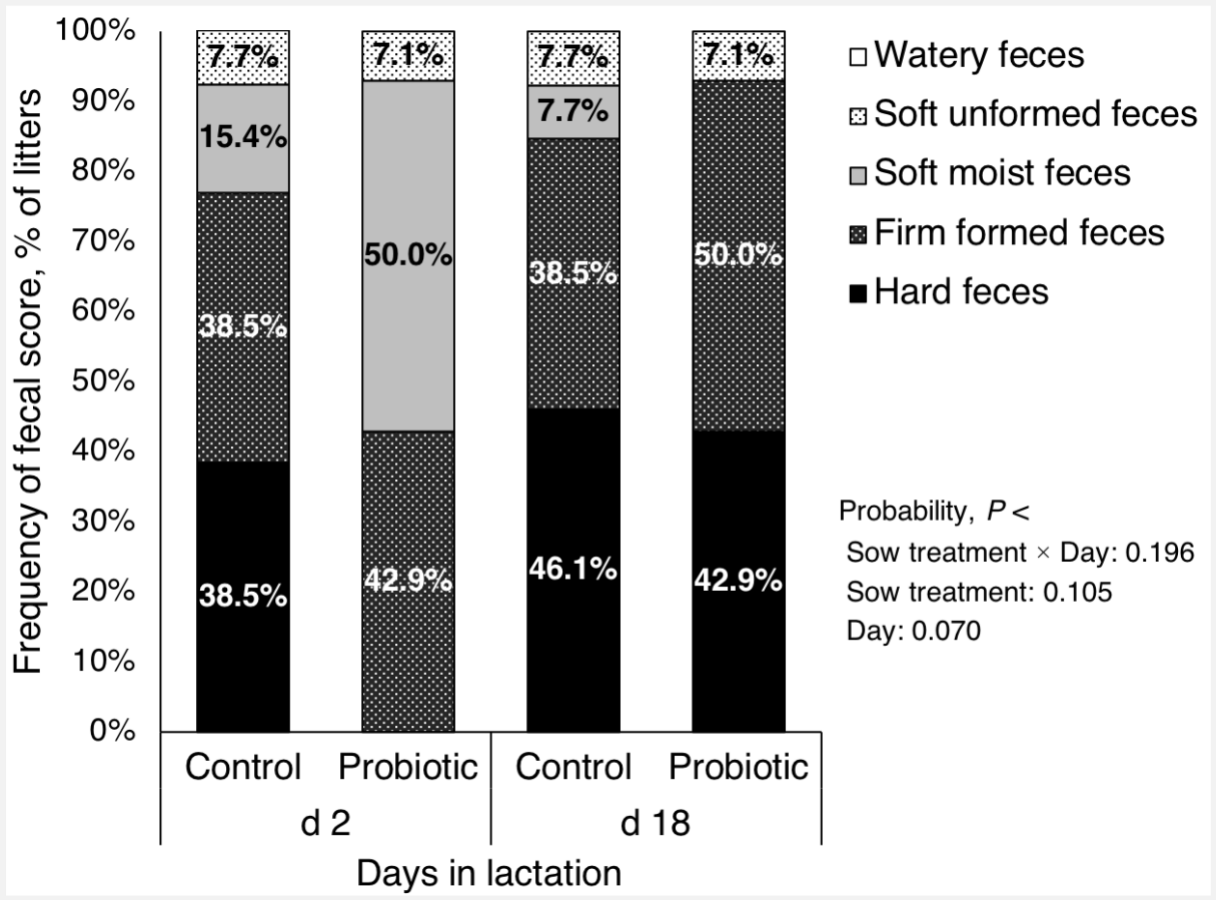

Figure 4.1 Effect of providing *Bacillus subtilis* C-3102 during gestation and lactation on fecal consistency of nursing piglets assessed by fecal score.

Graph bars show the frequency distribution of litters (n = 27 litters) within each fecal score category on days 2 and 18 of lactation according to sow dietary treatment. Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 of lactation).

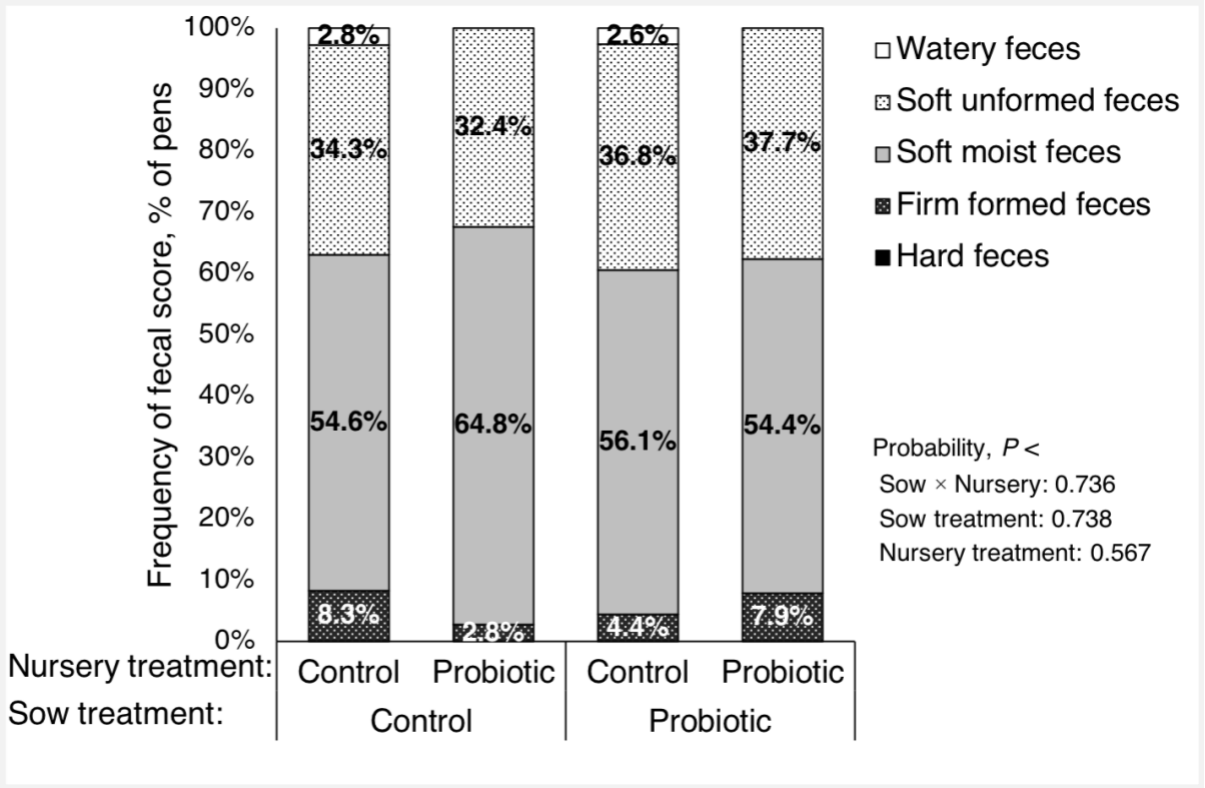

Figure 4.2 Effects of sow and nursery pig dietary treatment on fecal consistency of nursery pigs assessed by fecal score.

Graph bars show the frequency distribution of pens (n = 74 pens) within each fecal score category according to sow dietary treatment and nursery dietary treatment regardless of day in nursery. Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with a probiotic product containing viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis Co. Ltd., Tokyo, Japan) to achieve 500,000 CFU/g of gestation feed (d 30 gestation to farrowing) and 1,000,000 CFU/g of lactation feed (farrowing to d 19 of lactation). Nursery dietary treatments consisted of providing a control diet or a probiotic diet supplemented with viable spores of *Bacillus subtilis* C-3102 and prebiotics (BacPack ABF™, Quality Technology International, Inc., Elgin, IL) to nursery pigs to achieve 500,000 CFU/g of nursery feed.

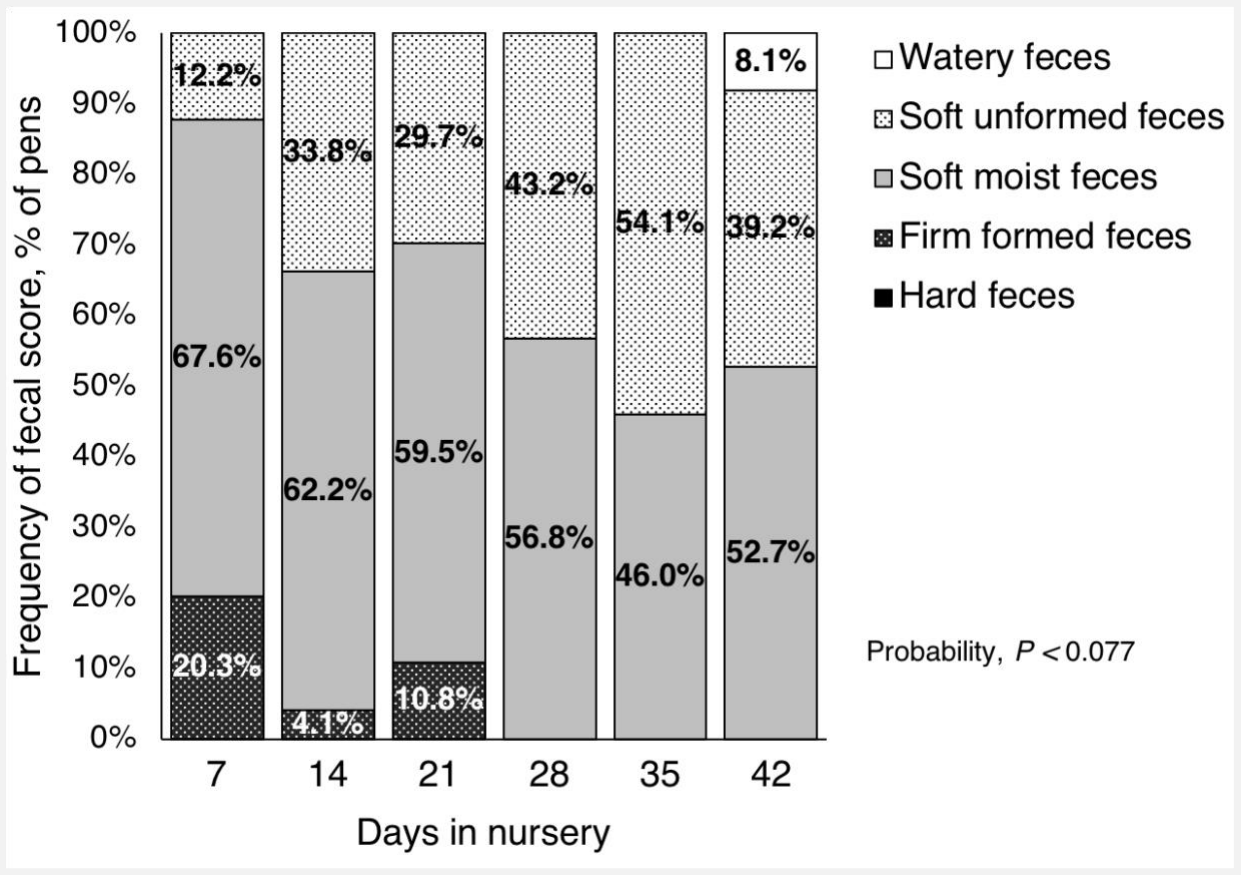

Figure 4.3 Effects of days into the nursery on fecal consistency of nursery pigs assessed by fecal score.

Graph bars show the frequency distribution of pens (n = 74 pens) within each fecal score category according to day in nursery regardless of dietary treatment.

Chapter 5 - Impact of added copper, alone or in combination with a feed-grade antimicrobial, on growth performance of nursery pigs

ABSTRACT: This study aimed to characterize the effect of added copper (**Cu**), alone or in combination with a feed-grade antimicrobial, on growth performance of nursery pigs. A total of 320 weaned barrows (DNA 200 × 400) initially 7.4 kg body weight (**BW**) were used in a 28-d study. Pigs were weaned at approximately 21 d of age and fed a common non-medicated diet for 7 d after weaning and then assigned to treatments in a randomized complete block design based on initial BW. Treatments were arranged in a 2 × 2 factorial with main effects of added Cu (0 vs. 200 mg/kg Cu from Cu sulfate) and chlortetracycline (0 vs. 440 mg/kg **CTC**). Diets were corn-soybean meal-based and fed in meal form. There were 5 pigs per pen and 8 replications per treatment with each replication consisting of a pair of adjoining pens. Growth performance was assessed on d 0, 14, and 28 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed (**G:F**). Data were analyzed using a linear mixed model with treatment as main effect and block as random effect. There was no evidence ($P > 0.05$) for a Cu by CTC interaction in growth performance throughout the study. Pigs fed diets with added Cu had increased ($P < 0.05$) ADG and ADFI from d 0 to 14, with no evidence for differences ($P > 0.05$) from d 14 to 28 and 0 to 28. Pigs fed diets with CTC had improved ($P < 0.01$) growth performance from d 0 to 14 and 14 to 28, leading to improved ADG, ADFI, and G:F from d 0 to 28. In conclusion, the results of this study show that supplementation of Cu or CTC in nursery diets exerts growth promotional effects on weaned pigs. The lack of interactive effects between Cu and CTC suggests the responses of Cu and CTC on growth performance of nursery pigs are as efficacious when fed alone or in combination.

Key words: chlortetracycline, growth promotion, swine, weaning

INTRODUCTION

Copper (**Cu**) is an essential chemical element required for basic cellular metabolism in eukaryotic and prokaryotic cells. In swine, Cu is included in trace amounts in diets (5 to 20 mg/kg) as part of a multi-mineral supplement to meet nutritional requirements (NRC, 2012). At elevated (pharmacological) concentrations in the diet, generally at 100 to 250 mg/kg, Cu has growth promotional benefits, which are attributed to alteration of gut microflora, retention of nutrients, reduction in gut fermentation losses, and reduced morbidity and mortality, particularly in weaned piglets (Hojberg et al., 2005). However, the exact mode of action of Cu on growth promotion remains unclear.

Studies performed in the 1980s have shown that pharmacological concentrations of Cu are effective even in combination with feed-grade antimicrobials (Stahly et al., 1980; Burnell et al., 1988). Recent studies on pharmacological additions of Cu have focused on sources and levels of Cu (Shelton et al., 2011; Ma et al., 2015; Carpenter et al., 2018), the interactive effect of Cu and Zn (Hill et al., 2000; Pérez et al., 2011), and Cu-associated antimicrobial resistance (Amachawadi et al., 2015). However, current studies on the addition of Cu in combination with feed-grade antimicrobials are scarce. In view of the potential similarity of modes of action and the perspectives on limiting the use of feed-grade antimicrobials, it is of interest to compare the effects of Cu and antimicrobials and to determine whether there are interactive or additive responses on growth performance of nursery pigs.

Therefore, the objective of this study was to characterize the effect of added Cu, alone or in combination with a feed-grade antimicrobial, on growth performance of nursery pigs.

MATERIALS AND METHODS

Animals, experimental design, and treatments

The Kansas State University Animal Care and Use Committee approved the use of animals and the experimental procedures for this research. The study was conducted at Kansas State University's Segregated Early Weaning Swine facility. A total of 320 weanling barrows (DNA 200 × 400, DNA Genetics, Columbus, NE) were used in a 28-d study. Pig were weaned at 21 days of age with an average initial body weight (**BW**) of 7.4 kg and were allocated into 64 pens with 5 pigs per pen (0.30 m^2 per pig) distributed in two barns. Specifically, each barn housed 32 pens that were oriented in 4 rows with 8 pens in each row. Row one adjoined row two and row three adjoined row four creating two 16-pen blocks per barn. Per 16-pen block, there were 8 pairs of pens that allowed pigs to make contact through vertical bars between rows and restricted within-row pig contact using solid polyvinyl boards. The availability of contact between rows resulted in the experimental unit expressed as a pair of pens. Therefore, each barn housed 16 pairs of pens, equaling a total of 16 experimental units per barn and totaling 32 experimental units in the study.

Treatments were arranged in a 2 × 2 factorial with main effects of added Cu (0 vs. 200 mg/kg Cu from Cu sulfate) and chlortetracycline (0 vs. 440 mg/kg **CTC**). Pairs of pens were randomly assigned to one of four treatments with a total of 8 replications per treatment. Treatment allocation followed a block design to ensure all treatments had equal adjacent contact with other treatments. Dietary treatments consisted of: a control group fed a basal diet with level of Cu to meet the NRC (2012) requirements of nursery pigs (17 mg/kg) and no antibiotic supplementation; a Cu-supplemented group fed the basal diet with 200 mg/kg added Cu from Cu

sulfate; an antibiotic-supplemented group fed the basal diet with CTC at 440 mg/kg (22 mg/kg BW); and a Cu and CTC group fed the basal diet with 200 mg/kg added Cu from Cu sulfate and CTC at 440 mg/kg.

Diets were corn-soybean meal-based formulated to meet or exceed the nutrient requirements of nursery pigs according to the NRC (2012) and fed in meal form (Table 5.1). Pigs were fed a common non-medicated pelleted diet for 7 d after weaning and then assigned to treatment diets. Ad libitum access to feed and water was provided in each pen with a four-hole, dry self-feeder and a nipple water. Treatments containing CTC were administered under two veterinary feed directives with the first fed from day 0 to 14 and the second fed from day 15 to 28 allowing for a one-day CTC withdrawal from day 14 to 15, thereby complying with the Food and Drug Administration (**FDA**) guidelines for CTC administration.

Pig and feeders were weighed on day 0, 14, and 28 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed (**G:F**).

Chemical analysis

Representative diet samples were obtained from all feeders and pooled by treatment in a composite sample. Samples were analyzed (Cumberland Valley Analytical Services, Inc., Waynesboro, PA) for dry matter (**DM**; method 935.29; AOAC International, 1990), crude protein (**CP**; method, 990.03; AOAC International, 1990), Ca (method 985.01; AOAC International, 1990), P (method 985.01; AOAC International, 1990), and Cu (method 925.56; AOAC International, 1990). Additionally, samples were submitted for CTC analysis (method 4438; FDA Laboratory Information Bulletin; Midwest Laboratories, Inc., Omaha, NE).

Statistical analysis

Data were analyzed using a linear mixed model with treatment as main effect and block as random effect. The pair of pens segregated by solid pen dividers served as the experimental unit. Preplanned contrast statements were built to evaluate the main effects and interactions of Cu and CTC. Statistical models were fitted using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

RESULTS

Analyzed Cu and CTC concentrations in diets reflected the addition of 200 mg/kg Cu and 440 mg/kg CTC to respective treatment diets (Table 5.1). Analyzed levels of DM, CP, Ca, and P were consistent with calculated values.

There was no evidence for Cu by CTC interaction in nursery growth performance throughout the study ($P > 0.05$), only main effects of Cu and CTC (Table 5.2). From day 0 to 14, pigs fed diets with added Cu had increased ($P < 0.05$) ADG and ADFI, and pigs fed diets with CTC had improved ($P < 0.01$) ADG, ADFI, and G:F. From day 14 to 28, there was no evidence ($P > 0.05$) for an effect of added Cu on growth performance, but pigs fed diets with CTC had increased ($P < 0.0001$) ADG and ADFI. For the overall treatment period, from day 0 to 28, there was no evidence ($P > 0.05$) for effect of added Cu on growth performance, but pigs fed diets with CTC had improved ($P < 0.01$) ADG, ADFI, and G:F. The addition of either Cu or CTC increased ($P < 0.05$) BW on day 14 and 28.

DISCUSSION

In swine, Cu is included in trace amounts (5 to 20 mg/kg) as part of a multi-mineral supplement in diets (NCR, 2012). At elevated, or pharmacological, levels of 100 to 250 mg/kg, Cu has growth promotional benefits. These are attributed to alteration of gut microflora, retention of nutrients, reduction in fermentation losses, and reduced morbidity and mortality, particularly in weanling pigs (Hojberg et al., 2005). The growth benefits of feeding added Cu to weaned pigs are well established (Cromwell et al., 1998; Hill et al., 2000; Ma et al., 2015; Bikker et al., 2016; Carpenter et al., 2018) and seem to be largely driven by increased feed intake, although efficiency of gain can also be improved (Stahly et al., 1980; Hill et al., 2000; Bikker et al., 2016). In the present study the addition of 200 mg/kg Cu from Cu sulfate improved rate of gain of nursery pigs by 8% during the initial period up to day 14, mostly driven by increased feed intake, and resulted in heavier pigs at the end of the 28-day feeding period, in agreement with recent study by Carpenter et al. (2018). Interestingly, the improvement in growth rate was mostly observed in the first 14-day period (7.5 to 11.5 kg BW) of Cu addition with no effect in the late (11.5 to 19.5 kg BW) or overall (7.5 to 19.5 kg BW) nursery periods.

The addition of in-feed antibiotics in nursery diets has also been shown to be efficacious at improving rate of gain and feed intake in weaned pigs (Stahly et al., 1980; Dritz et al., 2002; Williams et al., 2018; Puls et al., 2019). In the present study, the addition of 440 mg/kg CTC in nursery diets improved growth rate, feed intake, and efficiency of gain in the overall 28-day feeding period, resulting in heavier pigs at the end of the treatment period. The improvement in efficiency of gain has not been consistently associated with addition of in-feed antibiotics in nursery diets, as some studies have found improvement in feed efficiency similar to the present study (Stahly et al., 1980; Gottlob et al., 2004), whereas others have not (Dritz et al., 2002;

Williams et al., 2018; Puls et al., 2019). The lack of interactive effects between Cu and CTC suggests that the responses of Cu and CTC on growth performance of weaned pigs are as efficacious when added alone or in combination in nursery diets (Hill et al., 2001).

In conclusion, the results of this study show that 200 mg/kg added Cu or 440 mg/kg CTC in nursery dies exerts growth promotional effects on weaned pigs and are as efficacious when added alone or in combination in nursery diets.

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Table 5.1 Diet composition (as-fed basis)[1]

Item	Control	Cu	CTC	Cu + CTC
Ingredients, %				
Corn	56.05	55.97	55.62	55.54
Soybean meal, 47% crude protein	24.76	24.77	24.79	24.80
Dried whey	10.00	10.00	10.00	10.00
Enzyme-treated soybean meal[2]	5.00	5.00	5.00	5.00
Monocalcium phosphate, 21.5% phosphorus	1.20	1.20	1.20	1.20
Calcium carbonate	1.05	1.05	1.05	1.05
Sodium chloride	0.60	0.60	0.60	0.60
L-Lysine HCl	0.45	0.45	0.45	0.45
DL-Methionine	0.20	0.20	0.20	0.20
L-Threonine	0.20	0.20	0.20	0.20
L-Tryptophan	0.02	0.02	0.02	0.02
L-Valine	0.05	0.05	0.05	0.05
Trace mineral premix[3]	0.15	0.15	0.15	0.15
Vitamin premix[4]	0.25	0.25	0.25	0.25
Phytase[5]	0.02	0.02	0.02	0.02
Copper sulfate[6]	-	0.08	-	0.08
Chlortetracycline[7]	-	-	0.40	0.40
Total	100.0	100.0	100.0	100.0
Calculated analysis				
SID[8] amino acids, %				
Lysine	1.35	1.35	1.35	1.35
Isoleucine:lysine	57	57	57	57
Leucine:lysine	115	115	115	115
Methionine:lysine	36	36	35	35
Methionine and cysteine:lysine	57	57	57	57
Threonine:lysine	64	64	64	64
Tryptophan:lysine	18.5	18.5	18.5	18.5
Valine:lysine	65	65	65	65
NE, kcal/kg	2,436	2,434	2,425	2,423
Crude protein, %	21.1	21.1	21.1	21.1
Total calcium, %	0.79	0.79	0.79	0.79
STTD[9] phosphorus, %	0.53	0.53	0.53	0.53
Analyzed composition				
Dry matter, %	88.2	88.1	88.1	88.2
Crude protein, %	20.6	20.7	20.9	21.1
Calcium, %	0.85	0.91	0.93	0.91
Phosphorus, %	0.63	0.65	0.69	0.66
Copper, mg/kg	24	202	31	194
Chlortetracycline, mg/kg	ND[10]	0.06	539	309

[1] Diets were fed in meal form from 7.4 to 19.7 kg BW. Copper (Cu) sulfate and chlortetracycline (CTC) were included in the diet at the expense of corn.

[2] HP 300 (Hamlet Protein, Inc., Findlay, OH).

[3] Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[4] Provided per kg of premix: 3,527,399 IU vitamin A; 881,850 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[5] Ronozyme® Hiphos 2700 (DSM Nutritional Products, Inc., Basel, Switzerland) provided 476 phytase units per kg of feed.

[6] Copper sulfate provided 200 mg/kg of Cu.

[7] Aureomycin® 50 (Zoetis Services, LLC., Parsippany, NJ) provided 440 mg/kg CTC.

[8] SID = Standardized ileal digestible.

[9] STTD = Standardized total tract digestible.

[10] ND = Not detected above detection limit of 0.05 mg/kg.

Table 5.2 Effects of diets with or without added copper (Cu) and/or chlortetracycline (CTC) on growth performance of nursery pigs[1,2]

Cu, mg/kg[3]	0	200	0	200		Probability, $P <$		
CTC, mg/kg[4,5]	0	0	440	440	SEM	Cu × CTC	Cu	CTC
Day 0 to 14								
ADG, g	249	275	294	313	10.41	0.702	0.029	0.001
ADFI, g	345	374	378	401	10.22	0.755	0.013	0.005
G:F, g/kg	722	734	777	779	12.10	0.668	0.559	0.001
Day 14 to 28								
ADG, g	575	574	617	627	10.54	0.586	0.697	0.001
ADFI, g	857	856	910	934	14.68	0.332	0.386	0.001
G:F, g/kg	671	670	678	671	5.75	0.596	0.523	0.491
Day 0 to 28								
ADG, g	412	424	457	470	8.93	0.946	0.171	0.001
ADFI, g	602	614	647	668	10.97	0.686	0.122	0.001
G:F, g/kg	684	690	706	703	5.10	0.435	0.793	0.002
BW, kg								
Day 0	7.4	7.4	7.4	7.4	0.06	0.865	0.717	0.262
Day 14	10.9	11.3	11.5	11.8	0.15	0.702	0.011	0.001
Day 28	18.9	19.4	20.1	20.5	0.25	0.868	0.035	0.001

[1] A total of 320 weanling barrows (DNA 200 × 400, DNA Genetics, Columbus, NE) were used in a nursery study with 5 pigs per pen and 8 replications (pairs of pens) per treatment.

[2] Pig were weaned at 21 days of age and fed a common non-medicated diet for 7 days and then assigned to treatment diets for 28 days.

[3] Cu from Cu sulfate.

[4] CTC from Aureomycin® 50 (Zoetis Services, LLC., Parsippany, NJ).

[5] Treatments containing CTC were administered under two veterinary feed directives with the first fed from days 0 to 14 and the second fed from days 15 to 28 allowing for a one-day CTC withdrawal from day 14 to 15, thereby complying with the Food and Drug Administration guidelines.