THE EFFECTS OF A YEAST PROBIOTIC, *SACCHAROMYCES CEREVISIAE BOULARDII*, ON COMMON SOW STRESSORS AND THEIR EFFECT ON MATERNAL-FETAL PROGRAMMING

BY

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THESIS

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ABSTRACT

To date, antibiotics have been widely used in conventional animal medicine, as they are non-selective, as well as effective in killing pathogenic bacteria (Conly and Johnston, 2004). However, there is mounting concern over the increasing emergence of antibiotic-resistant bacteria due to the use of less innovative antibiotics; and moreover, the overuse of antibiotics in general (Llor and Bjerrum, 2014). Since antibiotics have been shown to kill-off beneficial bacteria in the gut, the development of possible alternatives to antibiotics has therefore come to the forefront as a solution to this agricultural-human health related issue. Maternal stress during late gestation may impair the development and reactivity of the sow's offspring which impacts disease susceptibility and mortality (Chu et al., 2016). The European-wide ban on antibiotics as growth promoters initially has had detrimental effects with reported increased morbidity and mortality in pigs, increases in enteric infections leading to increases in clinical diarrhea, and reduced weight gains (Hao et al., 2014). The utilization of probiotics as a method of promoting healthy gut bacteria may potentially restore the composition of the gut microbiome and may result in amelioration or prevention of gut inflammation (Hemarajata and Versalovic, 2012). Whether or not these potential alternatives are suitable to replace antibiotics as growth promoters or preventatives, or act as a co-product to these dietary supplements, is still unclear.

Feeding modified gestation diets, specifically the yeast probiotic, *Saccharomyces cerevisiae boulardii* (*Scb*), to sows throughout pregnancy could improve the immune status and stress responsiveness of both the dam and her progeny. The objectives of the sow study were to (1) assess the effects of the yeast probiotic treatment, *Scb*, on the immune status and cortisol levels of pregnant sows during late gestation (d 84 – d 112 of gestation); and to (2) assess the effects of the yeast probiotic treatment, *Scb*, on the stress responsiveness of sows at farrowing and weaning during the lactation period (d 112 – d 135). The objectives of the piglet study were to (1) assess the effects of feeding yeast probiotics, *Scb*, to sows during late gestation through lactation on the immune status of her piglets from birth till weaning (d 0 – d 21 of age); and to (2) assess the stress responsiveness of her piglets in response to farrowing and processing stressors, as well as the effects on the stress responsiveness of piglets to weaning stress in short- and long-term periods (d 21 – d 35 of age).

A total of eighteen pregnant sows, derived from the Genetiporc maternal line, were used in this study. Sows were randomly assigned to either the control treatment (CON-sows) or the

probiotic treatment (PRO-sows) starting on d 84 of gestation (baseline for the experiment). Sows were hand-fed either two boluses of the CON (placebo) or PRO (probiotic) daily at 0700 h starting on d 84 of gestation and ending on day of weaning (d 135). Sows were nose-snared to collect blood samples on gestational days 84, 91, 98, 105, and 112, and again on lactation days 115 (24 hours post-farrowing) and 135 (weaning). A total of eighty-four female piglets, born to sows derived of the Genetiporc maternal line were used in the piglet study (n = 84 piglets; 42 piglets/treatment). Fixed-effects parameters of treatment (control or probiotic-treated sows) and day of age for piglets were used for this study. Piglets were not directly fed the probiotic treatment during this research; rather, the maternal-fetal interaction was analyzed to assess the effects of the treatment given to the sows on the resulting immune status and stress responsiveness of the progeny.

When presented with a challenge, such as farrowing and weaning stress in sows or farrowing, processing, and weaning stress in piglets, the yeast probiotic, *Scb* was shown to have a treatment x day interaction ($p \leq 0.05$) on stress responsiveness, which included changes in immune status as well. The data imply that the increased immune status and stress response of the sows and piglets are interrelated in some cases, which can be changed with the inclusion of *Scb*. In general, few treatment x day interactions occurred for any measure assessed during gestation or lactation, except for plasma cortisol, natural killer cell cytotoxicity, neutrophil chemotaxis C5a and IL-8, Interleukin-12, and the leukocyte differential. Moreover, few treatment x day interactions occurred in piglets for any measure assessed during suckling or weaning, except for plasma cortisol, total white blood cell count, total neutrophil and lymphocyte counts, leukocyte differential, neutrophil phagocytosis, natural killer cell cytotoxicity, Interleukin-12, and LPS-induced mitogen proliferation.

Some aspects of innate immunity implied that the PRO-piglets' immune system was being affected by the *Scb* probiotic. The immune effects that occurred during the first 24-hours of age for the PRO-piglets were most likely due to the treatment that the probiotic-treated sows were given during late gestation. In addition, the immune effects that the PRO-piglets experienced from d 0 (birth) to d 7 of age were likely due to the PRO-sows' treatment during gestation and lactation. Therefore, it is possible that feeding PRO-sows the yeast probiotic supplementation during the periods of gestation and lactation, resulted in positive outcomes of both innate and adaptive immune responses.

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Upon enrolling as a graduate student, I was able to continue my studies in the field of immunophysiology and behavior, allowing me to further develop my passion for animal sciences, while sharpening my skills as a scientist in the laboratory as well. Having the opportunity to not only serve as a research assistant in the laboratory, but also as a teaching assistant to a course of 200 undergraduate students each spring semester, aided in the development of my public speaking, course development and organization, and professional networking skills and attributes. Therefore, not only did I have the opportunity to develop my research abilities, but I also had the distinct pleasure of furthering my character and leadership development.

Lastly, but most importantly, a very warm and loving "thank you" to my family. If it wasn't for their constant words of wisdom and encouragement, I would have never come to the realization that I had the potential to succeed in this academic field. As the youngest of five children, I have always had the distinct fortune of having the complete support of my parents (James and Mary Anne) and siblings (Sean, Derek, Ryan, and Mary) with me in times of success, and even in times of difficulty. After completing my sophomore year of college at the University of Illinois, I was lucky enough to travel to Ireland (the "old country" as my grandmother calls it) to see the place where my grandma and grandpa were born and raised, and where most of my mother's side of the family still resides. After experiencing farm life in Ireland that summer, I

came back to the U of I not as a Natural Resources and Environmental Sciences student as I had been before, but as an Animal Sciences major instead. Having had lost my Grandpa Kane when I was a senior in high school, I found that his legacy of hard work and determination still lives on in me. With that, I would like to dedicate this master's thesis, and the long hours of work and determination that went into it, to the man that I love and miss with each passing day. My decision to become an animal scientist stems from his love of farm animals (especially donkeys and horses, as you can probably imagine), and is the reason that I have accomplished what I have to date.

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INTRODUCTION

To date, antibiotics have been widely used in conventional animal medicine, as they are non-selective, as well as effective in killing pathogenic bacteria (Conly and Johnston, 2004). However, there is mounting concern over the increasing emergence of antibiotic-resistant bacteria due to the use of less innovative antibiotics; and moreover, the overuse of antibiotics in general (Llor and Bjerrum, 2014). The development of possible alternatives to antibiotics has therefore come to the forefront as a solution to this agricultural-human health related issue. The current use, and what is perceived by some as the misuse, of antibiotics in the food animal production industry is of particular interest to the accompanying literature review of research conducted on pig nutrition and management practices. It highlights a relatively new form of feed additive category, probiotics, which has the potential to supplement and/or replace antibiotics as a growth promotant or for immune enhancement. This review discusses the pros and cons of past and current antibiotic uses in animal agriculture, the pros and cons of the development and use of probiotics in the food-animal industry, as well as concerns associated with human and swine health and the possibility of emerging bacterial resistance.

The role of probiotics and antibiotics to maintain a healthy and balanced microbiota in swine production is of particular interest, including the use of a specific yeast probiotic, *Saccharomyces cerevisiae boulardii (Scb)*. Future research of alternatives to the use of antibiotics in animal agriculture may include the utilization of prebiotics, synbiotics, and/or phytogenics as well (Pandey et al., 2015). The gastrointestinal tract is of vital importance to the overall health and well-being of the animal, as it is the primary site of interaction between the host immune system, as well as both symbiotic and pathogenic microorganisms (Round and Mazmanian, 2014).With this in mind, research implies that direct-fed microbials provided in the diets of food animals intended for human consumption should meet four major criteria to be a viable alternative: (1) *treatment* of infectious diseases, (2) *control* of infectious diseases, (3) *prevention* of infectious diseases, and (4) antimicrobial compounds used for the *growth promotion* and *feed efficiency* of the food animals (FDA, 2012).

Probiotics are defined as living microbial adjuncts, which confer a health benefit onto the host, such as the prevention and treatment of pathological conditions (Czerucka et al., 2007).

Yeasts are a large and heterogeneous group of eukaryotic microorganisms, which are important when considering the probiotic properties of yeasts. These probiotics can elicit changes in the intestinal microbiota, and improve animal performance via host-specific alterations; however, the exact benefits that probiotics provide animals in production systems is unclear (O'Toole and Cooney, 2008). Potential mechanisms of *Scb* probiotics may include inhibition of certain bacterial toxins/subsequent pathogenic effects, trophic effects, anti-secretory activity, and immune-stimulatory effects on the intestinal mucosa (Stier and Bischoff, 2016).

There are vital factors that need to be considered when deciding which means of direct-fed microbial (antibiotics vs. probiotics in this case) is the most suitable practice to utilize in food animals. Cheng et al. (2014) and Cho et al. (2011) noted that reliable and efficient feed additives for animal production should result in a balanced gut microbiome that improves digestibility at a low cost to the producer, while reducing the impact (i.e., animal health, growth, and feed utilization) of unavoidable stressors. Increase in nutrient uptake and digestibility may improve animal welfare in terms of enhanced immune function and production efficiency, as well as reductions in waste management issues, overall mortality rates, and food borne pathogens (i.e., food safety concerns).

In Denmark, the ban on the use of subtherapeutic antibiotics in the industry was concluded as not having a positive effect on their pork industry. The ban resulted in a handful of unintended consequences, including: an increase in death and diseased animals; an increase in the use of antibiotics to treat sick animals (with the total use only slightly decreased); and while the resistance to some antibiotics decreased in animals, resistance to others increased (Hurd, 2011). Denmark's ban of all food animal antibiotics in 2000 showed very little positive impact on either human health or a decline in resistance. Consequently, there has been an increase in the use of antibiotics in order to treat sick animals (Hurd, 2011). Instead of Denmark's ban on antibiotics as growth promotants (AGPs) reducing the incidence of antibiotic resistance in humans, it was found to increase animal suffering, pain, and death (APUA, 2010).

Challenges associated with reducing the use of antibiotic growth promoters, requiring longer withdrawal phases or the outright elimination of their use, could exacerbate nutritional and environmental stressors. If probiotics were to become a central part of food animals' diets, then the manner in which these feed additives benefit health and are eventually excreted in the feces would need to be documented. Variables such as the timing in which the feeding of probiotics

begins (which would have to be started early in the animal's life to be effective) would affect the establishment of a healthy gut population (Yirga, 2015). Thereafter, in order for the probiotic supplements to continue to be effective, the continued feeding of this specific diet would need to be ensured throughout the production cycle. This is because probiotic species are diverse, and by consequence, vary in their modes of action and efficiency (Danisco Animal Nutrition, 2014).

Within the accompanying thesis, it is essential to analyze multiple immune and endocrine variables in order to better understand the responses that animals have to pathogenic challenges, as well as the resulting impact on growth. Past research has found that probiotics act largely by competing with enteric pathogens, balancing colonic microbiota, influencing the intestinal barrier, and modulating the systemic and mucosal immune systems (Hemarajata and Versalovic, 2012). During the period of lactation, it is necessary for the sow to activate body reserves. This is done in order to compensate for reduced feed intake, as well as to meet the nutritional demand for milk production. Maternal nutrition, as it relates to fetal growth and development, has the potential to influence metabolic, endocrine, and cardiovascular diseases in offspring (Delisle, 2002). Changes to the nutrition and the endocrine status of the offspring may result in permanent changes to structure, physiology, and metabolism of the offspring. Therefore, postnatal performance within pork production systems is crucial for solving future potential economic difficulties. Delisle (2002) described developmental programming as the long-term effects that "stressors" may have on fetal or neonatal development. The programming of organ systems during the developmental period can thereby alter offspring function, whether it occurs postnatal or later in life. Fetal programming further refers to the process by which an acute or chronic stimulus (i.e., in the uterus) establishes a permanent response in the fetus that impacts physiological function later in life. Optimal maternal nutrition and the intrauterine environment are the keys to ensuring ideal fetal development and a reduced risk of chronic disease in the later life of offspring (Lee, 2015). Therefore, maternal nutrition is important for body weight during pregnancy, overall health of the animal, fetal growth, and lactation performance.

THE ANTIBIOTIC VS. PROBIOTIC DEBATE

To date, pork is the most widely consumed and most in-demand meat protein in the world (Pork Checkoff, 2017). Since the World Health Organization's 2014 statement on antibiotic resistance, calling the topic a "major global threat" (WHO, 2014) to public health, the desire for

the use of antibiotic-free practices in food animal production industries has been emphasized. Since the 1940s, antibiotics have been widely used in food animals in the U.S. as a means of preventing animals from disease *and* improving performance efficiency (National Research Council (U.S.) Committee, 1980). Antibiotics have been used to prevent, control, and treat infectious diseases, but the benefits in the food animals have also resulted in enhanced feed efficiency, improved animal growth, and overall improvement in the quality of the animal product. Antibiotics inhibit the growth of, or destroy, microorganisms (US National Library of Medicine, 2016). By targeting bacteria in the body, antibiotics can cure bacterial infections; however, in the case of viral infections, antibiotics are ineffective. A probiotic is a microorganism that is introduced into the body for its beneficial qualities, often as a food or dietary supplement. Probiotics contain live organisms that either replace, or add to, the beneficial microbes already found in the gastrointestinal tract (Jo DiLonardo, 2016). Since probiotics are naturally-occurring live microbes intended to improve the gut flora, they are often administered as a directly-fed microbial that is added to feed. Moreover, probiotics are noted as having the ability to alter the pH (acidity) of the gut in order to allow or prevent the growth of other types of bacteria (Prebiotin, 2011).

Antibiotic therapy may disrupt normal bacterial population of the digestive tract, resulting in the colonization of harmful bacteria in the gut (Langdon et al., 2016), which can irritate the gut and cause antibiotic associated diarrhea (AAD). Antibiotic associated diarrhea is one of the most common side effects associated with the use of antibiotics in swine production. Past studies have shown that feeding *Scb* to pigs in common production environments may improve gut function of the weaned pigs and prevent AAD (Kelesidis, 2012). There is speculation that utilizing alternatives to antibiotics could be the first and foremost step in slowing the progress of antibiotic resistance Cheng et al. (2014). Supplementing production animals' diets with a feed additive other than antibiotics would change the way we use and prescribe these antibiotics. It is the expectation that regulation of antibiotics in animal production would limit their use on farms, turning instead to healthy bacteria that can fill the gap: probiotics. With an ever-increasing demand for pork, coupled with the expanding list of food brands that want to eliminate the use of antibiotics in livestock due to welfare concerns, producers and retailers alike must look to the future for ways in which to assure disease prevention, growth promotion, and animal well-being, while incorporating these new approaches to ensuring a healthy animal gut flora.

Different species of probiotics currently in use include various lactic acid bacteria, *Bacillus* species, and yeasts (such as *Saccharomyces cerevisiae* and *Aspergillus oryzae*), to name a few. Of these species, *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast are noted as the most commonly used in the livestock feed industry (Cho et al., 2011). By using probiotics in addition to a reduced amount of antibiotics in animal feed, beneficial microbes would be able to repopulate the intestinal tract (Danisco Animal Nutrition, 2014). A healthy nutritional status would maintain optimal gut health and support absorption of nutrients, all while improving feed efficiency. Therefore, it can be argued that effective strains of probiotic products should be combined with effective anti-coccidial drugs, which have been effectively used for years as a preventative in feed medication.

Agricultural applications of probiotics include live microbial feed supplements that improve the intestinal microbial balance in the animal. Probiotics are often used in poultry, ruminants, pigs, and aquaculture, in order to improve animal health and food safety. For example, feeding Holstein calves Lactic acid bacteria (LAB) and yeast diets resulted in improved growth rate and suppressed incidence of diarrhea (Kawakami et al., 2010). While in pigs, feeding *Bacillus* species as a probiotic reduced both pathogen load and gastrointestinal disease symptoms (Gaggia et al., 2010). Probiotics can produce inhibitory compounds including volatile fatty acids and hydrogen peroxide which may result in enhanced host resistance to enteric pathogens, via the inhibition of harmful bacterial growth (Jin et al., 2000). The use of probiotics in animal health has resulted in a plethora of benefits to the host; such as providing animals with additional sources of nutrients and digestive enzymes, and stimulating B-vitamin synthesis (LeBlanc et al., 2011).

Probiotic feed supplements may improve pathogenic bacteria resistance in livestock, including defending the digestive system against harmful microorganisms such as enteropathogenic *E. coli*, *Salmonella*, *Listeria* species, and *Helicobacter pylori* (Conly and Johnston, 2004). It has been suggested that improvement in intestinal microbial balance results in enhanced efficiency and performance during times of stress and gut acclimation (FAO, 2016). With the anticipation that the addition of probiotics to animal feed would minimize the effects of common production stressors on animal health and well-being, this then would allow for an increase in nutrient uptake while simultaneously reducing waste (Dhama et al., 2008).

As reported by Jacela et al. (2010) in a review regarding feed additives for swine, the possibility of utilizing prebiotics, synbiotics, and/or phytogenics were also considered as alternatives to the use of antibiotics. Prebiotics benefit the gut of the animal as they are "non-digestible food substances that selectively stimulate the growth of favorable species of bacteria" (Gibson and Roberfroid, 1995). Research on the use of prebiotics has produced inconsistent results, with differences indicating that both the environment, as well as production practices, play a role in the lack of reliability of these feed additives. It has also been proposed that the prebiotics were insufficient in their ability to survive and become established within the gastrointestinal tract, which could be attributed specifically to the number of viable organisms that are found within each dose of prebiotic feed additive. The second alternative feed additive suggested from this research was synbiotics (the combination of both prebiotics and probiotics). Potential benefits of synbiotics include the improved survival rate and colonization of the probiotic microorganisms; however, inconsistent results again hinder this alternative from being utilized in the industry. Finally, phytogenic feed additives (e.g., phytobiotics or botanicals), which are plant-derived substances have also been considered. In swine production, the two most common phytogenic substances are oregano and thyme. These substances are proposed to improve performance by increasing feed intake, improving gut function, and providing swine with anti-oxidative and antimicrobial effects (Jacela et al., 2010).

FUNCTION OF PROBIOTICS IN THE GASTROINTESTINAL TRACT

The roles of probiotics in the Gastrointestinal Tract (GIT) are numerous, including their beneficial role within the livestock industry (Cho et al., 2011). Positive effects of probiotics in animal feed could include: competition between probiotics and pathogenic (harmful) bacteria for gut nutrients, as well as for intestinal epithelium binding sites; stimulation of the immune system; and the ability for probiotics to produce toxic compounds that could incapacitate pathogenic bacteria (Cho et al., 2011). Furthermore, certain strains of probiotics are selected based upon specific criteria, which includes the microbial supplement having a high resistance to stomach acids and bile salts, thereby being able to colonize in the intestine (Verdenelli et al., 2009). Healthy intestines colonized by beneficial microbes thereby enhance the ability of natural gut flora to fight intestinal infections (Hatoum et al., 2012). Therefore, feeding probiotics to animals may support the health of the existing microflora while also treating existing infections.

Probiotic yeast and bacteria work through different mechanisms, including stimulating growth and enhancing survival of good bacteria in the body. Probiotics can be used for several purposes—three of which will be highlighted here. First, digestive health is of importance due to its role in the balance and multiplication of beneficial microbial populations in the GIT (Gorbach, 1996). For example, the restoration of normal intestinal microflora after supplementation with the yeast probiotic, *S. boulardii* has been reported in past studies (Hatoum et al., 2012). Second, induction of the host response stimulates specific proliferation responses, which allows for probiotics to shape the immune system via physiological action in the intestines. Schierack et al. (2007) conducted an experiment in which the peripheral blood mononuclear cells in piglets (specifically, cytokine production) had an effect on vaccination responses. *Bacillus cereus* var. *toyoi* was shown to enhance systemic immune responses in piglets, due to changes in the ratios and functionalities of systemic immune cell population reported in the probiotic-treated animals. Third, probiotics aid in the inhibition of potential pathogenic bacteria, by producing substances that can inhibit Gram-positive and Gram-negative bacteria (Corcionivoschi et al., 2010).

Ojeda et al. (2012) described the importance of the gut microbiota in both the mother and her offspring; particularly its role in metabolizing indigestible dietary components, which can impact obesity pathology. The chosen diet given to a female during gestation may influence whether her offspring become severely overweight during their early life, as well as affecting other physiological and behavioral traits. These products of fermentative digestion can either be utilized for energy, or even impact different host cellular processes. It is speculated that a high fat diet has the potential of altering the offspring's epigenetic programming, which would result in inflammation. Moreover, fetuses of sows fed high fat diets during the gestational period may be at a higher risk of pathogenic bacterial infections (Ojeda et al., 2012). Upon weaning, the intestinal microflora of piglets is altered due to dietary changes and environmental challenges, allowing for probiotics to have a greater and more influential role at this time. In fact, the beneficial effects of probiotics occur in the weaned pig more than the grow-finish pig (Falaye et al., 2016). This could potentially offset colonization of negative strains such as the enterotoxigenic *E. coli* that expresses K88 fimbrial antigens, resulting in diarrhea and even death in both neonatal and weaned pigs (Baker et al., 1997).

MATERNAL-FETAL PROGRAMMING IN SWINE PRODUCTION

Given the concept of maternal-fetal programming in swine production, research should be focused on determining the ways in which producers and farmers can improve the well-being of the sow, by transferring her immune status and/or stress responsiveness to her offspring. By looking at various measures such as stress response, immune system, behavior, performance, and productivity, a better understanding of the biological consequences of these changes can be gained; thereby allowing research to be focused on the improvement and minimization of possible negative aspects of animal production. Further research is necessary in this field, as the GIT of the pig is comprised of not only a wide range of beneficial bacteria, but an array of harmful microorganisms as well (Hardy et al., 2013). This delicate balance of bacteria within the animal's body has the propensity to shift during times of stress—especially during the weaning phase in piglets. The negative outcomes of not being able to control the spread of these harmful microorganisms could result in prolonged poor performance of the animal, or even consequences as severe as disease and/or mortality.

In a research review conducted by Chu et al. (2016), maternal nutrition during pregnancy and lactation was evaluated in order to determine the possible effects on offspring gut microbial composition and function. It was hypothesized that both the maternal gut nutrition and the offspring gut microbes would have an influence on offspring physiology and susceptibility to disease later in life. Moreover, this highlights the significance of the pregnancy period as a time in which the neonatal microbiome is undergoing essential development. The main objective of the review was to interlink the gut microbiota model with that of the Disease Origins of Health and Disease (DoHaD) hypothesis, which states that "adverse *in utero* conditions can influence developmental pathways in early life that results in long-term changes to offspring disease susceptibility."

Maternal nutrition during pregnancy has a significant influence on microbiota abundance and its associated functions, as well as its impacts on the physiology of the host itself. Within this review, it was noted that the intrauterine environment of the pregnant female is not sterile; therefore, the maternal-fetal transmission of microbiota can occur during pregnancy. This differs from previous research, which assumed that the offspring's first contact with microbiota occurs at the time of parturition. Therefore, the intrauterine environment is the key factor in determining fetal growth, with nutrition playing the most critical role in this process, and fetal genome also

altering these mechanisms. The organs that would be most affected by low birth weight (due to poor maternal nutrition) include the heart, liver, and spleen of developing offspring. This can be interpreted as the fetal programming of adult disease and reduced growth potential, with additional effects on neonatal growth, immune system maturation, neurodevelopment, and carcass quality (Chu et al., 2016).

"The maternal environment in which gilt fetuses develop plays a profound role in the development of the reproductive and other physiologic systems" (The Virginia Cooperative Extension, 2008). The formation of the placenta occurs at about day 18 post-fertilization of the ova. During this time (within the embryo), formation of the ectoderm, mesoderm, and endoderm have already occurred, allowing for cell specialization to continue. At 20 days post-mating, most of the major organs are established. Finally, during the last half of the gestational period, the size of the developing fetus rapidly increases (Estienne and Harper, 2008). Foxcroft and Town (2004) found that variation in growth performance after birth is actually pre-programmed during fetal development *in utero*. Furthermore, pre-programmed growth performance limitations can surface later in the production life of swine, even during the late grower or early finisher stages.

Eckhardt et al. (2014) measured the effects of a high-energy diet in late pregnancy, and hormone therapy at weaning, in order to create a plasma metabolite profile and assess litter performance and reproduction. The goal of the study was to better understand the maternal plane of nutrition and body condition during late gestation, and its effects on embryonic development. Results for sow performance in this study found that animals fed higher-energy diets presented the greatest loss in body weight during lactation ($P<0.05$). Alternatively, results from the piglet study found that there were no differences in piglet development, with similar body weight loss or gain consistently seen between treatments throughout the study (Eckhardt et al., 2014).

It is speculated that microbiota are capable of inhabiting the *in utero* environment, since the fetus may encounter exposure to bacteria for the first time during the gestational period, instead of at the time of parturition (Dong et al., 2015). Research shows that there are similarities between the neonate's meconium and amniotic fluid, with that of the placental microbiota. This suggests that microbiota move across the placenta and into the intrauterine space during the maternal-fetal interaction (Dong et al., 2015). A study by Collado et al. (2008) found that obesity and gestational weight gain were a result of alterations in the gut microbiome during pregnancy. Because diet plays a role in the type and abundance of microbiota that is produced, the

transmission of microbial bacteria via mother to offspring would substantially affect both the establishment and development of a healthy neonatal microbiome. Therefore, the interactions between diet, pregnancy, and the microbiome would result in impacting both maternal and fetal health (Collado et al., 2008).

Fetal programming and genomic imprinting are the bases on which nutrition in the intrauterine environment alters the expression of the fetal genome, leading to lifelong changes in the development of the offspring (Foxcroft et al., 2009). Genomic imprinting, which refers to the parent-of-origin-dependent expression of a single allele of a gene, further impacts fetal programming by means of maternal nutrition and metabolic state (Lawson et al., 2013). Therefore, determining the mechanisms of linking the maternal nutrition state to prenatal programming is needed to assess the relevance of nutrigenomics in the gilt and sow. Growth and development are two key aspects that should be assessed in the topic of fetal programming. For example, undernutrition of the fetus could affect both the health and performance of the offspring later in life (Weatherall et al., 2006). This means that maternal undernutrition, or even over nutrition, can have an effect on fetal and postnatal development, as it can reduce placental blood flow and stunt fetal growth. A study conducted by Blair et al. (2009) in which puberty in ewe offspring was assessed, and were later mated and a subset milked, showed how dam nutrition affects the yield and composition of milk in their offspring. Additionally, results of the study further indicated that dam nutrition can also affect the weight and reproductive capability of their grand-offspring. Depending on the species of animal, maternal malnutrition can reduce fetal growth, as well as protein and energy restrictions to offspring.

Intrauterine growth restriction (IUGR), or runting as it is also called, can be exacerbated by both undernutrition and over nutrition during the gestational period (Ashworth et al., 2001). Runt fetuses are more prevalent in the largest litters *in utero*, and are partly related to uterine capacity for normal placental and fetal development. Nutrient deficiencies, including proteins and micronutrients, during the fetal growth phase increase the vulnerability of the developing fetus to a multitude of developmental consequences. In the case of swine, Wu et al. (2004) reported that there is a disproportionate supply of nutrients found along the uterine horn, which results in 15-20% lower birth weight in piglets (<1.1 kg). Overall, this would severely affect growth performance, but more importantly, postnatal survivability. In maternal over nutrition, which involves an increased uptake of energy and/or protein, placental and fetal growth are at risk of

being restricted and causing mortality, especially in pigs (Wu et al., 2004). It has also been suggested that without influencing birth weights, maternal nutrition has the ability to influence progeny development.

YEAST PROBIOTICS

The main perceived threat to the use of antibiotics is the potential for resistant genes to be transferred to pathogenic bacteria. This is why yeast is of beneficial value, as the transfer of genetic material does not occur between bacteria and yeast. Yeasts contain nutrients such as enzymes (proteins), vitamins, and minerals that can be found in the enteric microflora of animals; furthermore, they are digestible and can be used for maintenance and production (Salyers et al., 2004). In pigs, probiotics improve growth performance, digestibility, and immunity. Increased nutrient uptake could improve the growth performance of the animals, via the feed additives' effect on the permeability of the gut (Baum et al., 2002). In cattle, yeast single cell protein can be used to accelerate the growth and improve well-being via rumen acetogen stimulation (Hatoum et al., 2012). Supporting research by Chiquette (2009), found that feeding *S. cerevisiae* to ruminants resulted in stabilization of the rumen pH, as well as prevention of acidosis (caused by rapid fermentation of large quantities of carbohydrates). Feeding yeast to animals allows for the stimulation of rumen microbial growth and oxygen, which allows for anaerobic microorganisms to persist in more favorable conditions (Chiquette, 2009). However, as has been stated previously, the mode of action of probiotic yeast in the rumen depends on yeast strain, viability, and diet composition.

The stability of probiotics as feed additives would have to prove dependable in terms of longevity when considering factors including: shelf life under storage conditions, survival through feed processing, animal ingestion, and surviving the animal's gastric barrier (Soccol et al., 2010). Commonly-used feed processing technologies have been found to kill probiotic cultures. Therefore, identifying the most suitable microbial strain is the first step in developing a probiotic for pigs that will garner a specific and more reliable effect within the swine production industry. Keeping in mind that not all probiotics work as effectively (or at all for that matter) with all pigs, the physiology of a pig needs to be considered in order to measure the reliability and consistency of the probiotic. Probiotic products must also be economical and produce measurable improvements in welfare and production.

In general, probiotics defend the cells of the body by receiving anti-inflammatory cytokines, while reducing proinflammatory cytokines from both enterocytes and intestinal immune cells (Cho et al., 2011). Stavric and Kornegay (1995) suggest that during microflora development, and also when microflora stability is impaired, probiotics prove to be the most effective. Supplementation of probiotics such as *L. sobrius*, for example, has been found to suppress pathogen levels and improve overall performance of weanling pigs that were affected with *E. coli* (Konstantinov et al., 2008). Adding probiotics to pig diets has been found to increase fermentative activity, while simultaneously stimulating digestion. In addition, lactobacilli are capable of colonizing in the GIT epithelium, thereby developing a protective membrane to combat harmful pathogenic microorganisms, while also stimulating epithelial lymphocytes (Yu et al., 2008; Hou et al., 2015).

Research conducted by Perry (2014) was the first to publish research regarding the use of probiotics in high-fiber diets; to increase fiber fermentation rates while reducing manure output. The high-fiber diets given to the tested pigs contained 10% soybean hulls and 20% corn DDGS, which were then supplemented with one of three bacterial supplements comprised of different strains of *Bacteroides ovatus*. The probiotic designated Bacterium B resulted in pigs with a 20% reduction of manure output, increased weight gain, improved blood cholesterol, and improved glucose levels. These outcomes enhanced overall energy status, while increasing producer profits and reducing the environmental impact of pork production (Perry, 2014). Similarly, research conducted by Bhandari et al. (2010) demonstrated how probiotics, coupled with a low protein diet could increase the overall performance of weanling pigs, resulting in improvement in growth rate and reduction of pathogenic bacteria.

A European efficacy study (Jorgensen and Hansen, 2016), utilizing gilts and sows on a commercial farrow-to-finish pig farm, supplemented the diet of sows with probiotic BioPlus2B two weeks prior to the farrowing-to-weaning phase. The study found a 42% reduction in pre-weaning mortality for the piglets of sows administered the designated probiotic. Additionally, there was an increase of about one piglet born and weaned per treated sow/year overall. The probiotic group also had an improved diarrhea score, as well as piglets having a 5% greater weaning weight than the control group. In terms of the effects of the probiotic BioPlus2B on the treated sows, there was an average of about 21% lower body weight loss during the lactation period (Jorgensen and Hansen, 2016).

SACCHAROMYCES CEREVISIAE BOULARDII

Saccharomyces cerevisiae boulardii (*Scb*) is non-pathogenic yeast used in human medicine to both prevent and treat intestinal disorders, including infectious and antibiotic-associated diarrhea (Llopis et al., 2014). *Scb* belongs to the group of simple eukaryotic cells (i.e., fungi and algae), which is why it is different from bacterial probiotics, which are prokaryotes. It is unique in its ability to survive in the GIT (with an optimal temperature of 37 degrees Celsius) while simultaneously inhibiting microbial pathogens (Llopis et al., 2014). The gastrointestinal microflora, or microbiota as it is also called, coexists in equilibrium with the host. A probiotic must be able to withstand the host's natural barriers to ingested microorganisms, so that they may be viable and biologically active microorganisms at the target site. It is essential that these probiotics be resistant to local stresses, including the presence of GI enzymes, stomach acids, bile salts, pancreatic juices, organic acids, and variations in pH and temperatures (i.e., thermo-tolerant) (Czerucka et al., 2007). These probiotic supplements must adhere to the internal epithelium, while also stabilizing the balance of gut flora. Microbial colonization is dependent upon the number and species of bacteria due to environmental conditions (Czerucka et al., 2007). In microbial ecology, yeast are a part of the microflora making up <0.1% of microbiota.

Product background studies have been conducted by Lallemand Inc. (Canada) in which the yeast probiotic, *Scb*, was supplemented in sow diets during the peripartum (in intestinal transit) and farrowing periods. These studies found that upon product administration, the probiotic-supplemented diet is capable of maintaining a healthy and balanced gut microflora in the sow. Additional benefits to the sow include colostrum and milk production/quality improvements, which directly affects the sows' piglets as well. Piglet benefits included reduced neonatal diarrhea and mortality (at birth and during lactation). Piglets were found to be stronger and more active (i.e., higher energy level) at birth, with continued improved growth development and performance during the lactation phase (Lallemand Animal Nutrition, 2011). Lallemand Inc. has indicated that strain-specific yeast and bacteria probiotics positively influence the digestive function and balance (throughout the animal's life), including the health of food production animals treated with the feed additive. More specifically, the benefits of incorporating probiotics into the diet of food animals include: enhancement of rumen/gut function, digestibility improvement, and protection against harmful bacteria (Lallemand Inc., Canada).

There are many purported benefits of feeding *S. boulardii* (*Sb*), as it may directly or indirectly suppress populations of potentially pathogenic or growth-suppressing microorganisms. Additional benefits, depending on the animal model used, as previously described by Collier et al. (2014), include: production of trophic factors to increase gut-associated lymphoid tissue-derived IgA production, improved mucosal barrier function, and improved brush border membrane integrity. For example, *in vitro*, it has also been found that *Sb* can stimulate the digestion of cellulose and promote the utilization of lactate (Qamar et al., 2001), by stimulating an increase in intestinal IgA secretion in mice when challenged with *C. difficile* toxin A. Results showed that *Sb* can be used against *C. difficile* infection, because the probiotic binds the pathogen in order to deactivate the bacteria and inhibit attachment to the epithelium. Another study that added *Sb* to diets found improved immune defenses, digestion, and absorption of nutrients (Buts and de Keyser, 2006). A considerable amount of polyamines can be found in *Sb*, and is said to be a mediator of trophic effects (Fioramonti et al., 2003). Important physiological effects of these polyamines include: cell maturation, enzyme expression, and membrane transport mechanisms (Buts et al., 1994). Finally, studies conducted on the effects of *Sb* in poultry have found in increase in bird weight when their feed was supplemented during the early stages of life. This is due to the fact that whole yeast improved the growth rate, meat tenderness, and oxidative stability in the animal (Zhang et al., 2005).

Research conducted by Lipinski et al. (2012) found positive effects of the probiotic yeast, *Scb*, on the reproductive performance of pregnant and lactating sows when supplemented into the animals' diets. For the purposes of the research indicated, the strain Levucell SB 10 was used to determine the health status and productivity of both the sows and their respective litters. Using a total of 243 gilts and sows, which included the 125 untreated control animals, treatment spanned from day 1 of gestation to day 28 of lactation. The results of the experiment found that sows (noted initially as having a poor health status) fed a diet that included the probiotic supplement, had better fertility and mating effectiveness compared to the control group sows. Moreover, the sows that had poor health prior to receiving the probiotic supplement benefited from the probiotic once the treatment began, as the sows' reproductive problems (including abscesses and abortions) improved over time in accordance with being fed the supplement. However, it was also noted that sows fed the probiotic supplement did not change the condition or the length of their parturiency (Lipinski et al., 2012).

It has been noted in some studies that supplementation of *Scb* did not modify the intake and digestibility of dry matter, organic matter, EE, and CP. First, Kimse et al. (2012) concluded that adding live yeast to the diets of rabbits did not modify the total digestibility of nutrients. Second, it was found that oral administration of yeast culture did not have an effect on the digestibility of nutrients in diets with different fiber content (Chaudary et al., 1995), although past research has found that *Sb* can cause an increase in secretory IgA. Lastly, Oso et al. (2013) reported that ADF, NDF, and other nutrient digestibility values were unaffected by the addition of probiotics of bacterial origin into the diets.

A study conducted by Collier et al. (2011), evaluated the effects of active dry yeast, *Scb*, on the immune and cortisol response was assessed in newly weaned piglets. The objective of the study was to determine whether oral administration of *Scb* reduces mortality of newly weaned piglets as it is associated with immune and cortisol responses to *Escherichia coli* endotoxin. It was hypothesized that before challenging the piglets (at weaning), a beneficial immune and/or neuroendocrine interaction profile would be established, thereby resulting in acute responses to pathogenic challenges in order to prevent the diversion of energy (i.e., used for growth-promotion instead). Results of the study indicated that mortality rates in LPS-induced piglets were reduced by 20%. In *Scb*-treated animals, prior to LPS dosing, there was an increase in white blood cells, lymphocytes, and neutrophils. This suggests that *Scb*-induced immune-neuroendocrine LPS response thus functions to facilitate short-term prevention of the pathogen. Within this study, *Scb*-treated pigs not only had reduced LPS-induced mortality, but increased average daily gain as well. *Scb* supplementation to newly weaned piglets resulted in differential cytokine production profiles that were taken from a larger pool of immune cells. Therefore, *in vitro*, *Sb* may alter the signaling pathways that were once implicated in proinflammatory cytokine synthesis. This was in the presence of reduced cortisol concentrations, before and during the LPS challenge response. With the potential of *Scb* to prevent LPS-induced mortality, benefits related to animal growth are promising across a multitude of animal models (Collier et al., 2011). In conclusion, *Sb* was found to have a unique immune/cortisol profile, with effects that are similar to the use of subtherapeutic antibiotics.

PIGLET WEANING PHASE

The first weeks of life constitute the most critical period for piglets, since they can be exposed to conditions that negatively influence neonatal immune responses, thereby compromising their ability to resist and combat pathogenic challenges (Tuchscherer et al., 2002). Factors such as environmental stressors, husbandry practices, and antigenic exposures of sows each may negatively affect the early development of the piglet's immune system, with a probable increase in susceptibility to infection or disease, and a reduction in growth and performance. Moreover, prenatal maternal stress during late-gestation reportedly impairs the development and reactivity of the immune system of a sow's offspring and impacts the frequency of disease and mortality (Tuchscherer et al., 2002).

At weaning time, piglets make the transition from liquid feeding (i.e., milk) to dry feeding, with the potential for digestive upset. In order to reduce the colonization or presence of pathogenic bacteria like *Salmonella* within the intestine, competitive exclusion of pathogenic bacteria by non-pathogenic microbes is a feeding option (Steer et al., 2000). Probiotic use in swine production has been found to improve gut microbiota balance, integrity of the intestinal epithelium, and maturation of gut-associated tissue (Hemarajata and Versalovic, 2012). The most commonly used probiotics are said to be bacterial-derived species. Coming from either the genus of *Bacillus* or *Streptococcus*, a mixture of these two probiotics has been reported to improve both average daily gain and feed conversion ratio in piglets subjected to weaning and mixing stress (Taras et al., 2005 & 2006).

In a study by Jorgensen and Hansen (2016), the weaning phase was pinpointed as the most traumatic event that piglets will be subjected to upon leaving the nursery (and in their life in general). It is in the first three weeks of a piglet's life that its GIT is under continuous development, yet still measurably immature, while the gut flora are being established. The transition phase (identified here as the first week *after* weaning) occurs when the highly stressed GIT and the maturing immune system are delicately interacting. Possible negative effects of this interaction during the transition phase include challenges to the sub-clinical disease status of the piglet. This could result in reduced feed intake, as well as increased illness and mortality, since gut microflora have "stimulating and/or depressing" effects on one another. The interaction of the gut micro-flora on the host animal differs depending on many factors, including age, feed consumption, stress level, and the environment (Jorgensen and Hansen, 2016).

Gastrointestinal disorders occur most prevalently immediately post-weaning. In a study conducted by Baum et al. (2002), weaned pigs were administered the live yeast, *Scb*, for a period of 3-4 weeks. This study found improvements for post-weaning growth performance, villus height, epithelial cell proliferation, and macrophage count at multiple sites within the small intestine. The incidence of diarrhea in piglets during the first week post-weaning was reduced when sows were fed *Escherichia faecium*. Moreover, the level of cytotoxic T-cells in the jejunal epithelium of these piglets was also reduced. Similarly, gastrointestinal disorders influenced by the physiological state, were assessed post-birth in the digestive tracts of piglets. It was reported that colonization of useful microorganisms (i.e., lactic acid bacteria, *Enterobacteriaceae*, and *Streptococcus*) occurred via contamination from the maternal environment, including increased numbers and density of anaerobes (Zoetendal et al., 2004). This newly created microbiota can then protect against pathogens due to defense at the mucosal level.

Giang et al. (2010) conducted a 35-day feeding trial in which weaned piglets were given a feeding trial that was supplemented with 0.2% yeast and a mixture of lactic acid bacteria. The results of this study suggest that the administered diet improved the total tract digestibility of crude protein, crude fiber, and organic matter. The mixture of lactic acid bacteria complex, along with *Sb*, helped to improve overall performance of the animal, since no growth parameters were thus affected by live yeast addition. *S. boulardii* further aided in increasing the concentration of short-chain fatty acids, as well as in populations of lactic acid bacteria (LAB). The increased content of organic acids acidifies the intestine and exerts an antibacterial effect. Piglets fed LAB had reduced incidences of diarrhea; additionally, average daily feed intake and average daily gain were increased, with a lower feed conversion ratio. Since yeast probiotics can cause proliferation of LAB, thereby having an effect on inflammation in the intestinal tract, this can potentially lead to secretion of immunosuppressive cytokines (i.e., IL-10). The utilization of *Sb* in this study was beneficial, as weaning stress is capable of decreasing good bacteria, while increasing the population of *E. coli*, thereby increasing susceptibility to disease (Giang et al., 2010).

A study of the effects of injectable antibiotics and a probiotic on suckling pigs (including growth and death loss) administered within 24-hours post-farrowing; and the effects of a probiotic on nursery pig performance administered at weaning, was conducted by Estienne et al. (2005). The objective of the first experiment was to specifically determine the effects of various

antibiotics (i.e., oxytetracycline, erythromycin, penicillin G procaine, and tylosin) and probiotics (i.e., *lactobacillus* and *streptococcus*) on suckling pig performance (pig body weights and survival during pre-weaning period), which were given within 24-hours post-farrowing. The study found that the sows treated in this experiment did not have different piglet survival rates from birth-to-weaning, nor did the groups have different body weights (i.e., birth, 7-day, 14-day, and 21-days of age) when compared to the control group. The second experiment of this study determined the effects of the probiotics *lactobacillus* and *streptococcus* on nursery pig performance (growth performance) which was given at the time of weaning. Here, the use of probiotic feed additives did increase average daily gain and feed consumption of non-littermate weaned pigs. Overall, the inclusion of antibiotics or probiotics in the sows' diets benefited pre-weaning performance (Estienne et al., 2005). It was suggested that if therapeutic antibiotic injections are to be used in the food animal industry, that it should be limited to use for *specific* disease-treatment scenarios.

CONCLUSION

The previous research cited within clearly demonstrates the impact that maternal nutrition has on the offspring gut microbiome. This interaction could potentially occur *in utero* in certain species, as well as beyond pregnancy, into the postnatal period and throughout lactation. Maternal diet, during pregnancy and throughout lactation, affects the microbiota by potentially changing the abundance and type of bacteria that can be transferred from mother to offspring during the periods of gestation, and even in the early life of offspring (Nuriel-Ohayon, 2016). A potential mechanism of great importance to this topic is the microbial transmission across the maternal-fetal interface within the placenta. Furthermore, a clear understanding of the sow's metabolic status throughout pregnancy is crucial to defining the specific maternal-fetal interactions that occur *in utero* and during the early life of offspring (pre-weaning phase). Further research as it relates to a variety of species models include, but are not limited to, the following: maternal and neonatal nutrition (i.e., energy, protein, etc.), number of fetuses, maternal age, maternal environmental stress, and maternal and fetal genotype (Langley-Evans, 2006). Favorable traits for litter quality include ovulation rate, embryonic and fetal survival, and enhanced uterine capacity. Hence, the avoidance of prenatal programming of undesirable litter

phenotypes will ensure improved lifetime performance of the offspring selected for breeding in future practices. (Langley-Evans, 2006).

Past research has reported both benefits and drawbacks of incorporating probiotic feed additives into animal agriculture operations (either as a supplement to, or in replacement of, antibiotics). Factors include: the low survival rate of strains, interactions with other medicines including antibiotics and antimicrobials, insufficient (or low) probiotic dosages, possible stressors it induces or alleviates on the animals, varying stabilities of strains, differing animal genetics, frequency of administration, health and nutritional status of the animal, etc. (Cheng et al., 2014). As suggested in a review by Turner et al. (2001), the specific production environment significantly influences the growth performance of pigs. Even when food animals are given performance-enhancing agents though, factors such as the cleanliness of the facility, the history of disease on that specific housing location, and the overall health status of the current group of animal housed there, can result in adverse effects for the animals being raised for human consumption (Turner et al., 2001). Criteria for effective strains of bacteria (e.g., probiotics) that are selected include the supplement having a high resistance to stomach acids and bile salts, as well as being able to colonize in the intestine (Cho et al., 2011).

Human health, animal health, and the environment are three key areas that must be considered when evaluating long-range planning to limit antibiotic resistance. As quoted by Dr. Robin Ganzert (CEO of American Humane Association, American Humane Certified program), "An outright ban [of antibiotics] would be inhumane to sick animals, and would violate one of the five freedoms (freedom from pain, injury or disease) that serves as the internationally accepted social contracts with animals" (Ganzert, 2015). In the food animal industry, both socioeconomic and health related issues are important factors driving the discussion of this topic, and must therefore be at the forefront of creating viable solutions to the perceived problem of continued antibiotic use in food animals. It is likely that the most viable option in this debate would be the *partial* replacement of antibiotic growth promoters, such as supplementing the use of both antibiotics and probiotics as feed additives in the diets of swine. Hence, determining how probiotic feed additives that are supplemented into that of the sows' diets impacts not only the health and overall performance of these sows, but that of their piglets (especially during the periods of gestation and lactation) needs to be further assessed.

CHAPTER 2. THE EFFECTS OF FEEDING PROBIOTIC, *SACCHAROMYCES CEREVISIAE BOULARDII*, TO SOWS DURING GESTATION AND THROUGH LACTATION ON IMMUNE AND CORTISOL RESPONSES TO FARROWING AND WEANING STRESS

INTRODUCTION

Feeding antibiotics to healthy food animals has become a global concern due to the increased occurrence of antibiotic resistant bacteria. A potential alternative to consider is the use of probiotics. Probiotics are microorganisms that, when ingested in adequate amounts, confer health benefits on the host (Czerucka et al., 2007) possibly by restoring the composition of the gut microbiome and reducing gut inflammation (Hemarajata and Versalovic, 2012). Evidence exist suggesting that bacterial probiotics (i.e., *Lactobacillus*) can non-specifically modulate the host immune system by enhancement of phagocytic activity (Roessler et al., 2008). But, probiotics have also been shown to have immunosuppressive properties by suppressing inflammation, primarily through disrupting pro- and anti-inflammatory cytokines. Specifically, *Saccharomyces cerevisiae boulardii* (*Scb*) has been shown to block the secretion of inflammatory cytokines, e.g., IL-8 and IL-6, which are released in response to infection (Dalmasso et al., 2006).

Most research conducted on feeding the probiotic *Scb* to swine has been in piglets. Feeding probiotics to piglets during lactation reduces the incidence of pathogen-induced diarrhea post-weaning by preventing adherence of pathogens to the intestinal wall and neutralizing bacterial endotoxins (Perdigon et al., 2002; Gad et al., 2011). Early studies conducted by Lallemand Inc. (France) found that there were benefits to supplementing food animal diets with *Scb* which included: enhancement of gut function, digestibility improvement, and protection against harmful bacteria (Chevaux and Guillou, 2015). More specifically, the use of *Scb* supplemented in sow diets during the peripartum (in intestinal transit) and farrowing periods is capable of maintaining a healthy and balanced gut microflora and improvement in colostrum and milk production/quality, which directly affects the well-being of her piglets. Thus, if the benefits of probiotics are also transferable from dam to piglets, probiotics may be a viable option for both the sow and her offspring. It is hypothesized that pregnant sows fed the yeast probiotic treatment (PRO-sows), *Scb*, during late gestation will have an overall improved immune status and cortisol levels. Treated sows will also have greater innate and adaptive immune measures when

compared to the control sows (CON-sows) during the gestation and lactation periods. Therefore, the objectives of this research were to evaluate the effects of feeding a yeast probiotic, *Scb*, to pregnant sows during late gestation (d 84 – d 112 of gestation) and a three-week lactation period (d 115 – d 135) on innate and adaptive immune status of the sow and the effects of farrowing and weaning stresses on immune and stress responsiveness.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures were approved by the University of Illinois and the Institutional Animal Care and Use Committee (IACUC). A total of eighteen pregnant sows, derived from the Genetiporc maternal line, were used in this study. Sows were all parity two, and vaccinations were used following farm standard operating procedures and health plan. The experiment was conducted across two phases of production (gestation and lactation). Days of gestation (phase one) included d 84 (baseline: began feeding placebo or yeast probiotic boluses to sows; d 0 of treatment), d 91 (d 7 of treatment), d 98 (d 14 of treatment), d 105 (d 21 of treatment), and d 112 (sows moved into farrowing crates on this day; d 28 of treatment). This resulted in a four-week gestational treatment period, with immune and blood measures being collected every seven days. Days of lactation (phase two) included gestational day 112 (baseline: sows moved into farrowing crates on this day; d 28 of treatment), and days 115 (24-hours post-farrowing; d 31 of treatment) and 135 (weaning; d 51 of treatment).

Sows were randomly assigned to either the control treatment (CON-sows) or the probiotic treatment (PRO-sows) starting on d 84 of gestation (baseline for the experiment). Sows were maintained in treatment groups (CON or PRO sows) throughout the study (n = 18 sows; 9 sows/treatment). Either the placebo or the probiotic boluses were fed daily starting on d 84 of gestation and ending on day of weaning of sows (d 135). Day 84 of gestation was chosen as the first day of treatment based on data that supports pig fetus immune cell development, and was also recommended by Dr. Ken Mellits at the University of Nottingham. *In utero*, research conducted by Salmon (1984) states that the pig fetus becomes immunocompetent at around 80 days of fetal life, which is indicative of the immune cell development that is of particular interest to this study. In addition, d 90 of gestation coincides with all other work from the Salak-Johnson laboratory for sow immune status.

Sows were hand-fed either two boluses of CON or PRO every morning at 0700 h. The probiotic bolus contained 2.0 x 10^9 (CFU/g) of *Saccharomyces cerevisiae* var. *boulardii* (*Scb*) CNCM I-1079 per bolus (Lallemand Inc., Canada). *Scb* is noted as being non-toxic and harmless to humans and animals, and has no withdrawal period. The control bolus was sugar based, and anatomically the same shape and size as the probiotic bolus.

Gloves were worn at all times during the feeding of placebo and probiotic boluses to prevent residue transfer. To avoid the possibility of cross contamination from the probiotic bolus, the CON boluses were always fed to the nine control sows first. Gloves were discarded and hands were washed after feeding the control sows. Then, the PRO boluses were fed to the nine treatment sows. Again, gloves were discarded and hands were washed after each daily feeding of the probiotic boluses.

Sows were individually fed a diet formulated to meet or exceed established nutrient allowances (NRC, 2016). During gestation, each sow was fed 2.5 kg/d of corn-soy based diet having a calculated composition (as fed) of 12.5% CP and providing a calculated ME density of 3,300 kcal/kg. All sows were fed between 0700 and 0730 h each day. Each stall was equipped with one nipple waterer. Lactating sows were fed ad libitum, a corn-soy based diet with a calculated composition (as fed) of 16% CP and 3,426 of ME/kg.

Housing

Sows were housed at the University of Illinois Swine Research Center in Urbana-Champaign. During gestation, sows were housed in standard gestation stalls (2 ft. x 7 ft.) in a mechanically-ventilated, insulated gestation building. On day 112 (± 2 days) of gestation, sows were moved to farrowing rooms, and kept in farrowing stalls, where they stayed until the end of the lactational period (d 135). All sows were kept on a 10 h light: 14 hour dark schedule, in which lights were on at 0700 h and turned off at 1700 h.

Blood Sample Collection and Leukocyte Differentials

Sows were nose-snared, and 10 mL of blood was collected via jugular venipuncture with vacutainers containing sodium heparin or EDTA (the procedure lasted <2 min) at gestational d 0 (gestational d 84), 7 (d 91), 14 (d 98), 21 (d 105), and 28 (d 112); and then again at 24 h post-

farrowing (d 115) and weaning (d 135). All control sows were bled first and then the probiotic-treated sows.

Heparin-treated whole blood was used to determine total white blood cell (WBC) counts and leukocyte differential counts (DIFF). Total WBC counts were made electronically using a Coulter Z1 particle counter (Beckman Coulter, Miami, FL). Ten microliters (10 µl) of whole blood was added to 10 mL of Beckman Coulter, Isoflow®, red blood cells were lysed with Beckman Coulter lytic reagent, ZAP-OGLOBIN® and then samples were placed in the counting chamber to determine total BBC count. To determine DIFF percentages, whole blood smears were made, fixed in methanol, and then stained with Hema-3 staining system (Fisher Scientific, Houston, TX). Slides were viewed under a light microscope, and 100 cells per slide were visually counted.

Cell Isolation and Plasma Analysis

Whole blood was collected and centrifuged at 700 × g for 30 min at 4℃. Plasma was aspirated and transferred to Eppendorf tubes and stored at -80°C until further analysis. Whole blood was diluted with Roswell Park Memorial Institute (**RPMI**; Gibco, Carlsbad, CA) medium, layered over Histopaque-1077 (density = 1.077g/mL; Sigma) and -1119 (density = 1.119 g/mL; Sigma), and centrifuged at 700 x g for 30 min at 25°C. Lymphocytes were removed from the top of the second layer and neutrophils from the top of the third layer. Red blood cells were lysed from the neutrophil fraction, which was then washed in RPMI and counted. Cell concentrations were adjusted with RPMI based on the immune assay requirements. Whole blood was diluted using Roswell Park Memorial Institute (RPMI) media.

Plasma cortisol and IL-12 were analyzed following manufacturer's protocols. Commercial radioimmunoassay validated for porcine cortisol was measured. Plasma samples from heparin-treated whole blood were assayed for CORT using a Coat-A-Count cortisol kit, following the manufacturer's protocol (Diagnostic Products Corp., Los Angeles, CA). Briefly, in duplicate, 25 µL of sample or standard were added to antibody-coated tubes. Radiolabeled (I125) CORT was added to tubes and incubated for 45 min at 37°C in a water bath. The liquid phase was decanted and radioactivity counted with a gamma counter. A standard curve based on 0, 10, 50, 100, 200, and 500 µg/mL was used. Intra- and inter-assay CV were 7.0 and 16.5%, respectively. Minimal detectable concentration of CORT using this assay was approximately 2

ng/mL. Porcine IL-12/IL-23 Quantikine kit was used to measure IL-12 in plasma samples (R & D Systems, Minneapolis, MN). Minimal detectable concentration of IL-12/IL-23 using this kit was on average 9.0 pg/mL.

Immune Assays

Neutrophil chemotaxis was measured using an assay previously described by Salak-Johnson et al. (1993). Neutrophils at a concentration of 3×10^6 cells/mL were used in order to evaluate the ability of cells to migrate toward, (a) assay medium, with the control being random migration, or (b) recombinant human complement -5a (1×10^{-7} M) and recombinant human IL-8 (100 μg/mL) with chemotaxis being directed migration in this case. Neutrophil phagocytosis measurements were gathered using a flow cytometry-based assay previously described by Heinzelmann et al. (1999), along with slight modifications described by Niekamp et al. (2006). First, fluorescent beads along with heat-inactivated porcine serum, were pre-incubated together for thirty minutes. Next, beads were added to the samples at a 10:1 ratio (beads: neutrophils). Finally, both cells and beads were incubated for forty-five minutes at room temperature. Flow cytometry allowed for the total percentage of beads that were engulfed by cells to be evaluated.

Using Promega CellTiter®, a colorimetric method for determining the number of viable cells in proliferation, cytotoxicity, and chemo sensitivity assays, with slight modifications described by Sutherland et al. (2005), a mitogen-induced lymphocyte proliferation assay was performed. First, lymphocytes from the sow were used at a concentration of 5×10^6 cells/mL, and placed in a sterile 96-well flat-bottom plate (sample run in triplicate per sow). Concanavalin A (ConA) and lipopolysaccharide (LPS), both acquired from Sigma Aldrich, were used as mitogens in order to stimulate T and B cells. ConA and LPS were both used at measurements of 0.2, 2.0, and 20 μg/mL. Plates were incubated for sixty-eight hours at a temperature of 37℃ in a 5% CO_2 humidified incubator. Next, 15μL of Promega Dye was added to each well, and the plates were incubated for an additional four hours. Promega Stop solution (100 μL) was added to each well, and the plates were incubated overnight at a temperature of 37℃. Finally, using BIO-TEK Instruments® microplate reader at a wavelength of 550 nm with reference wavelength 690 nm, results can be expressed as a proliferation index (PI).

$$PI = \frac{\text{Optical Density (550/690 nm) stimulated cells}}{\text{Optical Density (550/690 nm) non-stimulated cells}}$$

A nonradioactive cytotoxicity detection kit acquired from Roche Diagnostics®, also described previously by Sutherland et al. (2005), was used to measure natural killer (NK) cell cytotoxicity. First, sow lymphocytes were used as effector cells, while K-562 chronic human myelogenous leukemia cells (American Tissue Type Culture Collection, Manassas, VA) were used as target cells. Lymphocytes were adjusted to 1 x 10^7 cells/mL, and K-562 cells were adjusted to a constant 10,000 cells per wall. Second, samples were run in triplicate at effector to target-cell ratios of NK 12.5:1, 25:1, 50:1, and 100:1. Third, plates were read using BIO-TEK Instruments® microplate reader at a wavelength of 490 nm with reference wavelength 690 nm, after an eighteen hour incubation period. Finally, percent cytotoxicity was calculated as previously described by Lumpkin and McGlone (1992). An assay was thus considered valid as long as the maximum release, divided by spontaneous release, was ≤ 20%.

Sow Body Weight

Sow body weight for this study was recorded on d 84 of gestation (baseline) and on d 135 end of lactation (weaning). For this study no other body weight measures were taken in an attempt to minimize additional stress on the sows during gestation and lactation.

Fecal Enumerations

Both fecal samples and blood samples were collected on the same days; on gestational days 84, 91, 98, 105, and 112, as well as on lactation days 115 (24 hours post-farrowing) and 135 (weaning). This additional test was done in order to confirm that the nine control sows did not ingest a significant amount (if any at all) of the probiotic that would be needed in order to maintain a colony in the GI tract. Yeast fecal enumerations, obtained via rectal palpation, were conducted via standard methods using Rose Bengal Chloramphenicol Agar produced by Oxoid Limited®. The initial 10:1 dilution was made by adding 9 volumes of Maximum Recovery Diluent (MRD) of the original sample weight. Serial dilutions thereafter made with MRD, by putting 100 µL into 900 µL. Next, 100 µL was plated (using the spread plate method) onto duplicate agar plates in two different concentrations, 1 x 10^1 and 1 x 10^9. After the RBC agar

plates were incubated aerobically for a period of three days at a temperature of 30℃, then the plates could be read. Positive identification was based upon the typical colony morphology of *Saccharomyces cerevisiae* var. *boulardii* control stain. Results of this test (at the seven specified sample dates) showed yeast colonies were only successfully grown on RBC agar that were streaked with samples collected from the nine probiotic treatment sows, thus ensuring that there were no implications of cross-contamination in this study.

Statistical Analysis

Data were analyzed using PROC MIXED with repeated measures SAS (SAS Inst. Inc., Cary, NC), mixed linear models contained both fixed- and random-effects parameters. Baseline days 84 and 112 of gestation were used as covariates when values were reported as significant. The first set of data was analyzed for gestational d 0 (baseline; gestational d 84), 7 (d 91), 14 (d 98), 21 (d 105), and 28 (d 112) days post-treatment. The second set of data was analyzed for gestational d 28 (baseline; d 112), including lactational days 115 (24 h post-farrowing) and 135 (weaning of sows). All traits were tested for departures from a normal distribution. The model included fixed-effects parameters of treatment, control or probiotic, as well as day of gestation for sows. In addition, random effect of parity was evaluated for sows. Significance of data was set at ($p \leq 0.05$), and trends were also discussed at ($p > 0.05$) to ($p \leq 0.10$).

RESULTS

Experiment 1: Treatment x Day Effects during the Gestation Period

During gestation, at d 7 and d 14 post-treatment (d 91 and d 98 of gestation), CON-sows had lower plasma cortisol than did PRO-sows; while at d 21 post-treatment (d 105 of gestation), PRO-sows had lower plasma cortisol than did CON-sows ($p = 0.002$). At d 28 post-treatment (d 112 of gestation), both CON and PRO sows had similar plasma cortisol concentrations (Figure 2.1).

Leukocyte Differentials. At d 7 and d 28 post-treatment (d 91 and d 112 of gestation), PRO-sows had greater percentage of eosinophils than did CON-sows ($p \leq 0.05$). At d 14 and d 21 post-treatment (d 98 and 105 of gestation) CON-sows had greater eosinophils (Table 2.1). Banded neutrophils (immature) were greater in CON-sows at d 7, 14, and 28 post-treatment (d 91, 98,

and 112 of gestation), but by d 21 post-treatment (d 105 of gestation), PRO-sows had greater percentages of banded neutrophils (Table 2.1). Segmented neutrophils were greater in PRO-sows during gestation at days 7, 14, and 21 post-treatment (d 91, 98, and 105 of gestation), while at d 28 post-treatment (d 112 of gestation), CON-sows had greater percentages of segmented neutrophils (Table 2.1). Across all days of treatment during gestation, CON-sows had greater percentages of lymphocytes than did the PRO-sows (Table 2.1). Percentages of monocytes were greater for PRO-sows during gestation at all days post-treatment (d 14, 21, and 28); except for d 7 post-treatment (d 91 of gestation), CON-sows had greater percentage of monocytes (Table 2.1).

Natural Killer Cell Cytotoxicity. During gestation, at all days post-treatment (d 14, 21, and 28) PRO-sows had greater ($p = 0.001$) natural killer (NK) cell cytotoxicity than did CON-sows. However, at d 7 post-treatment (d 91 of gestation), CON-sows had greater NK cytotoxicity (Figure 2.2).

Neutrophil Chemotaxis C5a and IL-8. Across gestation ($p = 0.01$), at d 7 and d 21 post-treatment (d 91 and d 105 of gestation), PRO-sows had greater C5a-induced neutrophil chemotaxis than did CON-sows. However, at d 14 post-treatment (d 98 of gestation) PRO-sows had lower chemotaxis than CON-sows. Among the CON-sows, neutrophil chemotaxis remained unchanged until d 28 post-treatment (d 112 of gestation), when the CON-sows had greater chemotaxis than did PRO-sows (Figure 2.3). During gestation, PRO-sows had greater IL-induced neutrophil chemotaxis at d 7 and d 28 post-treatment (d 91 and d 112 of gestation) than did CON-sows ($p = 0.02$); while at d 14 and d 21 post-treatment (d 98 and d 105 of gestation), CON-sows had greater neutrophil chemotaxis than did PRO-sows (Figure 2.4).

Interleukin-12. During gestation ($p = 0.001$), at d 7 and d 14 post-treatment (d 91 and 98 of gestation), PRO-sows had greater IL-12 than did CON-sows. However, by d 21 and d 28 post-treatment (d 105 and d 112 of gestation), CON-sows had greater IL-12 than did PRO-sows (Figure 2.5).

Experiment 2: Treatment x Day Effects during the Lactation Period

Plasma Cortisol. On d 31 post-treatment (24-hours post-farrowing) PRO-sows had lower plasma cortisol than did CON-sows ($p = 0.001$). At d 51 post-treatment (at weaning), plasma cortisol was lower regardless of treatment. PRO-sows had lower plasma cortisol (43.3 ± 6.9, ng/mL) than did the CON-sows (65.5 ± 8.5, ng/mL) at the end of lactation (Figure 2.6).

Leukocyte Differentials. Leukocyte differentials were also affected by treatment during lactation ($p \leq 0.05$). Once sows were moved to farrowing, PRO-sows had greater percentages of eosinophils and banded neutrophils (immature) on d 31 and d 51 post-treatment (24-hours post-farrowing and at weaning) when compared to the CON-sows (Table 2.3). Segmented neutrophils and monocytes were greater in CON-sows on d 31 and d 51 post-treatment (24-hours post-farrowing and at weaning) when compared to the PRO-sows (Table 2.3). PRO-sows had a greater percentage of lymphocytes on d 31 and d 51 post-treatment (24-hours post-farrowing and at weaning) compared to the CON-sows (Table 2.3).

Natural Killer Cell Cytotoxicity. Once sows moved into farrowing ($p = 0.02$), on d 31 post-treatment (24-hours post-farrowing) the PRO-sows had lower NK cytotoxicity than the CON-sows, but by d 51 post-treatment (at weaning), PRO-sows had greater NK cytotoxicity than did CON-sows (Figure 2.7).

Neutrophil Chemotaxis C5a. At d 31 post-treatment (24-hours post-farrowing) during lactation, CON-sows had greater chemotaxis than did PRO-sows ($p = 0.001$) (Figure 2.8).

Interleukin-12. During lactation ($p = 0.001$), PRO-sows had greater IL-12 on d 31 and d 51 post-treatment (24-hours post-farrowing and at weaning) when compared to the CON-sows (Figure 2.9).

Sow Body Weight

Sow body weight (kg) on d 0 of treatment (d 84 of gestation), was similar between CON-sows (224.28 ± 6.11, kg) and PRO-sows (224.19 ± 6.46, kg); and similar on d 51 post-treatment (at weaning) between CON-sows (218.90 ± 6.66, kg) and PRO-sows (214.35 ± 10.5, kg) (Figure 2.10).

DISCUSSION

Results of the sow study imply that feeding yeast-derived probiotics to sows during gestation and lactation may affect innate immune status of the sow and her biological responses to farrowing and weaning stresses, but have minimal effects on adaptive immunity with the exception of cytokine IL-12. During gestation, innate immune measures, especially neutrophil chemotaxis and natural killer (NK) cell cytotoxicity were enhanced after 3-weeks of feeding *Saccharomyces cerevisiae boulardii* (*Scb)*, to gestating sows when compared to the control sows, but adaptive immune measures were unchanged. Plasma cortisol of the probiotic-treated sows was also lower than the control sows by day 115 of gestation. In response to farrowing and weaning stressors, the sows fed probiotics still had greater innate immune responsiveness and less activated acute stress responses (e.g., reduced cortisol), especially in response to weaning stress. These data imply that probiotics enhanced the innate immune responsiveness of the sows while reducing stress responsiveness to acute stressors (or in the short-term).

Interestingly, both neutrophil function and NK cell cytotoxicity were the primary innate immune parameters affected by feeding sows probiotics. Innate immune cells such as NK cells act as the first line of defense against viral pathogens (Friberg, 1996), and can be affected by changes in cortisol concentrations. As a subset of lymphocytes, NK cells are capable of controlling the immune response through the secretion of cytokines, with the primary effector function being the lysis of their targets. During gestation, PRO-sows had greater NK starting at 14 days post-treatment and until sows were moved into farrowing crates. This may imply that feeding yeast probiotics to sows during gestation may have beneficial effects on innate immune response of a pregnant and/or lactating sow. Past research using the probiotic *Lactobacillus casei* strain Shirota (*Lc*S) indicated that depletion of monocytes greatly reduced the effect of *Lc*S on lymphocyte activation, cytokine production, and natural killer cell activity (Dong et al., 2010), whereas, we found an increase in monocytes and increase in NK cytotoxicity when sows were fed a yeast-probiotic (*Scb*). It can be speculated that NK cytotoxicity was enhanced among probiotic treated sows because of stimulatory effects of IL-12. Therefore, it is possible that continuing to feed probiotics during lactation may have also minimized the suppressive effects of weaning stress on NK cytotoxicity since these sows had greater NK cytotoxicity and less cortisol following weaning. For example, once sows moved on to the lactation period, PRO-sows had greater NK at d 51 post-treatment (at weaning), which may indicate that the probiotic treatment

benefited the PRO-sows not only during gestation but in response to weaning stress, with enhanced innate immune response and reduced stress responsiveness. Interestingly, there was no effect of either stressor on neutrophil chemotaxis even though during gestation probiotics affected this measure and at times neutrophils were increased in the periphery.

As was stated previously, it is possible that the increase in IL-12 among probiotic treated sows may have also had a stimulatory effect on NK cytotoxicity. Even though adaptive immune measures were not affected by treatment, IL-12, a pro-inflammatory cytokine that activates T-helper 1 cells which play a role in cell-mediated immunity (Trinchieri, 1995), was greater among the probiotic sows during gestation; but more importantly was stimulated in response to both farrowing and weaning stress. An *in vitro* study conducted by Shida et al. (2006), found that heat-killed *Lactobacillus casei* strain Shirota, **(***Lc*S) stimulated the IL-12 production. This study may suggest that the bacterial supplemented *Lc*S probiotic modulates cytokine production, and that its effects appear to be strain-specific. The study by Shida et al. indicates that heat-killed bacterial probiotic preparations are capable of stimulating IL-12, which is also true of the yeast probiotic supplement, *Scb*, which was fed to PRO-sows in this study.

Elevated plasma cortisol is indicative of an activated stress axis (hypothalamic-pituitary-adrenal (HPA) axis) or a need to mobilize energy due to low glucose concentrations. Greater concentrations of plasma cortisol may suppress the immune system, decrease bone formation/muscle wasting, and/or lead to proteolysis (breakdown of proteins) in the body (Herman et al., 2016). In this study, plasma cortisol was lower for the control sows until d 21 post-treatment (d 105 of gestation) when the probiotic treated sows had lower plasma cortisol; these sows also had lower plasma cortisol in response to farrowing and weaning stresses. This may indicate that feeding yeast probiotics to sows may diminish the natural increase in plasma cortisol at the time of farrowing and weaning, while lowering baseline cortisol in pregnant sows (Anil et al., 2005; Tsuma et al., 1995). It should also be noted that despite the differential stress response between the probiotic and control sows, that on the day probiotic treatment was initiated (d 84 of gestation), the PRO-sows had significantly higher cortisol; however, it is plausible that this was due to control sows always being bled first and that early on in the study, the commotion and handling may affected the PRO-sows. Previous work by McGlone et al. (1993) found that pigs in adjacent pens were more affected by bleeding than pigs that were housed in the same pen. Regardless, these data imply that feeding sows probiotics during

gestation and lactation did affect plasma cortisol throughout gestation and lactation because PRO-sows did not have a greater stress response at weaning, even though the control sows were weaned and bled before the PRO-sows.

Finally, feeding probiotics to sows also affects total white blood cell counts and differential leukocyte populations, especially neutrophils and monocytes during gestation. Neutrophils serve as the primary defense against bacterial infection and physiological stress (Black, 2011); while blood monocytes, once migrated into the tissue, serve as a secondary line of defense against infection and some inflammatory diseases. During gestation, mature neutrophils were increased during the first 3-weeks of feeding probiotics to sows during gestation, while during the last 3-weeks of gestation, monocytes were greater among probiotic treated sows. This may partly explain the lack of effects on adaptive immune measures in response to the probiotic treatment. Interestingly, greater percentages of immature neutrophils were prevalent among the control sows, often seen early in the response to infection and stress (Black, 2011), which may indicate that these sows were activating early immune defenses during the first 3-weeks of the gestational treatment.

Previous research by Luke (1953) on the differential leukocyte count in the normal pig suggested that the overall increase in the total white cells may be associated with both the older age of the pig and the time of bleeding, thereby causing a variation in the total white blood cell count. This may explain why the CON and PRO sows had differing leukocyte differentials, in addition to higher plasma cortisol concentrations, in response to the beginning and end of the gestational treatment period. It implies that at the different days of treatment during the gestational period, the sows had to elicit specific immune responses to cope with potential baseline stressors, whether that occurred earlier or later in the gestation period. During the lactation period, at d 31 and d 51 post-treatment (24-hours post-farrowing and at weaning), PRO-sows had greater percentages of eosinophils, banded neutrophils, and lymphocytes than CON-sows did. Feeding sows the probiotic boluses may have aided the PRO-sows in having less responsiveness to farrowing and weaning stress, as can be seen by the greater percentages of the leukocyte differential. Segmented neutrophils and monocytes were not affected by the probiotic treatment during the lactation period. However PRO-sows did have greater effects during the middle of the gestation treatment.

Conclusion

During the sow study, it was hypothesized that feeding the yeast probiotic, *Saccharomyces cerevisiae boulardii* (*Scb*), may enhance both innate and adaptive immune measures of the gestating sow and affect both her immune response and stress-responsiveness to farrowing and weaning stressors. After three weeks of feeding probiotics, innate immune measures differed, with PRO-sows having more activated responses by d 105 of gestation compared with the control sows. Overall, feeding sows yeast probiotics during late gestation and through lactation resulted in greater innate immune status and reduced baseline plasma cortisol throughout gestation and lactation compared with the control sows. More specifically, at d 31 post-treatment (24-h post-farrowing), the PRO-sows immune profile was different than that of the CON-sows, with greater cytokine IL-12, and leukocyte differential (percentage of eosinophils, banded neutrophils, and lymphocytes), as well as lower plasma cortisol in response to farrowing stress; while CON-sows had greater natural killer cell cytotoxicity, neutrophil chemotaxis C5a, and leukocyte differential (percentage of segmented neutrophils and monocytes) at 24-hours post-farrowing. Again at d 51 post-treatment (at weaning), PRO-sows had a greater overall immune profile, including natural killer cell cytotoxicity, cytokine IL-12, and leukocyte differential (percentage of eosinophils, banded neutrophils, and lymphocytes), as well as lower plasma cortisol in response to weaning stress.

It is possible that feeding PRO-sows the yeast probiotic supplementation during the periods of gestation and lactation may positively impact innate immune responses and diminish the stress responsiveness to common stressors that are unavoidable during gestation and lactation. Greater responses to innate and adaptive measures, including having lower plasma cortisol during the lactation period, further expresses the interactive effects that may have aided the PRO-sows in having a decreased stress response to farrowing and weaning stressors. However, results varied between the CON and PRO sows in regards to the measures that were assessed. Feeding modified gestation diets to sows throughout pregnancy may reduce the incidence of infectious disease outbreaks, thereby optimizing health so that the need for sub-therapeutic antibiotics declines. Therefore, the supplementation of the yeast probiotic boluses, *Scb*, during the period of gestation to weaning did have an impact on the immune status and stress response of PRO-sows during the gestation and lactation periods.

TABLES

Table 2.1 Effect of treatment on sow plasma cortisol, total WBC counts, and differential WBC during gestation[1]

Item	Control (n = 9)	Probiotic (n = 9)	P-Value
Plasma cortisol, ng/mL			0.002*
d7	31.3 ± 4.60	36.5 ± 7.90	
d14	29.9 ± 4.50[a]	47.1 ± 6.30[b]	
d21	45.0 ± 6.00[a]	36.9 ± 4.90[b]	
d28	45.6 ± 5.00	47.5 ± 6.20	
White Blood Cell (WBC), 10^7			0.99
d0	1.89 ± 0.05	2.00 ± 0.10	
d7	2.17 ± 0.15	2.39 ± 0.22	
d14	2.07 ± 0.11	2.45 ± 0.30	
d21	2.01 ± 0.21	2.43 ± 0.36	
d28	2.23 ± 0.22	2.28 ± 0.25	
Neutrophils,10^6			0.99
d0	3.83 ± 0.31	4.00 ± 0.42	
d7	3.68 ± 0.36	3.33 ± 0.55	
d14	3.47 ± 0.30	3.21 ± 0.27	
d21	3.60 ± 0.37	3.44 ± 0.58	
d28	3.39 ± 0.30	3.79 ± 0.38	
Lymphocytes, 10^6			0.98
d0	2.63 ± 0.30	2.34 ± 0.32	
d7	2.78 ± 0.20[a]	3.62 ± 0.63[b]	
d14	2.47 ± 0.21	2.37 ± 0.17	
d21	2.36 ± 0.16	2.39 ± 0.37	
d28	2.34 ± 0.20	1.83 ± 0.12	
Eosinophils, %			0.05*
d0	6.00 ± 0.33	6.11 ± 0.53	
d7	5.22 ± 1.19	5.56 ± 1.16	
d14	5.44 ± 0.85[a]	3.89 ± 0.63[b]	
d21	5.44 ± 1.04[a]	4.22 ± 0.61[b]	
d28	3.89 ± 0.77[a]	5.44 ± 0.84[b]	
Banded Neutrophils, %			0.001*
d7	3.00 ± 1.48	2.89 ± 1.49	
d14	1.78 ± 0.76[a]	1.11 ± 0.48[b]	
d21	3.44 ± 1.75	3.67 ± 2.13	
d28	5.00 ± 2.51	4.22 ± 2.16	
Segmented Neutrophils, %			0.001*
d0	28.4 ± 3.88	31.1 ± 2.49	
d7	31.3 ± 1.78[a]	44.4 ± 5.12[b]	
d14	32.8 ± 3.40[a]	41.4 ± 2.26[b]	
d21	33.7 ± 2.58[a]	39.1 ± 2.19[b]	

Table 2.1 (Continued)

d28	40.2 ± 3.40[a]	37.7 ± 2.77[b]	
Lymphocytes, %			0.001*
d7	56.0 ± 2.40[a]	44.4 ± 4.32[b]	
d14	58.6 ± 4.24[a]	51.1 ± 2.44[b]	
d21	53.4 ± 3.58[a]	48.7 ± 2.40[b]	
d28	47.0 ± 2.59	46.3 ± 2.58	
Monocytes, %			0.001*
d0	2.89 ± 0.87	2.89 ± 0.59	
d7	3.33 ± 0.91	2.67 ± 0.7[b]	
d14	1.33 ± 0.37[a]	2.44 ± 0.80[b]	
d21	3.78 ± 0.96	4.33 ± 1.01	
d28	3.78 ± 0.72	4.22 ± 0.63	

[*] Treatment x Day effect across gestation phase.
[a,b] Within a row, means without a common superscript differ ($p \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE
Days of gestation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).

Table 2.2 Effect of treatment on sow functional measures during gestation[1]

Item	Control (n = 9)	Probiotic (n = 9)	P-Value
Phagocytosis, %			0.33
d0	48.0 ± 4.66	49.2 ± 2.65	
d7	41.8 ± 4.13	43.8 ± 4.71	
d14	59.7 ± 4.01[c]	56.8 ± 3.48	
d21	59.1 ± 3.87[a]	54.5 ± 2.40[b]	
d28	49.1 ± 3.64[a]	63.1 ± 3.71[b]	
Natural killer cells (NK) 12.5, %			0.001*
d7	8.04 ± 1.99	7.47 ± 1.56	
d14	29.3 ± 5.72[a]	52.1 ± 15.0[b]	
d21	30.5 ± 5.19[a]	48.6 ± 5.45[b]	
d28	43.3 ± 10.7[a]	59.1 ± 19.9[b]	
Chemotaxis C5a, Number of cells/4 fields			0.01*
d7	7.75 ± 3.53[a]	14.1 ± 2.66[b]	
d14	7.92 ± 2.36[a]	5.08 ± 0.49[b]	
d21	5.42 ± 2.05[a]	9.33 ± 2.46[b]	
d28	9.75 ± 2.51	7.25 ± 1.89	
Chemotaxis IL-8, Number of cells/4 fields			0.02*
d7	12.3 ± 3.38[a]	19.4 ± 3.93[b]	
d14	11.7 ± 1.75[a]	7.06 ± 1.34[b]	
d21	10.7 ± 2.11	9.33 ± 1.26	
d28	10.7 ± 2.73	13.1 ± 2.37	
Interleukin-12 (IL-12 Elisa), pg/mL			0.001*
d7	174.40 ± 21.3	183.63 ± 21.8	
d14	185.21 ± 14.7	198.16 ± 20.5	
d21	210.14 ± 32.8[a]	180.42 ± 19.1[b]	
d28	180.44 ± 19.9	180.30 ± 12.0	
Concanavalin A Proliferation (ConA), 20.0			0.97
d7	1.41 ± 0.26[a]	1.22 ± 0.08[b]	
d14	1.28 ± 0.19	0.92 ± 0.11	
d21	1.90 ± 0.34[a]	1.34 ± 0.07[b]	
d28	1.81 ± 0.22[a]	1.31 ± 0.10[b]	
Lipopolysaccharide Proliferation (LPS), 20.0			0.98
d7	1.51 ± 0.33	1.83 ± 0.36	
d14	1.95 ± 0.49	1.24 ± 0.20	
d21	1.39 ± 0.20	1.28 ± 0.18	
d28	1.33 ± 0.09[a]	1.13 ± 0.02[b]	

[*] Treatment x Day effect across gestation phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE

Table 2.2 (Continued)

Days of gestation are equivalent to experimental days: d84 = d 0 of treatment (baseline, began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).

Table 2.3 Effect of treatment on sow plasma cortisol, total WBC counts, and differential WBC during lactation[1]

Item	Control (n = 9)	Probiotic (n = 9)	P-Value
Plasma cortisol, ng/mL			0.001*
d28	45.6 ± 5.00	47.5 ± 6.20	
d31	79.5 ± 9.80[a]	64.6 ± 7.10[b]	
d51	65.5 ± 8.50[a]	43.3 ± 6.90[b]	
White Blood Cell (WBC), 10^7			0.37
d28	2.23 ± 0.22	2.28 ± 0.25	
d31	1.38 ± 0.16[c]	2.39 ± 0.42[d]	
d51	1.92 ± 0.22	2.30 ± 0.24	
Neutrophils, 10^6			0.56
d28	3.39 ± 0.30	3.79 ± 0.38	
d31	3.20 ± 0.63	4.28 ± 0.59	
d51	3.56 ± 0.30	3.58 ± 0.35	
Lymphocytes, 10^6			0.66
d28	2.34 ± 0.20	1.83 ± 0.12	
d31	2.00 ± 0.35	2.24 ± 0.33	
d51	2.25 ± 0.39	1.64 ± 0.15	
Eosinophils, %			0.001*
d31	3.11 ± 1.18	3.22 ± 1.05	
d51	4.22 ± 0.62[a]	5.11 ± 1.06[b]	
Banded Neutrophils, %			0.001*
d28	5.00 ± 2.51	4.22 ± 2.16	
d31	12.7 ± 5.40	12.9 ± 5.66	
d51	1.89 ± 0.87	2.33 ± 1.11	
Segmented Neutrophils, %			0.001*
d31	38.9 ± 7.06[a]	33.3 ± 3.80[b]	
d51	45.4 ± 2.06[a]	37.7 ± 3.21[b]	
Lymphocytes, %			0.001*
d28	47.0 ± 3.10	46.3 ± 2.58	
d31	42.1 ± 3.35	45.6 ± 3.18	
d51	45.7 ± 2.62	51.6 ± 4.63	
Monocytes, %			0.001*
d28	3.78 ± 0.72	4.22 ± 0.63	
d31	3.33 ± 0.76	3.00 ± 1.14	
d51	3.11 ± 0.99[a]	2.67 ± 0.49[b]	

[*] Treatment x Day effect across lactation phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE

Table 2.3 (Continued)

Days of lactation are equivalent to experimental days: d 112 = d 28 of treatment (sows moved into farrowing crates; baseline), d 115 = d 31 of treatment (24-hours post-farrowing), and d 135 = d 51 of treatment (piglets weaned from sows).

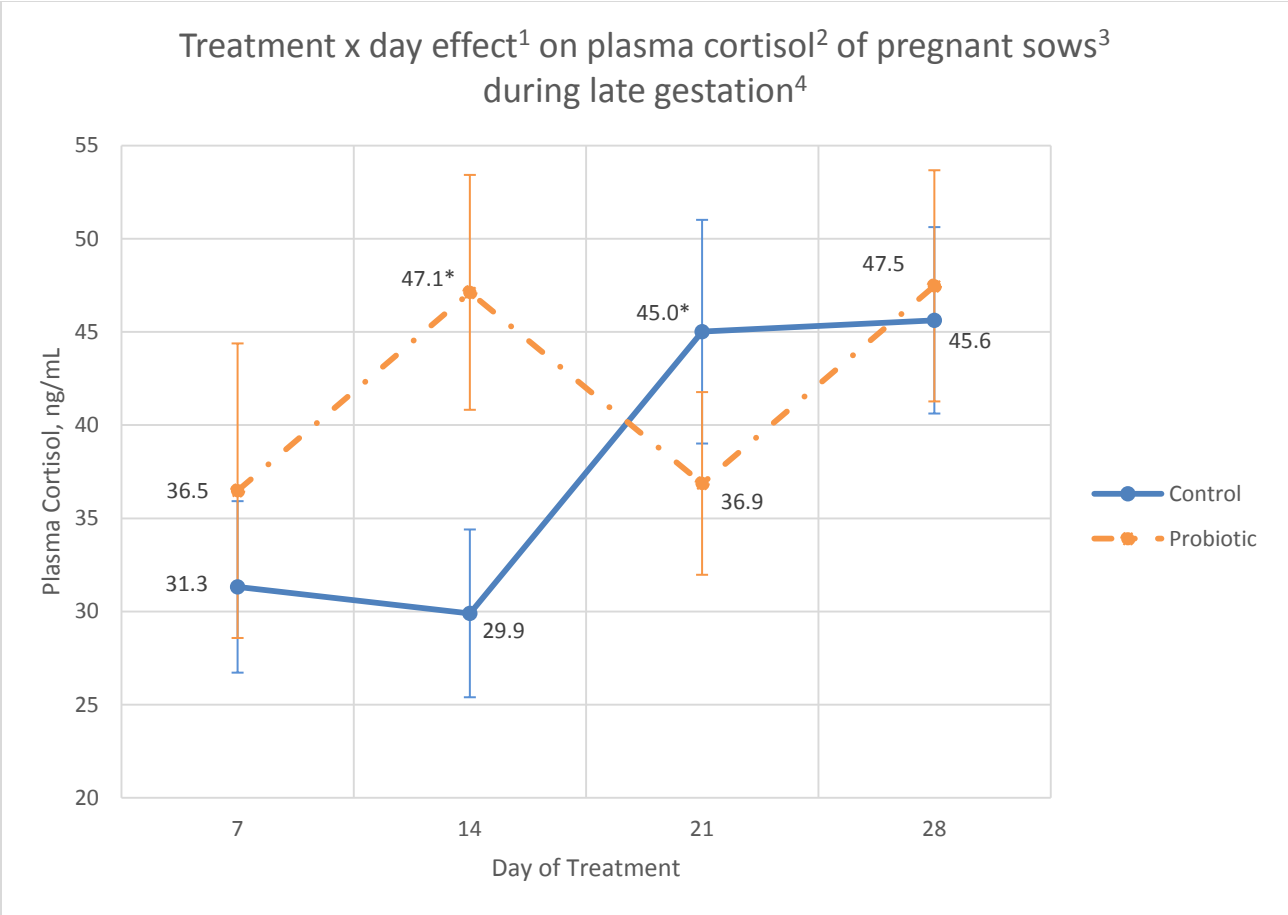
Table 2.4 Effect of treatment on sow functional measures during lactation[1]

Item	Control (n = 9)	Probiotic (n = 9)	P-Value
Phagocytosis, %			0.61
d31	60.1 ± 5.45	60.4 ± 6.48	
d51	54.9 ± 3.89	54.0 ± 5.28	
Natural killer cells (NK) 12.5, %			0.02*
d31	30.8 ± 3.32[a]	19.8 ± 1.14[b]	
d51	67.8 ± 20.3	78.9 ± 15.0	
Chemotaxis C5a, Number of cells/4 fields			0.001*
d28	9.75 ± 2.51	7.25 ± 1.89	
d31	32.8 ± 11.0[a]	22.4 ± 3.04[b]	
Interleukin-12 (IL-12 Elisa), pg/mL			0.001*
d28	180.44 ± 19.9	180.30 ± 12.0	
d31	147.82 ± 17.2	149.96 ± 9.73	
d51	201.67 ± 18.2	219.92 ± 19.3	
Concanavalin A Proliferation (ConA), 20.0			0.88
d28	1.81 ± 0.22	1.31 ± 0.10	
d31	1.83 ± 0.22	2.00 ± 0.30	
d51	1.48 ± 0.13	2.08 ± 0.26	
Lipopolysaccharide Proliferation (LPS), 20.0			0.86
d28	1.33 ± 0.09[a]	1.13 ± 0.02[b]	
d31	1.16 ± 0.06	1.34 ± 0.09	
d51	1.17 ± 0.12	1.23 ± 0.20	

[*] Treatment x Day effect across lactation phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE
Days of lactation are equivalent to experimental days: d 112 = d 28 of treatment (sows moved into farrowing crates; baseline), d 115 = d 31 of treatment (24-hours post-farrowing), and d 135 = d 51 of treatment (piglets weaned from sows).

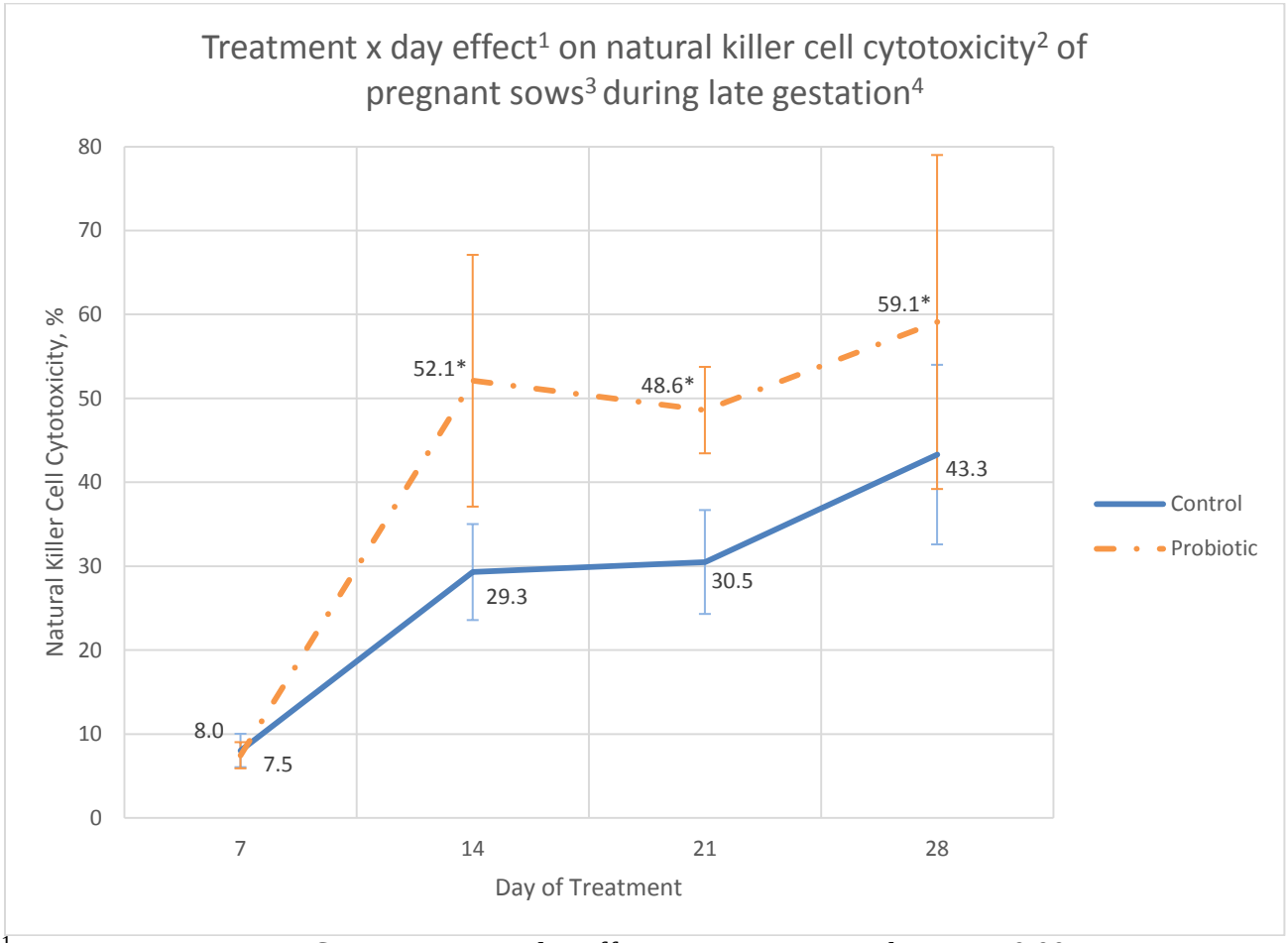
FIGURES

Figure 2.1 Treatment x day effect[1] on plasma cortisol[2] of pregnant sows[3] during late gestation[4]



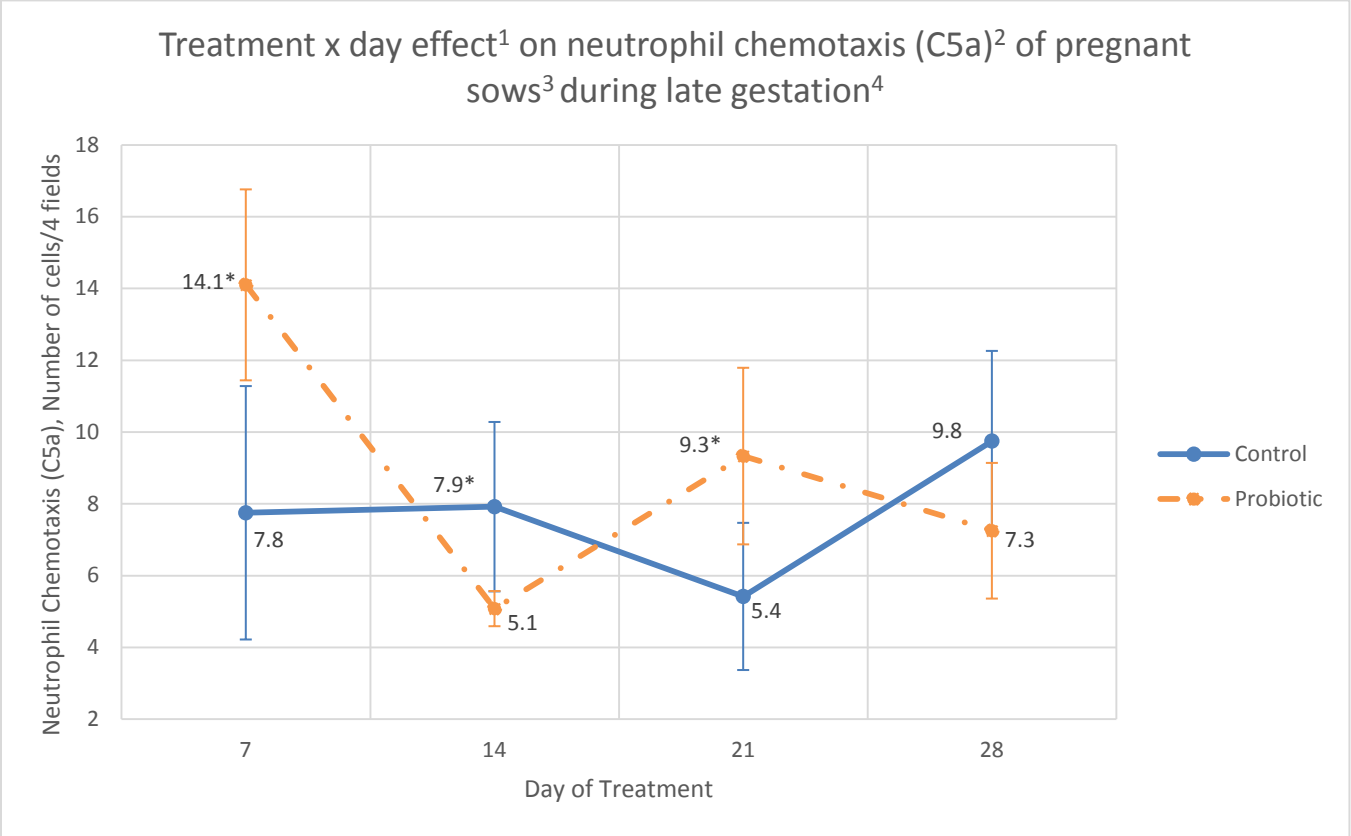
[1] Least squares means ± SE; Treatment x day effect across gestation phase; p = 0.002
[2] Plasma cortisol, ng/mL
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of gestation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).
*Significant ($p \leq 0.05$) at d 14 and d 21 post-treatment.

Figure 2.2 Treatment x day effect[1] on natural killer cell cytotoxicity[2] of pregnant sows[3] during late gestation[4]



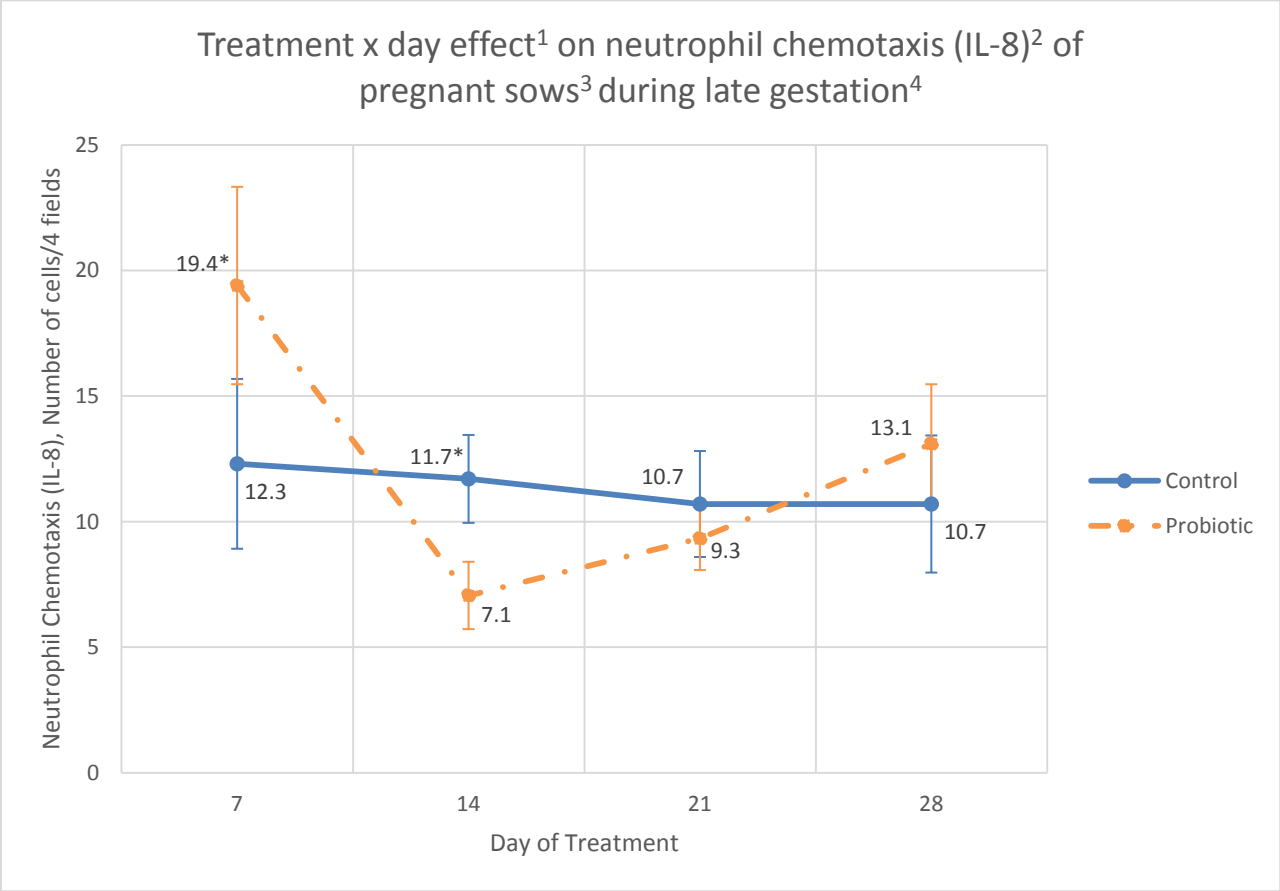
[1] Least squares means ± SE; Treatment x day effect across gestation phase; p = 0.001
[2] Cytotoxicity %, 12.5:1 effector to target
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of gestation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).
*Significant ($p \leq 0.05$) at d 14, d 21, and d 28 post-treatment.

Figure 2.3 Treatment x day effect[1] on neutrophil chemotaxis (C5a)[2] of pregnant sows[3] during late gestation[4]



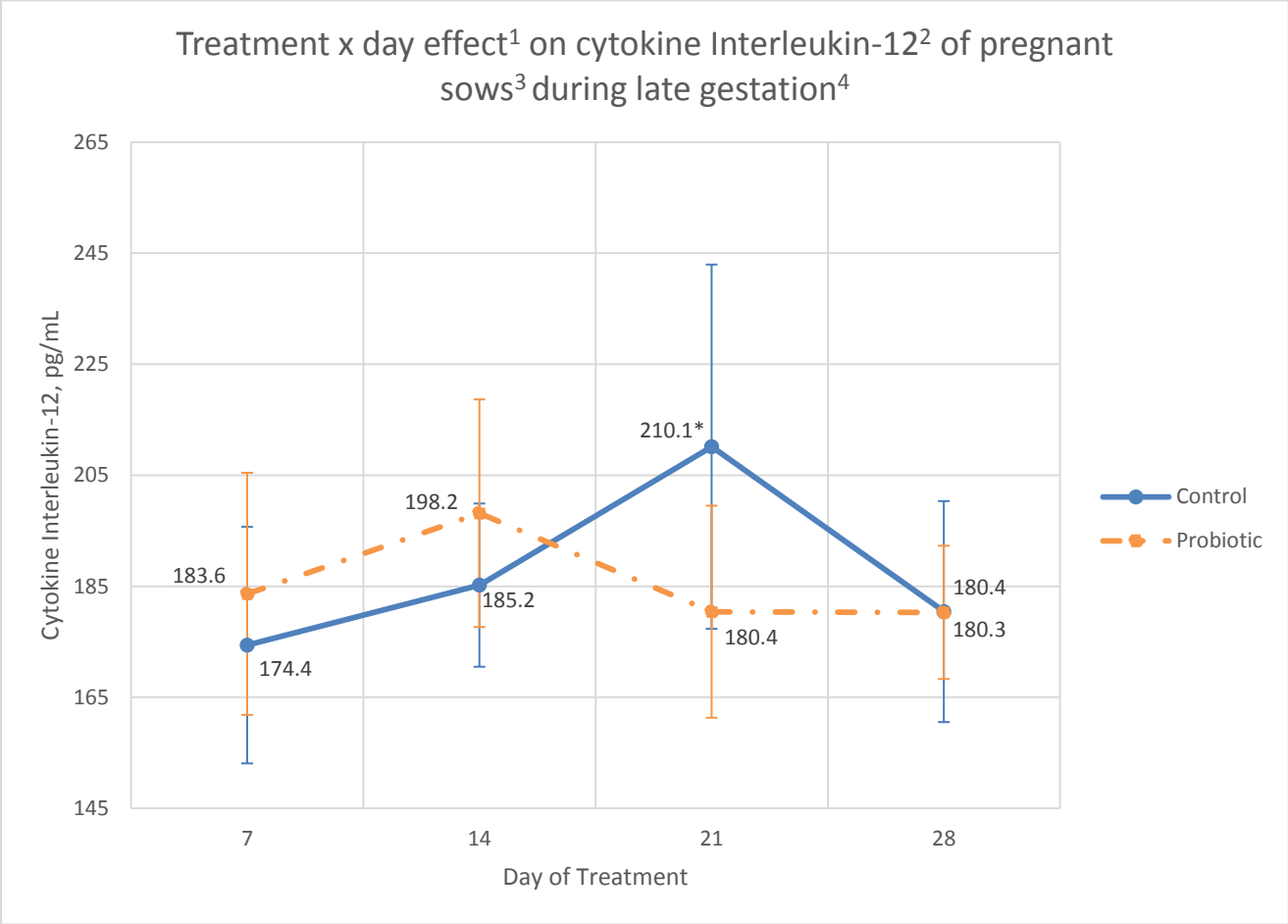
[1] Least squares means ± SE; Treatment x day effect across gestation phase; p = 0.01
[2] Neutrophil chemotaxis (C5a), number of cells/4 fields
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of gestation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).
*Significant ($p \leq 0.05$) at d 7, d 14, and d 21 post-treatment.

Figure 2.4 Treatment x day effect[1] on neutrophil chemotaxis (IL-8)[2] of pregnant sows[3] during late gestation[4]



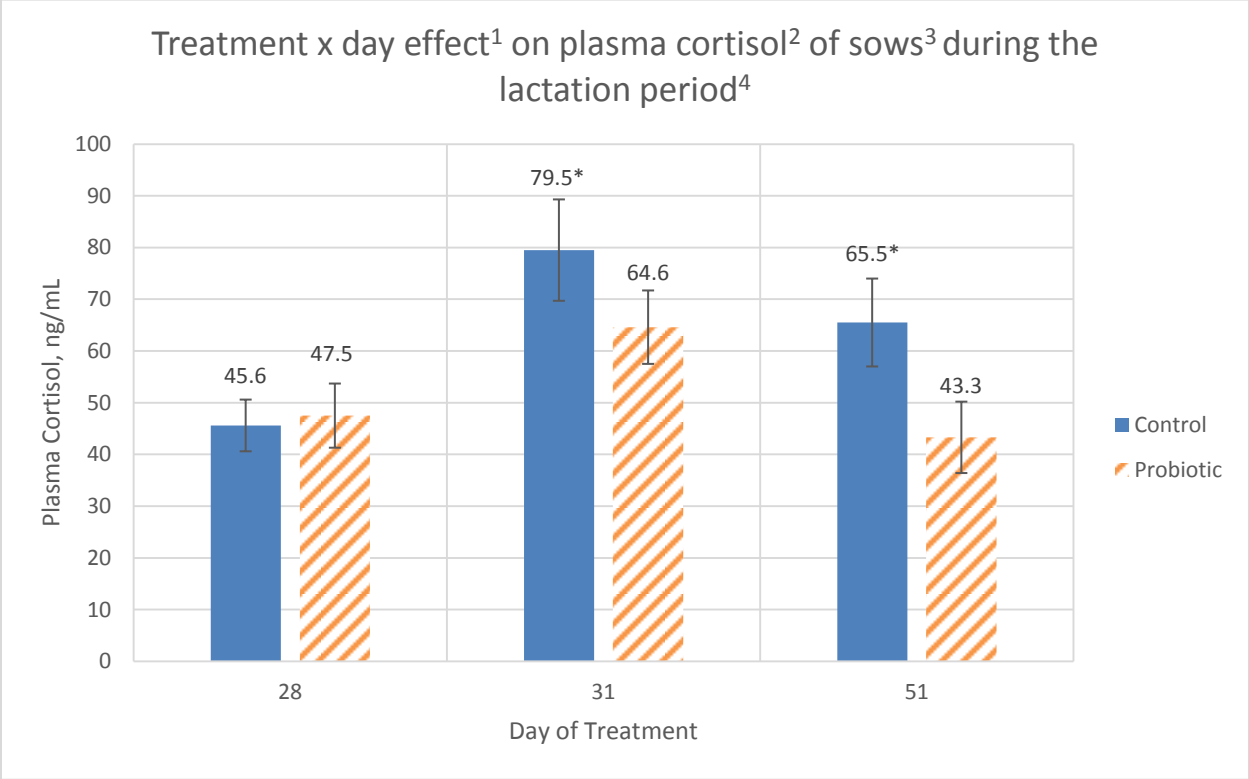
[1] Least squares means ± SE; Treatment x day effect across gestation phase; p = 0.02
[2] Neutrophil chemotaxis (IL-8), number of cells/4 fields
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of gestation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).
*Significant ($p \leq 0.05$) at d 7 and d 14 post-treatment.

Figure 2.5 Treatment x day effect[1] on cytokine Interleukin-12[2] of pregnant sows[3] during late gestation[4]



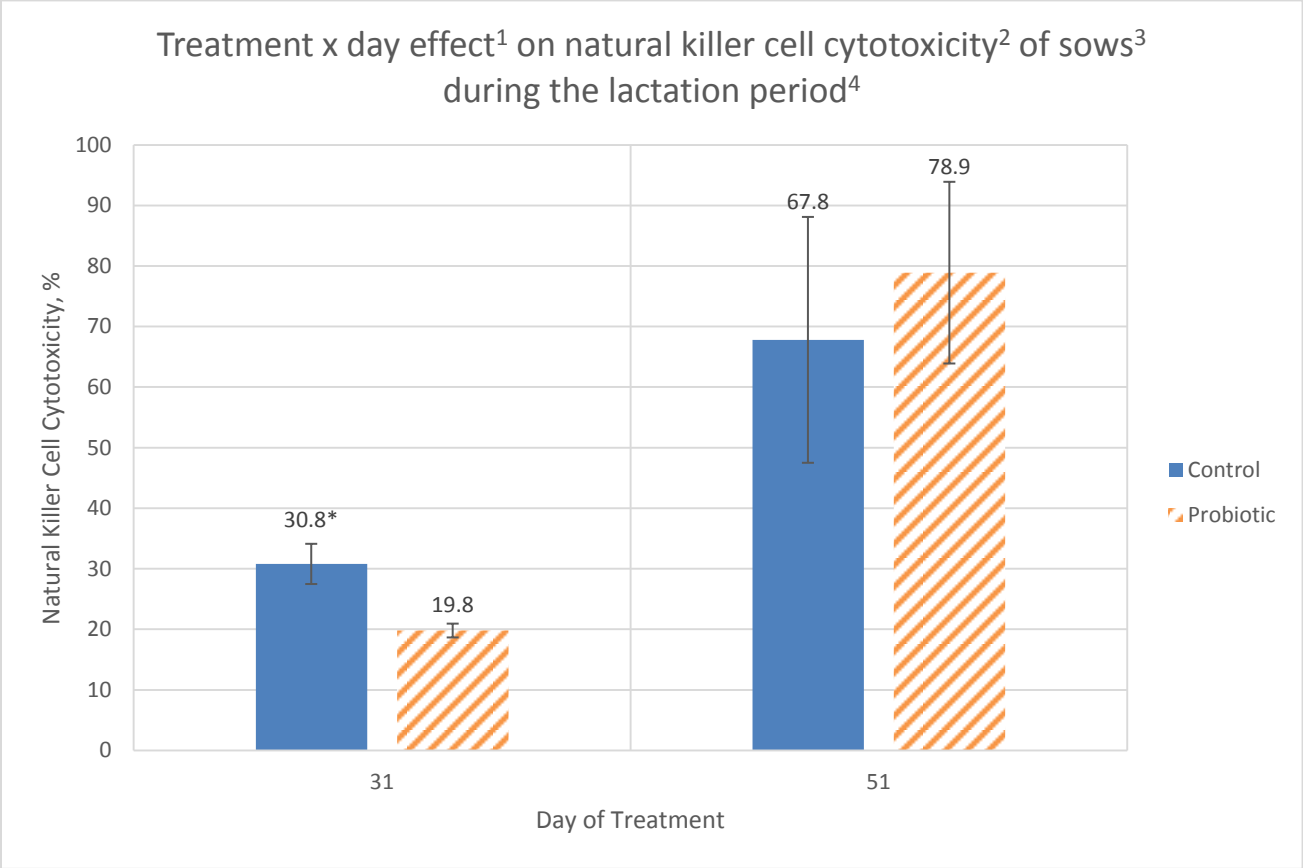
[1] Least squares means ± SE; Treatment x day effect across gestation phase; p = 0.001
[2] Interleukin-12 (IL-12), pg/mL
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of gestation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); d 91 = d 7 of treatment; d 98 = d 14 of treatment; d 105 = d 21 of treatment; d 112 = d 28 of treatment (sows moved into farrowing crates).
*Significant ($p \leq 0.05$) at d 21 post-treatment.

Figure 2.6 Treatment x day effect[1] on plasma cortisol[2] of sows[3] during the lactation period[4]



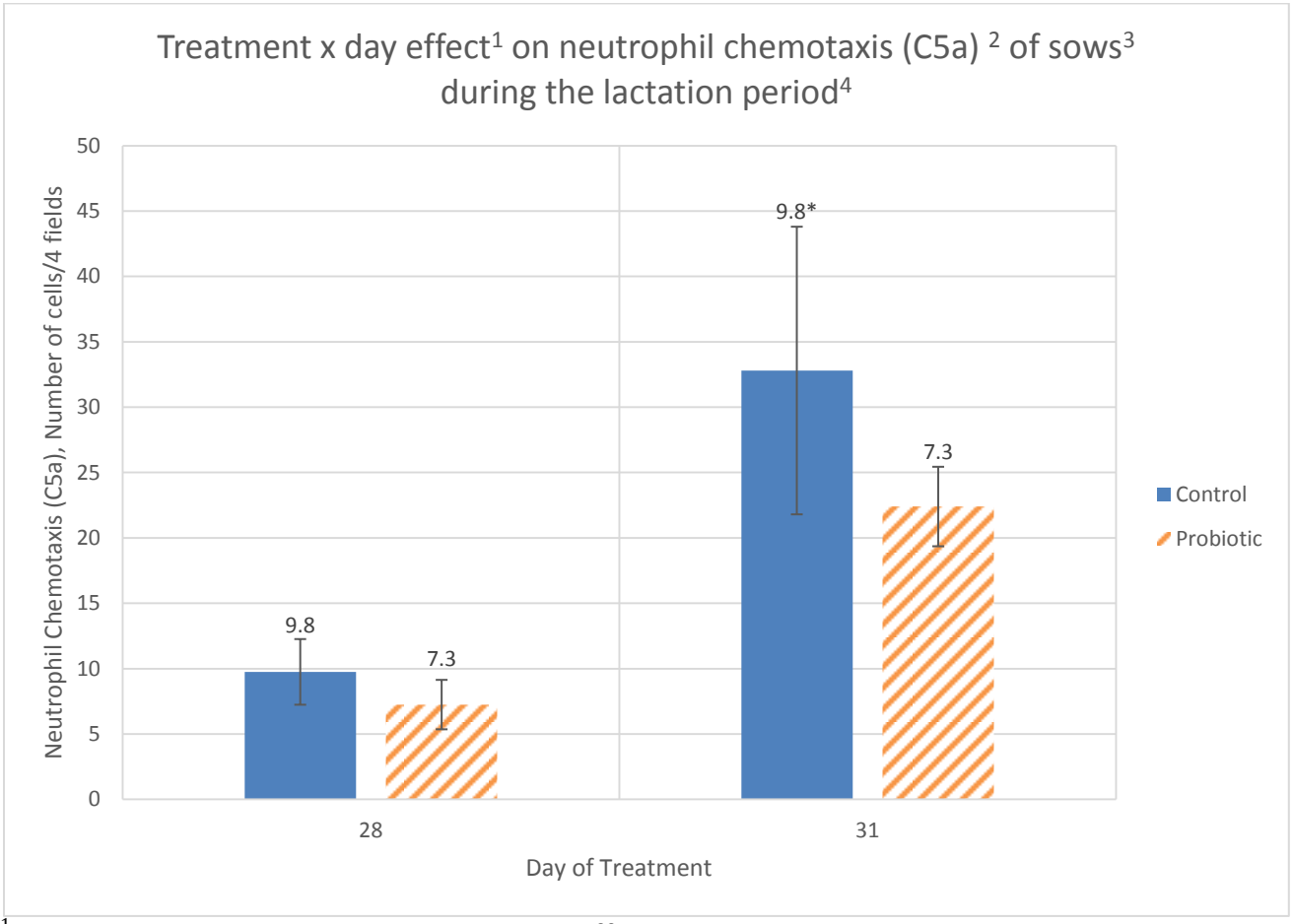
[1] Least squares means ± SE; Treatment x day effect across lactation phase; p = 0.001
[2] Plasma cortisol, ng/mL
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of lactation are equivalent to experimental days: d 112 = d 28 of treatment (sows moved into farrowing crates; baseline), d 115 = d 31 of treatment (24-hours post-farrowing), and d 135 = d 51 of treatment (piglets weaned from sows).
*Significant ($p \leq 0.05$) at d 31 and d 51 post-treatment.

Figure 2.7 Treatment x day effect[1] on natural killer cell cytotoxicity[2] of sows[3] during the lactation period[4]



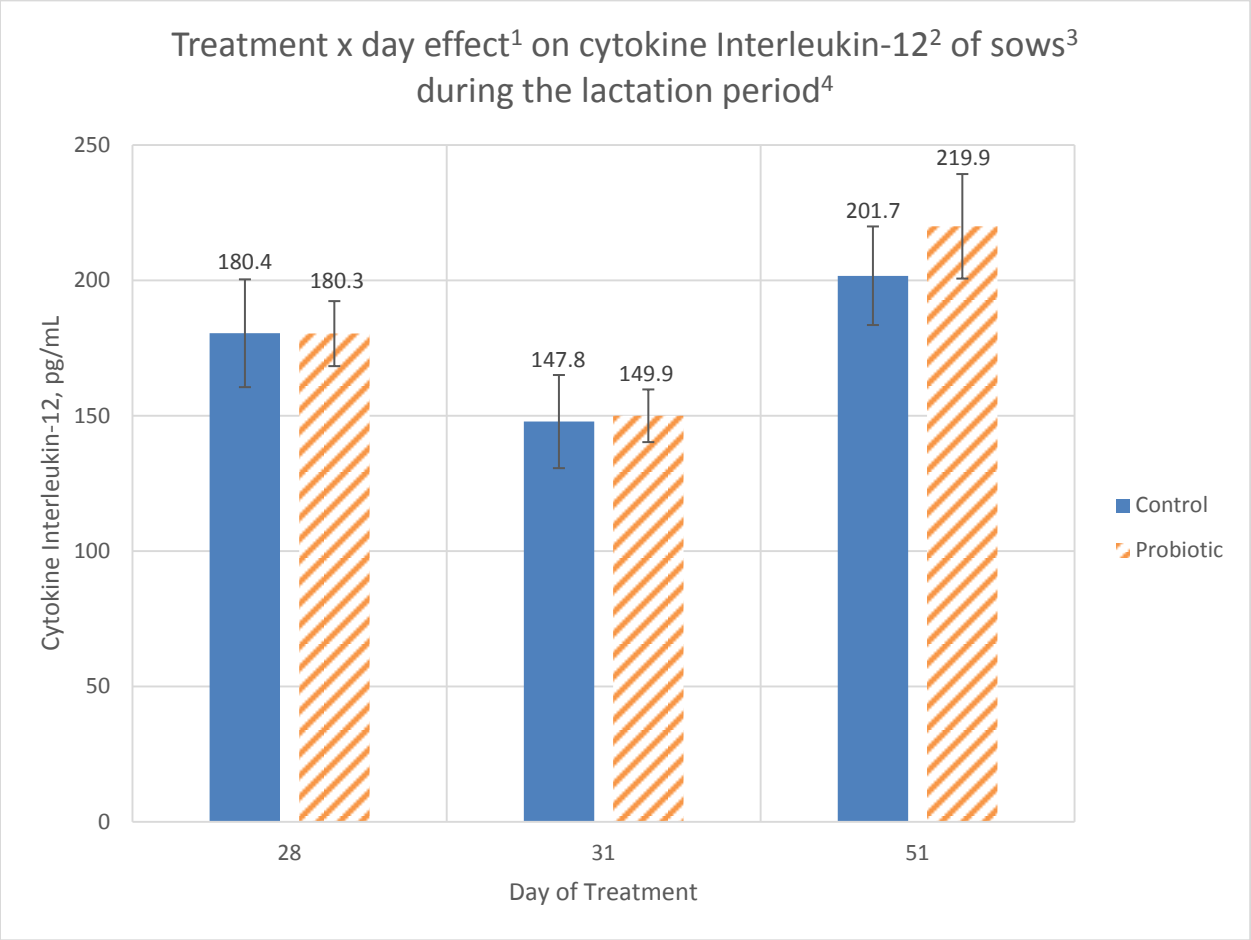
[1] Least squares means ± SE; Treatment x day effect across lactation phase; p = 0.02
[2] Cytotoxicity %, 12.5:1 effector to target
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of lactation are equivalent to experimental days: d 112 = d 28 of treatment (sows moved into farrowing crates; baseline), d 115 = d 31 of treatment (24-hours post-farrowing), and d 135 = d 51 of treatment (piglets weaned from sows).
*Significant ($p \leq 0.05$) at d 31 post-treatment.

Figure 2.8 Treatment x day effect[1] on neutrophil chemotaxis (C5a) [2] of sows[3] during the lactation period[4]



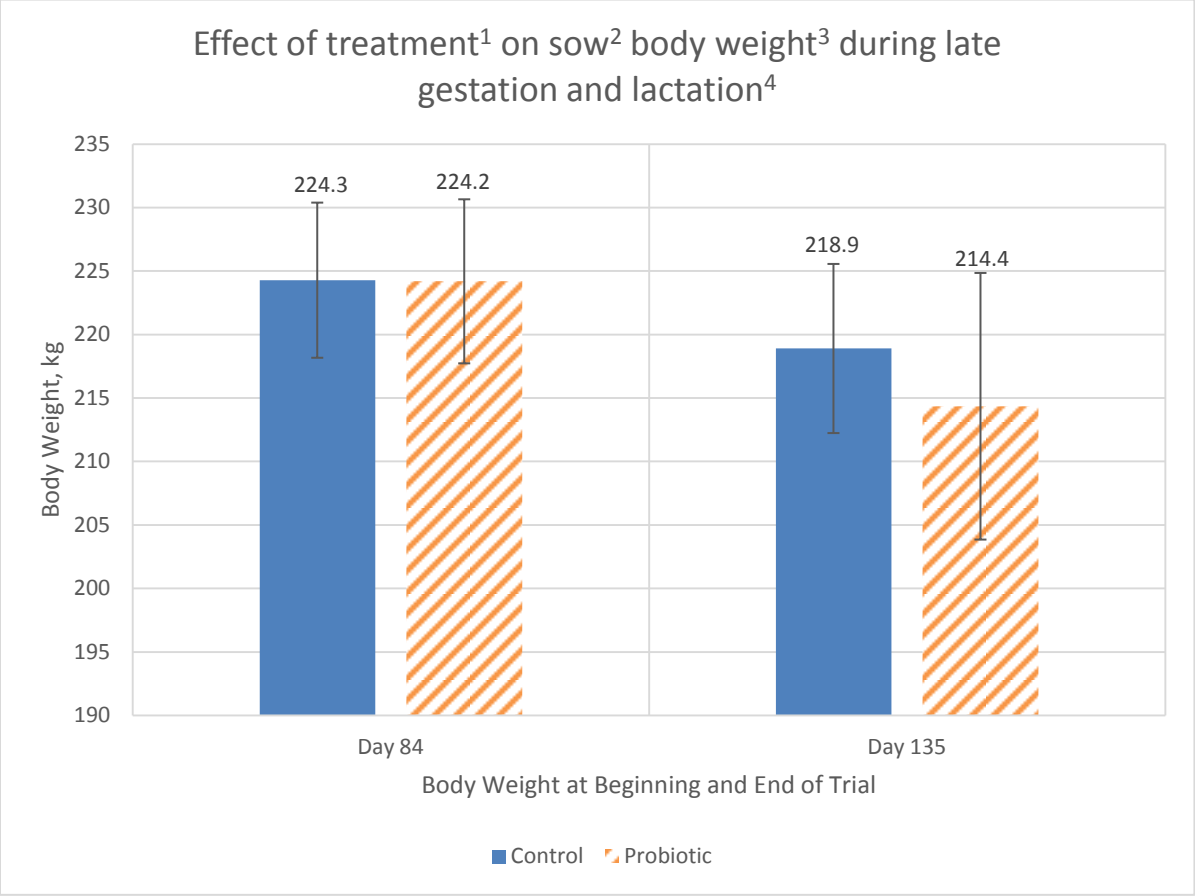
[1] Least squares means ± SE; Treatment x day effect across lactation phase; p = 0.001
[2] Neutrophil chemotaxis (C5a), number of cells/4 fields
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of lactation are equivalent to experimental days: d 112 = d 28 of treatment (sows moved into farrowing crates; baseline); d 115 = d 31 of treatment (24-hours post-farrowing).
*Significant ($p \leq 0.05$) at d 31 post-treatment.

Figure 2.9 Treatment x day effect[1] on cytokine Interleukin-12[2] of sows[3] during the lactation period[4]



[1] Least squares means ± SE; Treatment x day effect across lactation phase; p = 0.001
[2] Interleukin-12 (IL-12), pg/mL
[3] Control (CON) vs. Probiotic (PRO) sows
[4] Days of lactation are equivalent to experimental days: d 112 = d 28 of treatment (sows moved into farrowing crates; baseline), d 115 = d 31 of treatment (24-hours post-farrowing), and d 135 = d 51 of treatment (piglets weaned from sows).
*Significant ($p \leq 0.05$)

Figure 2.10 Effect of treatment[1] on sow[2] body weight[3] during late gestation and lactation[4]



[1] Least squares means ± SE
[2] Control vs. Probiotic sows
[3] Body Weight, kg
[4] Days of gestation and lactation are equivalent to experimental days: d 84 = d 0 of treatment (baseline; began feeding placebo or yeast probiotic boluses to sows); and d 135 = d 51 of treatment (piglets weaned from sows).
*Significant ($p \leq 0.05$)

CHAPTER 3. THE EFFECT OF MATERNAL-FETAL PROGRAMMING BY TRANSFERRING THE IMMUNE STATUS AND STRESS RESPONSIVENESS OF THE SOW TO THE OFFSPRING DURING FARROWING, PROCESSING, AND WEANING STRESS

INTRODUCTION

The establishment of microbiota in the neonate is dependent on the inter-relationship between the environment and maternal factors (i.e., nutrition). Piglets are born with a sterile, non-colonized gastrointestinal tract without a functional immune system (Romano-Keeler and Weitkamp, 2014). Early colonization events affected by the sow may have profound and long-term effects on the development of mucosal immunity, gut architecture, and microbiota composition of the neonate. Stress during late gestation may impair the development and reactivity of the sow's offspring which impacts disease susceptibility and mortality (Tuchscherer et al., 2002). It has been further speculated that other impingements (stressors) play a crucial role in the ability of the host to utilize innate and adaptive immune responses that may otherwise compromise animal health and lead to disease outcome, or resolve the challenges and achieve homeostasis. A study conducted by Clapperton et al. (2009) emphasized the need for genetic biomarkers capable of predicting offspring's resistance towards infectious diseases. Regardless of health status, the study concluded that innate and adaptive immune traits had strong genetic and phenotypic correlations between average growth rate and performance. In pig production, husbandry practices can cause suppression and/or enhancement of the immune system. Unavoidable production stressors within animal production systems tend to exacerbate the disease process, often by compromising the immune system of an animal (Salak-Johnson and McGlone, 2007). Therefore, the use of innate and adaptive immune biomarkers may serve as metrics for selecting offspring with increased resistance to infectious diseases or with an increased ability to maintain high performance levels during periods of unavoidable stress.

It is hypothesized that sows fed the supplemented yeast probiotic diet, *Saccharomyces cerevisiae boulardii* (*Scb*), during the periods of late gestation until weaning could potentially transfer beneficial immune and stress responsiveness to her offspring. Piglets born to the probiotic-treated sows (PRO-piglets) may have an enhanced responsiveness to farrowing and processing stressors when compared to the piglets born to the control sows (CON-piglets). More

specifically, piglets from PRO-sows will have a greater immune status during the period of suckling (d 0 – d 21 of age) and diminished stress responsiveness (e.g., lower cortisol). During the period of weaning, piglets from PRO-sows will have a greater responsiveness to both the short- and long-term effects of acute stress compared to the CON-piglets. Therefore, the objectives of this study were to assess the effects of feeding yeast probiotics, *Scb*, to sows during late gestation through lactation on the immune status of her piglets from birth till weaning; and stress responsiveness of her piglets in response to farrowing and processing stressors, and to assess the effects on the stress responsiveness of piglets to weaning stress in short- and long-term periods (d 21 – d 35 of age).

MATERIALS AND METHODS

Animals and Experimental Design

All procedures were approved by the University of Illinois at Urbana-Champaign and the Institutional Animal Care and Use Committee (IACUC). A total of eighty-four female piglets, born to sows derived of the Genetiporc maternal line were used in this study. The piglets were selected from 18 litters born to sows fed either probiotics (PRO; n = 9) or placebo (CON; n = 9) during gestation and lactation. All piglets were housed at the University of Illinois Swine Research Center in Urbana-Champaign. Piglets remained with their dams until d 21 of age. At weaning, piglets were moved to the nursery as littermates and penned together. In the nursery, piglets were fed ad libitum, a standard nursery diet, and each nursery pen contained one nipple waterer. All piglets were kept on a 10 h light: 14 h dark schedule, in which lights were on at 0700 h and off at 1700 h.

Piglets from dams that were fed either probiotics (PRO; n = 42) or placebo (CON; n = 42) were randomly selected from each treatment and assessed for immune and endocrine metrics at twoexperimental periods. Each experimental day is equivalent to day-of-age of piglet. At farrowing (d 0 of age) and until weaning (d 21 of age), piglets were housed with their dams and blood samples were taken at d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age (Phase 1). Phase 2 blood samples were taken at d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age. Fixed-effects parameters of treatment (control or probiotic-treated sows) and

day of age for piglets were used for this study; therefore, piglets were not fed a probiotic treatment during this research.

Blood Sample Collection and Leukocyte Differentials

Sows were observed continuously until they farrowed. A birth, piglets were dried off and 1 mL of blood was collected via jugular venipuncture with vacutainers containing sodium heparin or EDTA at (d 0) and 5-10 mL of blood was collected at the remaining time points. The procedure lasted <2 mins. At birth, blood samples and body weights were taken prior to suckling. Heparin-treated whole blood was used to determine total white blood cell (WBC) counts and leukocyte differential counts (DIFF). Total WBC counts were made electronically using a Coulter Z1 particle counter (Beckman Coulter, Miami, FL). Ten microliters (10 μl) of whole blood was added to 10 mL of Beckman Coulter, Isoflow®, red blood cells were lysed with Beckman Coulter lytic reagent, ZAP-OGLOBIN® and then samples were placed in the counting chamber to determine total BBC count. To determine DIFF percentages, whole blood smears were made, fixed in methanol, and then stained with Hema-3 staining system (Fisher Scientific, Houston, TX). Slides were viewed under a light microscope, and 100 cells per slide were visually counted.

Cell Isolation and Plasma Analysis

Whole blood was collected and centrifuged at 700 × g for 30 min at 4℃. Plasma was aspirated and transferred to Eppendorf tubes and stored at -80°C until further analysis. Whole blood was diluted with Roswell Park Memorial Institute (**RPMI**; Gibco, Carlsbad, CA) medium, layered over Histopaque-1077 (density = 1.077g/mL; Sigma) and -1119 (density = 1.119 g/mL; Sigma), and centrifuged at 700 x g for 30 min at 25°C. Lymphocytes were removed from the top of the second layer and neutrophils from the top of the third layer. Red blood cells were lysed from the neutrophil fraction, which was then washed in RPMI and counted. Cell concentrations were adjusted with RPMI based on the immune assay requirements. Whole blood was diluted using Roswell Park Memorial Institute (RPMI) media.

Plasma cortisol and IL-12 were analyzed following manufacturer's protocols. Commercial radioimmunoassay validated for porcine cortisol was measured. Plasma samples from heparin-treated whole blood were assayed for CORT using a Coat-A-Count cortisol kit,

following the manufacturer's protocol (Diagnostic Products Corp., Los Angeles, CA). Briefly, in duplicate, 25 µL of sample or standard were added to antibody-coated tubes. Radiolabeled (I125) CORT was added to tubes and incubated for 45 min at 37°C in a water bath. The liquid phase was decanted and radioactivity counted with a gamma counter. A standard curve based on 0, 10, 50, 100, 200, and 500 µg/mL was used. Intra- and inter-assay CV were 7.0 and 16.5%, respectively. Minimal detectable concentration of CORT using this assay was approximately 2 ng/mL. Porcine IL-12/IL-23 Quantikine kit was used to measure IL-12 in plasma samples (R & D Systems, Minneapolis, MN). Minimal detectable concentration of IL-12/IL-23 using this kit was on average 9.0 pg/mL.

Immune Assays

Neutrophil phagocytosis was measured using a flow cytometry-based assay previously described by Heinzelmann et al. (1999), with minor modifications described by Niekamp et al. (2006). Briefly, fluorescent beads were pre-incubated for 30 min with non-heat-inactivated porcine serum before adding the fluorescent beads to the samples at a 10:1 (beads: neutrophils) ratio. Cells and beads were incubated together for 45 min at room temperature. The percentage of engulfment of beads by neutrophils was evaluated using flow cytometer.

Using Promega CellTiter®, a colorimetric method for determining the number of viable cells in proliferation, cytotoxicity, and chemo sensitivity assays, with slight modifications described by Sutherland et al. (2005), a mitogen-induced lymphocyte proliferation assay was performed. First, lymphocytes from the sow were used at a concentration of 5×10^6 cells/mL, and placed in a sterile 96-well flat-bottom plate (sample run in triplicate per sow). Concanavalin A (ConA) and lipopolysaccharide (LPS), both acquired from Sigma Aldrich, were used as mitogens in order to stimulate T and B cells. ConA and LPS were both used at measurements of 0.2, 2.0, and 20 µg/mL. Plates were incubated for sixty-eight hours at a temperature of 37℃ in a 5% CO_2 humidified incubator. Next, 15µL of Promega Dye was added to each well, and the plates were incubated for an additional four hours. Promega Stop solution (100 µL) was added to each well, and the plates were incubated overnight at a temperature of 37℃. Finally, using BIO-TEK Instruments® microplate reader at a wavelength of 550 nm with reference wavelength 690 nm, results can be expressed as a proliferation index (PI).

$$PI = \frac{\text{Optical Density (550/690 nm) stimulated cells}}{\text{Optical Density (550/690 nm) non-stimulated cells}}$$

A nonradioactive cytotoxicity detection kit acquired from Roche Diagnostics®, also described previously by Sutherland et al. (2005), was used to measure natural killer (NK) cell cytotoxicity. First, sow lymphocytes were used as effector cells, while K-562 chronic human myelogenous leukemia cells (American Tissue Type Culture Collection, Manassas, VA) were used as target cells. Lymphocytes were adjusted to 1 x 10^7 cells/mL, and K-562 cells were adjusted to a constant 10,000 cells per wall. Second, samples were run in triplicate at effector to target-cell ratios of NK 12.5:1, 25:1, 50:1, and 100:1. Third, plates were read using BIO-TEK Instruments® microplate reader at a wavelength of 490 nm with reference wavelength 690 nm, after an eighteen hour incubation period. Finally, percent cytotoxicity was calculated as previously described by Lumpkin and McGlone (1992). An assay was thus considered valid as long as the maximum release, divided by spontaneous release, was ≤ 20%.

Piglet Body Weight

Piglet body weights were recorded at various time points throughout the study. During the suckling period (phase 1), piglet body weights were recorded on days 1A (pre-process), 1B (post-process), 7, 14, and 21 (at weaning) of age. During the weaning period (phase 2), body weights were recorded on d 21 (at weaning), d 22 (24-h post-weaning), d 28 (7 d post-weaning), and d 35 (14 d post-weaning) of age.

Statistical Analysis

Data were analyzed using PROC MIXED with repeated measures SAS (SAS Inst. Inc., Cary, NC), mixed linear models contained both fixed- and random-effects parameters. Data were analyzed for the suckling phase, including days 0, 1A, 1B, 7, 14, and 21 of age. Data were analyzed for the weaning phase, including d 21 (at weaning), d 22 (24-h post-weaning), d 28 (7 d post-weaning), and d 35 (14 d post-weaning) of age. The model included fixed-effects parameters of treatment, control or probiotic, as well as day of age for piglets. Significance of data was set at ($p \leq 0.05$), and trends were also discussed at ($p > 0.05$) to ($p \leq 0.10$).

RESULTS

Experiment 1: Treatment x Day Effects during the Suckling Period

Plasma Cortisol. Plasma cortisol ($p = 0.001$) at farrowing (d 0 of age) for CON-piglets (204.54 ± 10.8, ng/mL) was greater when compared to the PRO-piglets (111.17 ± 11.7, ng/mL). At d 1A (pre-processing of piglets), d 1B (post-processing of piglets), d 14, and d 21 (at weaning) of age, PRO-piglets had greater plasma cortisol than did CON-piglets. At d 7 of age PRO-piglets had lower plasma cortisol than did CON-piglets (Figure 3.1).

Total WBC and Neutrophil and Lymphocyte Counts. Total WBC counts were greater ($p = 0.03$) in CON-piglets at d 0 (birth) and d 7 of age when compared to the PRO-piglets. At d 1A of age (pre-process) and 1B (post-process) PRO-piglets had greater total WBC counts when compared to the CON-piglets (Figure 3.2). At d 14 of age, total WBC counts were similar between treatment groups, but at d 21 of age, PRO-piglets had greater total WBC counts when compared to the CON-piglets (Figure 3.2). At d 7 of age, CON-piglets had a greater ($p = 0.04$) neutrophil counts than did PRO-piglets. At d 14 and d 21 (at weaning) of age, PRO-piglets had greater neutrophil counts than did CON-piglets (Figure 3.3). PRO-piglets had greater ($p = 0.001$) lymphocyte counts on d 7 and d 14 of age when compared to the CON-piglets. At weaning (d 21 of age), the CON-piglets had greater lymphocyte counts than did PRO-piglets (Figure 3.4).

Leukocyte Differentials. At processing, At d 0, d 1A (pre-process), and d 1B (post-process) of age PRO-piglets had greater ($p = 0.001$) percentage of segmented neutrophils than did CON-piglets, but at d 7 and 14 of age, CON-piglets had greater percentage segmented neutrophils (Figure 3.5). By d 21 (at weaning) of age, both CON- and PRO-piglets had similar percentage of segmented neutrophils. The percentage of lymphocytes ($p = 0.001$) were greater at d 0, d 1A (pre-process), and d 1B (post-process) of age for CON-piglets when compared to PRO-piglets. However, PRO-piglets had a greater percentage of lymphocytes at d 7 and d 14 of age than did CON-piglets. But, by d 21 (at weaning) of age, both CON and PRO piglets had similar percentage of segmented neutrophils (Figure 3.6).

Neutrophil Phagocytosis and Natural Killer Cell Cytotoxicity. Neutrophil phagocytosis was similar at d 7 of age for both CON and PRO piglets, but at d 14 of age, the CON-piglets had

greater ($p = 0.011$) phagocytosis than did PRO-piglets. But, by d 21 (at weaning) of age, PRO-piglets had greater neutrophil phagocytosis when compared to the CON-piglets (Figure 3.7). Natural killer cell cytotoxicity at d 7 and d 14 of age (was greater ($p = 0.03$), in PRO- piglets when compared to CON-piglets (Figure 3.8). By d 21 (at weaning) of age, both CON and PRO piglets had similar NK. All other measures of innate immunity were not different.

Interleukin-12. At d 0 and d 7 of age, CON-piglets had greater ($p = 0.001$) IL-12 than did PRO-piglets. At d 1A (pre-process), d 1B (post-process), d 14, and d 21 (at weaning) of age, PRO-piglets had greater plasma IL-12 than did CON-piglets (Figure 3.9).

Lipopolysaccharides. At d 7 and 21 (at weaning) of age, LPS-induced lymphocyte proliferation index was greater ($p < 0.05$) in PRO-piglets when compared to CON piglets. At d 14 of age, CON-piglets had greater LPS-induced proliferation than did PRO-piglets (Figure 3.10). All other measures of adaptive immunity were not different.

Experiment 2: Treatment x Day Effects during the Weaning Period

Plasma cortisol. At weaning (d 21 of age) and 7 d post-weaning (d 28 of age), PRO-piglets had greater ($p = 0.026$) plasma cortisol than did CON-piglets. But, 14 d post-weaning (d 35 of age) PRO-piglets had a lower (0.03) plasma cortisol than did CON-piglets (Figure 3.11).

Total WBC and Neutrophil and Lymphocyte Counts. No treatment x day interaction occurred for total WBC during weaning. At weaning, PRO-piglets had greater neutrophil counts at d 21 of age than did CON-piglets. At days 22, 28, and 35 of age, the CON-piglets had greater ($p = 0.03$) neutrophil counts when compared to PRO-piglets (Figure 3.12). CON-piglets had greater ($p = 0.02$) lymphocyte counts on days d 21, 22, and 35 of age when compared to the PRO-piglets. But, 7 d post-weaning (d 28 of age), both CON and PRO piglets had similar lymphocyte counts (Figure 3.13).

Leukocyte Differentials. Only at d 28 and 35 of age did percentage of segmented neutrophils differ ($p \leq 0.05$) between CON- and PRO-piglets; with the CON-piglets having greater percentage of segmented neutrophils than the PRO-piglets (Figure 3.14). Only at d 35 of age, did

percentage of lymphocytes differ, with PRO-piglets having greater percentages than did CON-piglets (Figure 3.15).

Neutrophil Phagocytosis and Natural Killer Cell Cytotoxicity. At days 21, 28, and 35 of age, the PRO-piglets had greater ($p = 0.012$) neutrophil phagocytosis than did CON-piglets. At d 22 of age (24-h post-weaning), the CON-piglets had greater phagocytosis than did PRO-piglets (Figure 3.16). Natural killer cell cytotoxicity was similar for both CON and PRO piglets on d 21 (at weaning) of age, but at days 22, 28, and 35 of age, CON-piglets had greater ($p = 0.014$), NK cytotoxicity than did PRO-piglets (Figure 3.17). All other measures of innate immunity were not different.

Interleukin-12. At days 21, 22, and 35 of age, PRO- piglets had greater ($p = 0.001$) plasma IL-12 than did CON -piglets. However, at d 28 (24-hours post-weaning) of age, CON piglets had greater IL-12 than did PRO-piglets (Figure 3.18). All other measures of adaptive immunity were not different.

Piglet Body Weight

Piglet body weight (kg), for CON and PRO piglets was recorded during the suckling and weaning periods. During suckling, days 1B (post-process), 7, 14, and 21 (at weaning) of age were reported. During the weaning period, days 21 (at weaning), 22, 28, and 35 of age were reported. Throughout the study, piglet body weight was similar between the CON and PRO piglets. Therefore, for each time point taken, piglet body weight was similar, regardless of whether they were offspring from dams fed the control (CON-sows) or probiotic (PRO-sows) treatments (Figure 3.19).

DISCUSSION

Results of the piglet study imply that feeding yeast-derived probiotics to sows during gestation and lactation may affect innate immune status of the dams' offspring, as well as the piglets' biological responses to farrowing, processing, and weaning stresses; but have minimal effect on adaptive immunity with the exception of cytokine IL-12 and LPS-induced mitogen proliferation. During farrowing stress, innate immune measures, especially the subset of

neutrophils in the leukocyte differential was enhanced at birth (d 0 of age) in piglets from dams that were fed the probiotic treatment (PRO-piglets) when compared to the control piglets (CON-piglets). Also, plasma cortisol was lower in the PRO-piglets when compared to the CON-piglets at birth. During processing stress, innate and adaptive immune measures, especially total WBC count, leukocyte differential (subset of neutrophils), and cytokine IL-12 were enhanced at d 1A and d 1B of age (pre- and post-processing) in PRO-piglets when compared to CON-piglets. The immune effects that occurred during the first 24-hours of age for the PRO-piglets are most likely due to the treatment that the probiotic-treated sows were given during late gestation. In addition, the immune effects that the PRO-piglets experienced from d 0 (birth) to d 7 of age are likely due to the PRO-sows' treatment during both gestation and lactation. Finally, during weaning stress, innate and adaptive immune measures, especially total WBC count, neutrophil phagocytosis, cytokine IL-12, and LPS-induced mitogen proliferation were enhanced at d 21 of age (at weaning) in PRO-piglets when compared to the CON-piglets' response to acute weaning stress. These data imply that probiotics may enhance the innate and adaptive immune response of offspring from probiotic-treated dams, while reducing the stress responsiveness of these piglets to multiple stressors.

Contrasting to the results that were found for PRO-sows, PRO-piglets had an overall lower response to the leukocyte differential during the suckling and weaning periods. It has been suggested in previous studies that immune parameters (including: lymphocytes, leukocyte, and neutrophils) increase in supplementation of synbiotic when compared to the single administration of lactobacillus in weaned pigs, as was previously described by Rivera et al. (2002). However, results of this study did not find an overall apparent increase in total WBC count, neutrophil and lymphocyte counts, or leukocyte differential for the PRO-piglets. In the piglet study, total WBC counts only had an effect during the suckling period. PRO-piglets had greater WBC counts on d 1A and d 1B of age (pre- and post-process), indicating a greater responsiveness to processing stress when compared to the CON-piglets. By d 14 of age, both CON and PRO piglets had similar WBC counts. However, PRO-piglets had a greater WBC count on d 21 of age (at weaning), which may suggest that PRO-piglets had a greater responsiveness to weaning stress than did CON-piglets.

Treatment x day effects were reported for subsets of segmented neutrophils and lymphocytes in the leukocyte differential, as well as in total neutrophil and lymphocyte counts,

during the periods of suckling and weaning. Segmented neutrophils, which are the most common of the white blood cells (WBC) and serve as the primary defense against bacterial infection and physiological stress (Black, 2011), were greater in PRO-piglets on d 0 (at birth), d 1A, and d 1B of age (pre- and post-process) during the suckling period when compared to CON-piglets. Moreover, PRO-piglets had greater total neutrophil counts on d 14 of age during the suckling period, as well as on d 21 of age (at weaning). This may indicate that the probiotic treatment benefited the PRO-piglets in responsiveness to farrowing, processing, and weaning stressors. The subset of lymphocytes in the leukocyte differential, as well as in the total lymphocyte count, was greater at the end of the suckling period (d 7 and d 14 of age) for PRO-piglets when compared to CON-piglets. In addition, PRO-piglets had greater percentage of lymphocytes on d 22 of the weaning period (24-hours post-weaning). Lymphocyte results may suggest that the probiotic treatment may have positively affected the PRO-piglets during the second half of the suckling period, as well as at 24-hours post-weaning.

Research conducted by Kawakami et al. (2010) found that calves administered the probiotic, *Lactobacillus plantarum*, reported no leukocyte increases in the peripheral blood of the treated calves during the experimental treatment, which is not true of the results reported for the PRO-piglets. The author speculates that it is more likely that the innate aspect of the immune system is enhanced for challenges. It may initially be perceived that PRO-piglets have a greater stress response since increased neutrophils are often a sign of acute stress if the N:L ratio is higher; however, this may not be the case in conjunction with total white blood cells, indicating that this may be a positive effect and that the piglets aren't exhibiting stress. Results of this study on *Scb* reported different results from past studies on the use of prebiotic and probiotic supplemented diets in piglets. Shim (2005) reported that hematological traits (including: WBC, neutrophil, and lymphocyte counts) were unaffected by prebiotic, multi-strain probiotic, and synbiotic in weaned pigs. Similarly, the effects of probiotic and prebiotic on the immune system status of newborn female calves indicated no significant difference in WBC count, neutrophil, lymphocyte, and monocyte concentration at plasma concentration at any collection period taken, as previously conducted by Roodposhti and Dabiri (2012).

Interestingly, neutrophil phagocytosis and natural killer (NK) cell cytotoxicity were the primary innate immune parameters affected by feeding the offspring's dams probiotics. Innate immune cells such as NK cells act as the first line of defense against pathogens (Friberg, 1996),

and can be affected by elevated cortisol. As a subset of lymphocytes, NK cells are capable of controlling the immune response through the secretion of cytokines, with the primary effector function being the lysis of their targets. In a study conducted by Niekamp et al. (2007), it was hypothesized that weaning age has a marketed effect on the immune status of the piglet. Results showed that piglets weaned at 14-days-old had lower NK cell cytotoxicity when compared to piglets weaned between 21-28 days old. Older piglets were thus able to generate stronger immune responses when stimulated by environmental antigens during weaning. The authors concluded that piglets with high immune system activation thus had a decreased weight gain and food intake. PRO-piglets in this study showed dissimilar results to those reported by Niekamp et al., seeing as NK was higher in both CON and PRO piglets at d 35 of age, when compared to d 21 of age (at weaning). PRO-piglets only had greater NK cytotoxicity when compared to the CON-piglets on d 7 of age during the suckling period. NK results suggest that the probiotic treatment did not benefit PRO-piglets during this study overall. These results were very different to the results that were found for the PRO-sows that were directly fed the probiotic treatment.

Early weaning of piglets can cause oxidative stress by producing increased levels of reactive oxygen species, as was previously described by Dowarah and Agarwal (2016). The physiological concentration of reactive oxygen species is of particular importance when considering normal cell function, energy production, phagocytosis, and intercellular signaling regulation. The function of phagocytosis is to ingest and destroy pathogens. This process, by which a cell engulfs a solid particle in order to form a phagosome, results in the removal of pathogens and cell debris from the body. On d 21 (at weaning) of age, PRO-piglets had greater phagocytosis measures when compared to the CON-piglets. PRO piglets also had greater phagocytosis at days 28 and 35 of age. Phagocytosis results suggest that the probiotic treatment may have positively affected the PRO-piglets at weaning, as well as during the long-term assessment of the post-weaning period. Supporting research on the effect on the immune status of germ-free piglets of probiotics potentiated with polyunsaturated fatty acids was reported by Kastel et al. (2007). Results of this study at d 28 of age (7 d post-treatment) showed significant differences in the phagocytic activity of neutrophils and phagocytic activity of potentially phagocytizing cells of piglets, similar to the results seen for PRO-piglets in the weaning period.

Maternal stress and its impact on the offspring can occur both prenatal and postnatal, thereby affecting early life stressors. Prenatal stresses are those changes that occur in utero,

which also include functions of the HPA axis including basal activity and stress responsiveness. Moreover, postnatal stress can have excitatory or inhibitory effects on an animal which can alter cognitive ability and anxiety (Nuriel-Ohayon, 2016; Wu et al., 1998). At birth (d 0) and d 7 of age, plasma cortisol secretion was lower for the PRO-piglets when compared to the CON-piglets. Long-term effects of weaning found that at d 35 of age (14 d post-weaning), PRO-piglets had lower plasma cortisol levels as well. Plasma cortisol results suggest that the probiotic treatment did not substantially benefit PRO-piglets, except at birth (in response to farrowing stress), during the middle of the suckling period, and in response to long-term stress responsiveness. A study conducted by Wang et al. (2013) found that supplementing the probiotic agent lactic acid bacteria, *Lactobacillus reuteri* (noted as being one of the few endogenous *Lactobacillus* species that are found within the gastrointestinal tracts of the pig), thus alleviated oxidative stress and enhanced the performance of weanling pigs. This may be similar to results seen in this study, as PRO-piglets had lower plasma cortisol in response to farrowing stress, as well as in response to long-term weaning stress (d 35 of age). Another study by Heinrich et al. (2002) found that even though differences were not significant among control and treated weaned pigs, that plasma concentration tended to be greater in synbiotic and probiotic treated animals overall, which was similarly seen in the PRO-piglets of this study in general (across both periods of treatment). Again, plasma levels were reportedly highest after birth due to the passive transfer of colostrum antibodies, which is then reduced until the animal is capable to producing its own antibodies (Riddell et al., 2010). This was true of both the CON and PRO piglets at d 0 of age (birth); however, PRO-piglets did have a lower response than did CON-piglets.

Interestingly, both cytokine IL-12 and LPS-induced mitogen proliferation were the primary adaptive immune parameters affected by feeding the offspring's dams the probiotic treatment. Interleukin-12, a pro-inflammatory response which thereby induces an immune response, plays a key role in the induction of Th1 immune responses (Trinchieri, 1995). Deng et al. (2007) reported that dietary supplementation with polysaccharides may increase interleukin, since polysaccharides may enhance the cell-mediated immune response as well as the humoral immunity in early-weaned piglets. At d 1A, d 1B, and d 14 of age during the suckling period, PRO-piglets had greater IL-12 than did CON-piglets. Once piglets moved on to the weaning period, PRO-piglets had greater IL-12 on d 21, d 22, and d 35 of age. These results for IL-12 suggest that the probiotic treatment potentially had a positive effect on responsiveness to

processing (d 1A and d 1B of age) and weaning stress (d 21 of age) for PRO-piglets. These results were similar to the results that were found for the PRO-sows as well. The results found for PRO-piglets supports the above research by Deng at al., since the dietary supplementation of *Scb* may have affected the greater response to IL-12 reported. A review on parasites, probiotics, and piglets suggests that the underlying immunological mechanisms that ameliorate pathology showed no differences of T cells with a regulatory phenotype in probiotic-treated animals, as was previously described by Devaney (2014). However, results of the study on PRO-piglets found different results. On d 7 and d 21 of age during the suckling period, PRO-piglets had greater LPS measures than did CON-piglets. LPS (which function by eliciting strong immune responses in animals, thereby stabilizing the overall membrane structure (Black, 2011) results suggest that the probiotic treatment may have positively affected the PRO-piglets in response to short-term weaning stress (d 21 of age).

Conclusion

During the piglet study, it was hypothesized that sows fed the supplemented yeast probiotic diet, *Scb*, during the periods of late gestation until weaning could potentially transfer beneficial immune and stress responsiveness to her offspring. The maternal-fetal interaction that occurred *in utero* would likely be due to the *Scb*-supplemented diet fed to the PRO-sows during gestation, resulting in immune status and stress responsiveness effects on the offspring within 24-hours post-birth. Overall, PRO-piglets were found to have greater innate and adaptive immune status than did CON-piglets during periods of farrowing and processing stress. At birth (d 0 of age). PRO-piglets had lower plasma cortisol and greater leukocyte differential (percentage of segmented neutrophils) in response to farrowing stress; while CON-piglets had greater total WBC count, leukocyte differential (percentage of lymphocytes), and cytokine IL-12 at farrowing. In response to processing stress (d 1A and d 1B of age) PRO-piglets had greater total WBC count, leukocyte differential (percentage of segmented neutrophils), and cytokine IL-12; while CON-piglets had lower plasma cortisol, as well as greater leukocyte differential (percentage of lymphocytes).

During the weaning period, it was further hypothesized that piglets from PRO-sows would have a greater responsiveness to both the short- and long-term effects of acute stress compared to the CON-piglets. At d 21 of age (weaning), PRO-piglets had greater total WBC

count, neutrophil phagocytosis, cytokine IL-12, and LPS-induced mitogen proliferation index in response to acute farrowing stress; while CON-piglets had lower plasma cortisol and greater total lymphocyte count at weaning. Both CON and PRO piglets had similar responses to weaning (d 21 of age) in regards to the leukocyte differential (percentage of segmented neutrophils and lymphocytes) and natural killer cell cytotoxicity. However, results varied between the CON and PRO piglets in regards to the measures that were assessed. The fewest effects on stress responsiveness or immunity were seen during the post-weaning period (i.e., long-term). This suggests that the probiotic treatment that the piglets had first experienced *in utero* may have diminished over time in the bodies of the PRO-piglets, leading to less of an impact during the weaning period. It is possible that feeding PRO-sows the yeast probiotic supplementation during the periods of gestation and lactation, resulted in positive outcomes of both innate and adaptive immune responses, leading to a maternal-fetal interaction, thereby transferring the immune status and stress responsiveness effects from the dam to her offspring.

TABLES

Table 3.1 Effect of treatment on piglet plasma cortisol, total WBC counts, and differential WBC during suckling[1]

Item	Control (n = 42)	Probiotic (n = 42)	P-Value
Plasma cortisol, ng/mL			0.001*
d0	204.54 ± 10.8[a]	111.17 ± 11.7[b]	
d1A	30.8 ± 3.28	38.8 ± 5.52	
d1B	50.5 ± 5.42[a]	64.2 ± 7.50[b]	
d7	44.9 ± 12.1[a]	38.3 ± 3.43[b]	
d14	38.6 ± 3.76	46.3 ± 7.14	
d21	44.7 ± 3.48	50.5 ± 3.14	
White Blood Cell (WBC), 10^7			0.03*
d0	1.67 ± 0.17[a]	0.99 ± 0.18[b]	
d1A	2.40 ± 0.23	2.65 ± 0.22	
d1B	2.29 ± 0.21	2.42 ± 0.16	
d7	5.93 ± 0.72	5.86 ± 0.52	
d14	3.13 ± 0.35	3.10 ± 0.30	
d21	1.71 ± 0.11	2.13 ± 0.28	
Neutrophils, 10^6			0.04*
d7	4.16 ± 0.38[a]	3.72 ± 0.14[b]	
d14	2.22 ± 0.25	2.57 ± 0.35	
d21	1.88 ± 0.20	1.94 ± 0.15	
Lymphocytes, 10^6			0.001*
d7	2.70 ± 0.32[a]	4.19 ± 0.97[b]	
d14	2.45 ± 0.55	4.01 ± 1.34	
d21	8.02 ± 2.02[a]	3.85 ± 1.10[b]	
Eosinophils, %			0.85
d0	0.43 ± 0.08	0.29 ± 0.07	
d1A	0.48 ± 0.12	0.74 ± 0.15	
d1B	0.12 ± 0.06	0.48 ± 0.11	
d7	0.33 ± 0.11	0.52 ± 0.19	
d14	0.45 ± 0.12	0.52 ± 0.11	
d21	0.40 ± 0.13	0.36 ± 0.06	
Banded Neutrophils, %			0.35
d0	1.17 ± 0.20	1.21 ± 0.02	
d1A	1.29 ± 0.24	2.17 ± 0.36	
d1B	0.98 ± 0.21[a]	2.90 ± 0.60[b]	
d7	0.86 ± 0.19	1.02 ± 0.21	
d14	0.29 ± 0.10	0.36 ± 0.13	

Table 3.1 (Continued)

d21	0.14 ± 0.06	0.41 ± 0.13	
Segmented Neutrophils, %			0.001*
d0	51.0 ± 1.09[a]	58.0 ± 2.03[b]	
d1A	67.0 ± 1.43	67.8 ± 1.63	
d1B	58.5 ± 2.82[a]	64.9 ± 2.15[b]	
d7	54.3 ± 2.34[a]	43.5 ± 2.13[b]	
d14	37.1 ± 1.81[a]	32.3 ± 2.05[b]	
d21	32.4 ± 1.86	32.0 ± 2.17	
Lymphocytes, %			0.001*
d0	46.5 ± 1.06[a]	39.0 ± 2.04[b]	
d1A	29.5 ± 1.64[a]	25.7 ± 1.62[b]	
d1B	38.5 ± 2.73[a]	30.0 ± 2.51[b]	
d7	42.5 ± 2.44[a]	52.8 ± 2.19[b]	
d14	60.4 ± 1.91	64.6 ± 2.36	
d21	66.1 ± 1.71	65.1 ± 2.14	
Monocytes, %			0.86
d0	0.95 ± 0.16	1.57 ± 0.19	
d1A	1.64 ± 0.28	2.64 ± 0.41	
d1B	1.57 ± 0.32	1.71 ± 0.25	
d7	2.33 ± 0.37	2.21 ± 0.34	
d14	1.81 ± 0.28	1.24 ± 0.33	
d21	1.60 ± 0.29	2.14 ± 0.32	

[*] Treatment x Day effect across suckling phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE
Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.

Table 3.2 Effect of treatment on piglet functional measures during suckling[1]

Item	Control (n = 42)	Probiotic (n = 42)	P-Value
Phagocytosis, %			0.011*
d7	67.6 ± 2.29	67.5 ± 2.58	
d14	63.5 ± 1.75	60.3 ± 2.00	
d21	56.2 ± 1.40	62.5 ± 1.65	
Natural killer cells (NK) 12.5, %			0.03*
d7	64.8 ± 7.11	67.9 ± 6.91	
d14	98.12 ± 11.6	95.01 ± 12.7	
d21	89.9 ± 11.3	88.7 ± 10.3	
Interleukin-12 (IL-12 Elisa), pg/mL			0.001*
d0	172.42 ± 7.42	162.79 ± 7.64	
d1A	194.50 ± 11.2	212.65 ± 10.2	
d1B	182.11 ± 12.7	202.57 ± 14.1	
d7	167.33 ± 8.20	160.69 ± 7.10	
d14	259.74 ± 16.5	276.68 ± 13.5	
d21	298.00 ± 16.7	317.23 ± 16.6	
Concanavalin A Proliferation (ConA), 20.0			0.87
d7	2.29 ± 0.33	1.96 ± 0.28	
d14	1.92 ± 0.17	1.74 ± 0.20	
d21	2.53 ± 0.22	1.76 ± 0.18	
Lipopolysaccharide Proliferation (LPS), 20.0			0.05*
d7	1.48 ± 0.02[a]	1.75 ± 0.22[b]	
d14	1.31 ± 0.09	1.14 ± 0.05	
d21	1.46 ± 0.11[a]	1.72 ± 0.31[b]	

[*] Treatment x Day effect across suckling phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE
Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.

Table 3.3 Effect of treatment on piglet plasma cortisol, total WBC counts, and differential WBC during weaning[1]

Item	Control (n = 42)	Probiotic (n = 42)	P-Value
Plasma cortisol, ng/mL			0.026*
d21	44.7 ± 3.48	50.5 ± 3.14	
d22	37.0 ± 2.94[a]	53.0 ± 5.70[b]	
d28	25.2 ± 1.69[a]	30.8 ± 2.38[b]	
d35	26.4 ± 1.93	24.5 ± 1.54	
White Blood Cell (WBC), 10^7			0.55
d21	1.71 ± 0.11	2.13 ± 0.28	
d22	1.99 ± 0.22[a]	1.27 ± 0.07[b]	
d28	2.10 ± 0.19	1.99 ± 0.17	
d35	3.17 ± 0.15	3.44 ± 0.17	
Neutrophils, 10^6			0.03*
d21	1.88 ± 0.20	1.94 ± 0.15	
d22	1.68 ± 1.23[a]	1.64 ± 0.24[b]	
d28	2.18 ± 0.21	2.03 ± 0.19	
d35	6.15 ± 0.44[a]	4.90 ± 0.43[b]	
Lymphocytes, 10^6			0.02*
d21	8.02 ± 2.02[a]	3.85 ± 0.30[b]	
d22	2.53 ± 0.35[a]	1.39 ± 0.15[b]	
d28	2.31 ± 0.23	2.33 ± 0.15	
d35	4.34 ± 0.35	3.54 ± 0.24	
Eosinophils, %			0.94
d21	0.40 ± 0.13	0.36 ± 0.06	
d22	0.38 ± 0.11	0.36 ± 0.11	
d28	0.52 ± 0.15	1.19 ± 0.23	
d35	1.10 ± 0.17	0.91 ± 0.13	
Banded Neutrophils, %			0.76
d21	0.14 ± 0.06	0.41 ± 0.13	
d22	0.48 ± 0.16	0.45 ± 0.14	
d28	0.33 ± 0.11	0.52 ± 0.13	
d35	0.21 ± 0.06	0.48 ± 0.11	
Segmented Neutrophils, %			0.019*
d21	32.4 ± 1.86	32.0 ± 2.17	
d22	40.2 ± 1.85	39.6 ± 2.54	
d28	36.3 ± 2.04	34.0 ± 2.01	
d35	49.0 ± 2.03	41.1 ± 1.72	
Lymphocytes, %			0.04*
d21	66.1 ± 1.71	65.1 ± 2.14	

Table 3.3 (Continued)

d22	56.5 ± 1.98	57.2 ± 2.13	
d28	59.4 ± 2.00	60.8 ± 1.95	
d35	48.1 ± 2.17*	55.7 ± 1.71*	
Monocytes, %			0.67
d21	1.60 ± 0.29	2.14 ± 0.32	
d22	2.38 ± 0.36	2.31 ± 0.38	
d28	2.90 ± 0.40	3.57 ± 0.50	
d35	1.62 ± 0.23	1.90 ± 0.22	

[*] Treatment x Day effect across weaning phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE
Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.

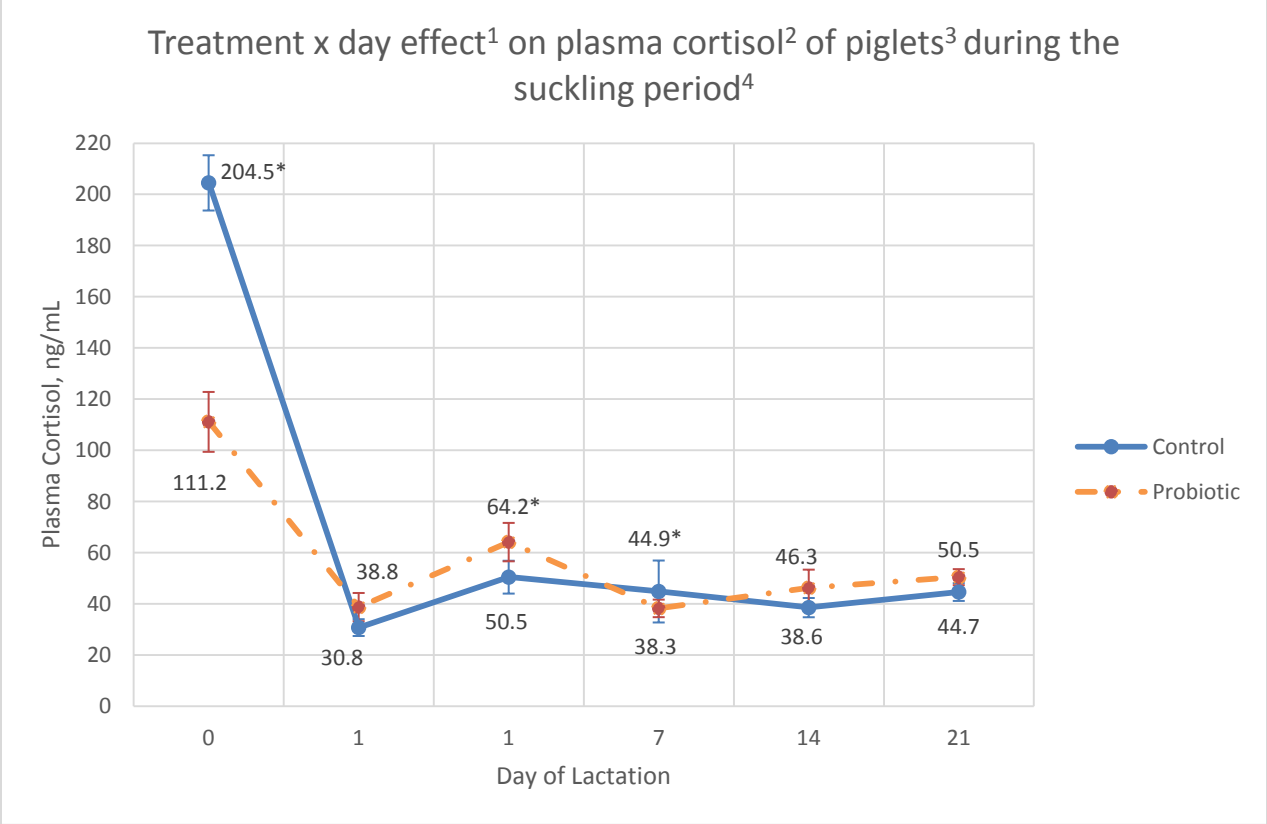
Table 3.4 Effect of treatment on piglet functional measures during weaning[1]

Item	Control (n = 42)	Probiotic (n = 42)	P-Value
Phagocytosis, %			0.012*
d21	56.2 ± 2.40	62.5 ± 1.65	
d22	70.1 ± 2.01[a]	65.5 ± 2.03[b]	
d28	60.7 ± 1.33	62.4 ± 1.16	
d35	66.4 ± 1.15	68.5 ± 1.13	
Natural killer cells (NK) 12.5, %			0.014*
d21	89.9 ± 11.3	88.7 ± 10.3	
d22	48.2 ± 8.53	45.1 ± 6.27	
d28	87.5 ± 5.53[a]	73.7 ± 4.37[b]	
d35	52.8 ± 3.84[a]	44.8 ± 3.07[b]	
Interleukin-12 (IL-12 Elisa), pg/mL			0.001*
d21	298.00 ± 16.7	317.23 ± 16.6	
d22	237.73 ± 10.2	243.13 ± 15.1	
d28	426.98 ± 30.2	417.63 ± 21.5	
d35	320.67 ± 10.8[a]	349.73 ± 24.8[b]	
Concanavalin A Proliferation (ConA), 20.0			0.54
d21	2.53 ± 0.22	1.76 ± 0.18	
d22	1.81 ± 0.28	2.32 ± 0.30	
d28	2.42 ± 0.20	2.38 ± 0.23	
d35	2.24 ± 0.27	1.98 ± 0.20	
Lipopolysaccharide Proliferation (LPS), 20.0			0.78
d21	1.46 ± 0.09[a]	1.72 ± 0.27[b]	
d22	1.66 ± 0.19	1.45 ± 0.19	
d28	1.10 ± 0.10	1.49 ± 0.21	
d35	1.65 ± 0.17	1.80 ± 0.24	

[*] Treatment x Day effect across weaning phase.
[a,b] Within a row, means without a common superscript differ ($P \leq 0.05$)
[c,d] Within a row, means without a common superscript differ (trend at $p > 0.05$ and $p \leq 0.10$)
[1] Least squares means ± SE
Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.

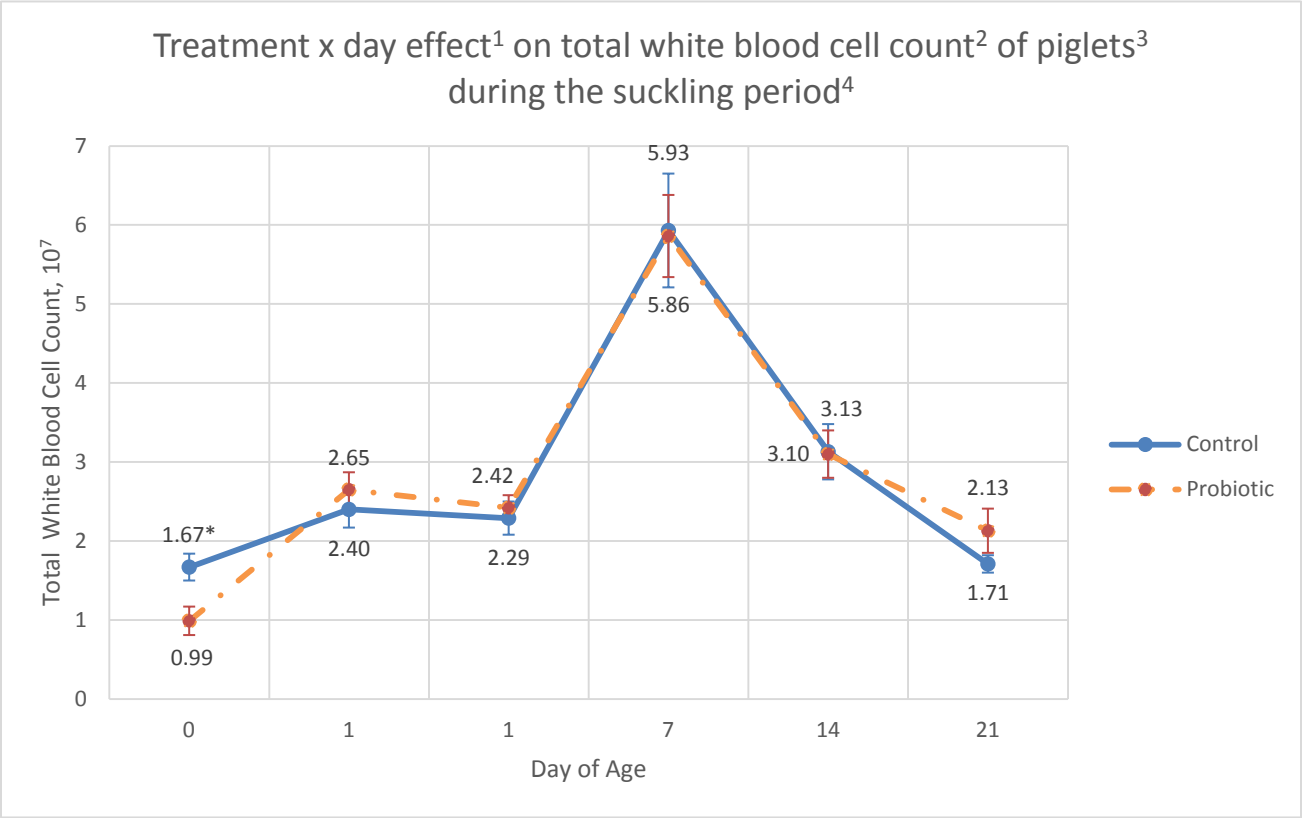
FIGURES

Figure 3.1 Treatment x day effect[1] on plasma cortisol[2] of piglets[3] during the suckling period[4]



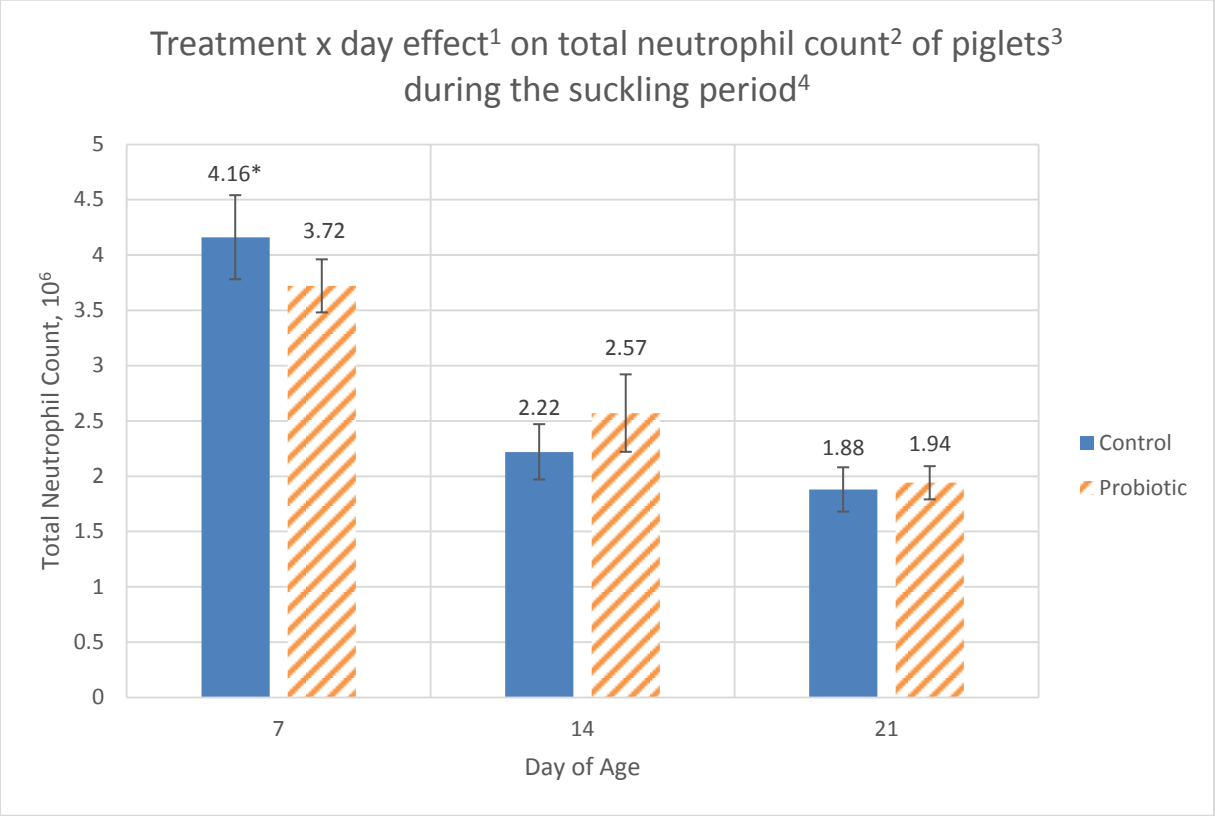
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.001
[2] Plasma cortisol, ng/mL
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 0, d 1B, and d 7 of age.

Figure 3.2 Treatment x day effect[1] on total white blood cell count[2] of piglets[3] during the suckling period[4]



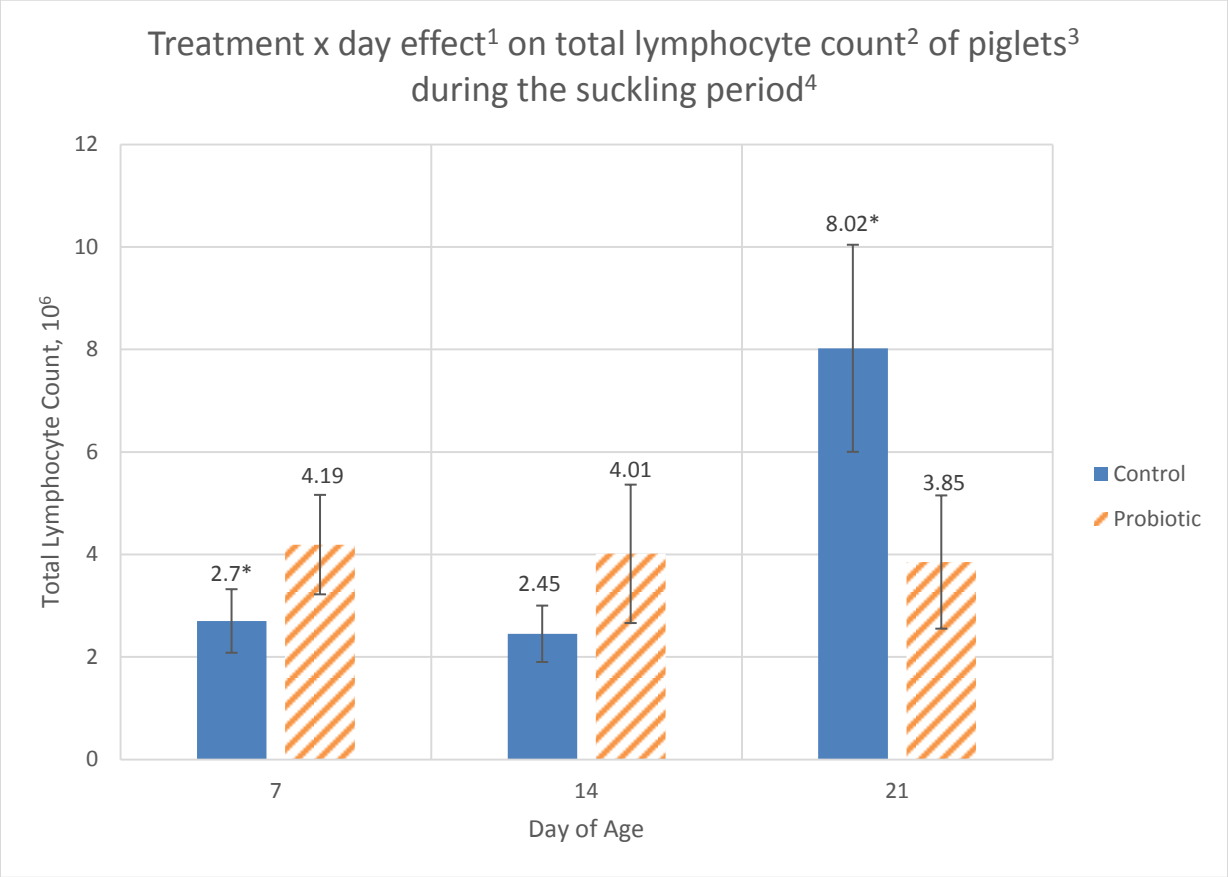
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.03
[2] Total White Blood Cell Count, 10^7
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 0 of age.

Figure 3.3 Treatment x day effect[1] on total neutrophil count[2] of piglets[3] during the suckling period[4]



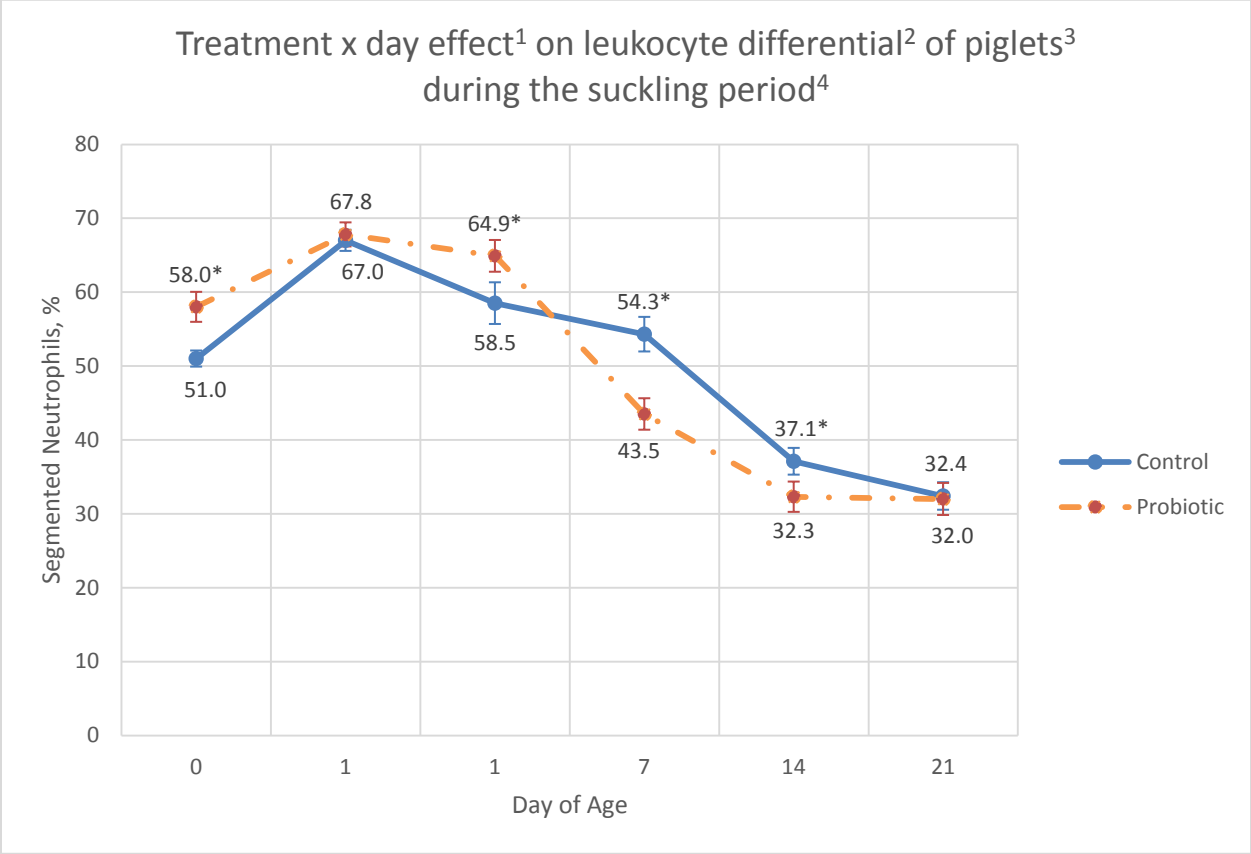
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.04
[2] Total Neutrophil Count, 10^6
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 7 of age.

Figure 3.4 Treatment x day effect[1] on total lymphocyte count[2] of piglets[3] during the suckling period[4]



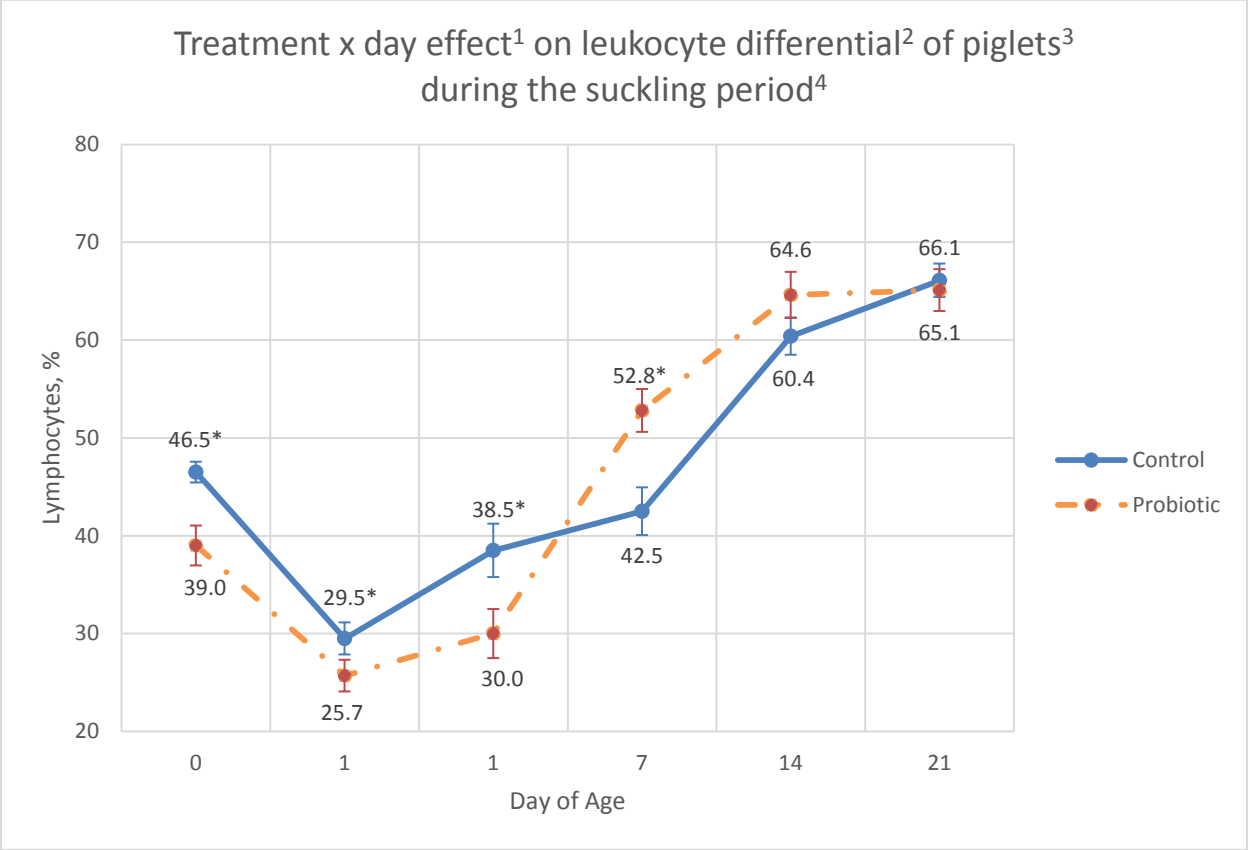
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.001
[2] Total Lymphocyte Count, 10^6
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 7 and d 21 of age.

Figure 3.5 Treatment x day effect[1] on leukocyte differential[2] of piglets[3] during the suckling period[4]



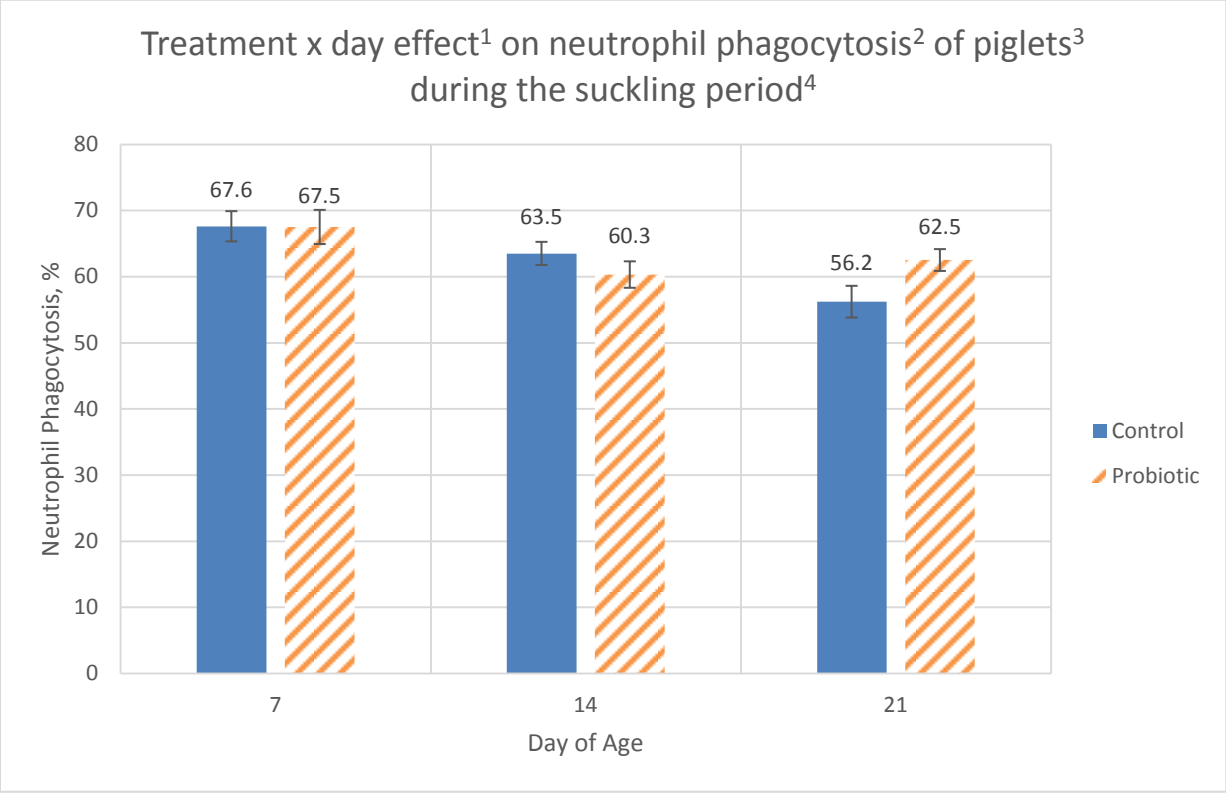
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.001
[2] Leukocyte Differential-Segmented Neutrophils, %
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 0, d 1B, d 7, and d 14 of age.

Figure 3.6 Treatment x day effect[1] on leukocyte differential[2] of piglets[3] during the suckling period[4]



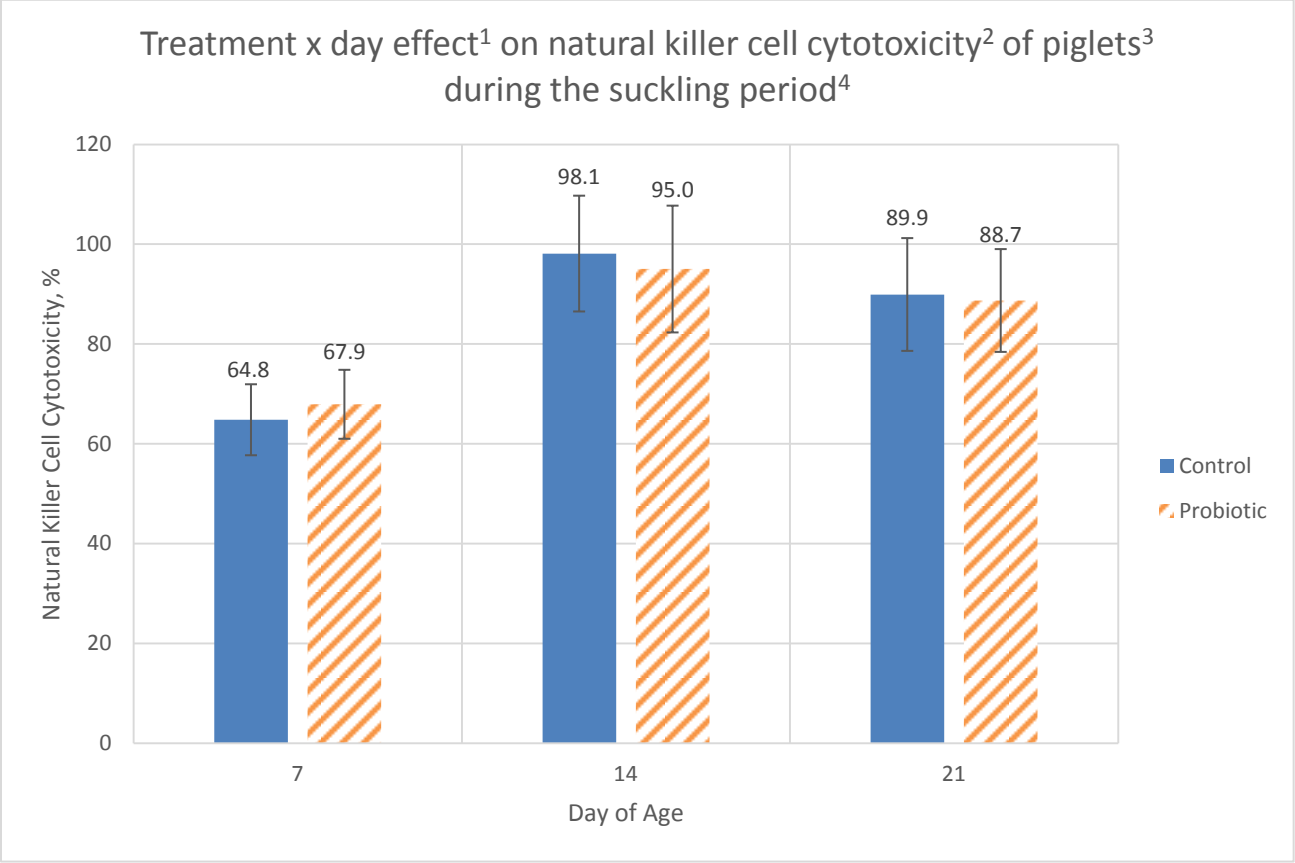
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.001
[2] Leukocyte Differential-Lymphocytes, %
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 0, d 1A, d 1B, and d 7 of age.

Figure 3.7 Treatment x day effect[1] on neutrophil phagocytosis[2] of piglets[3] during the suckling period[4]



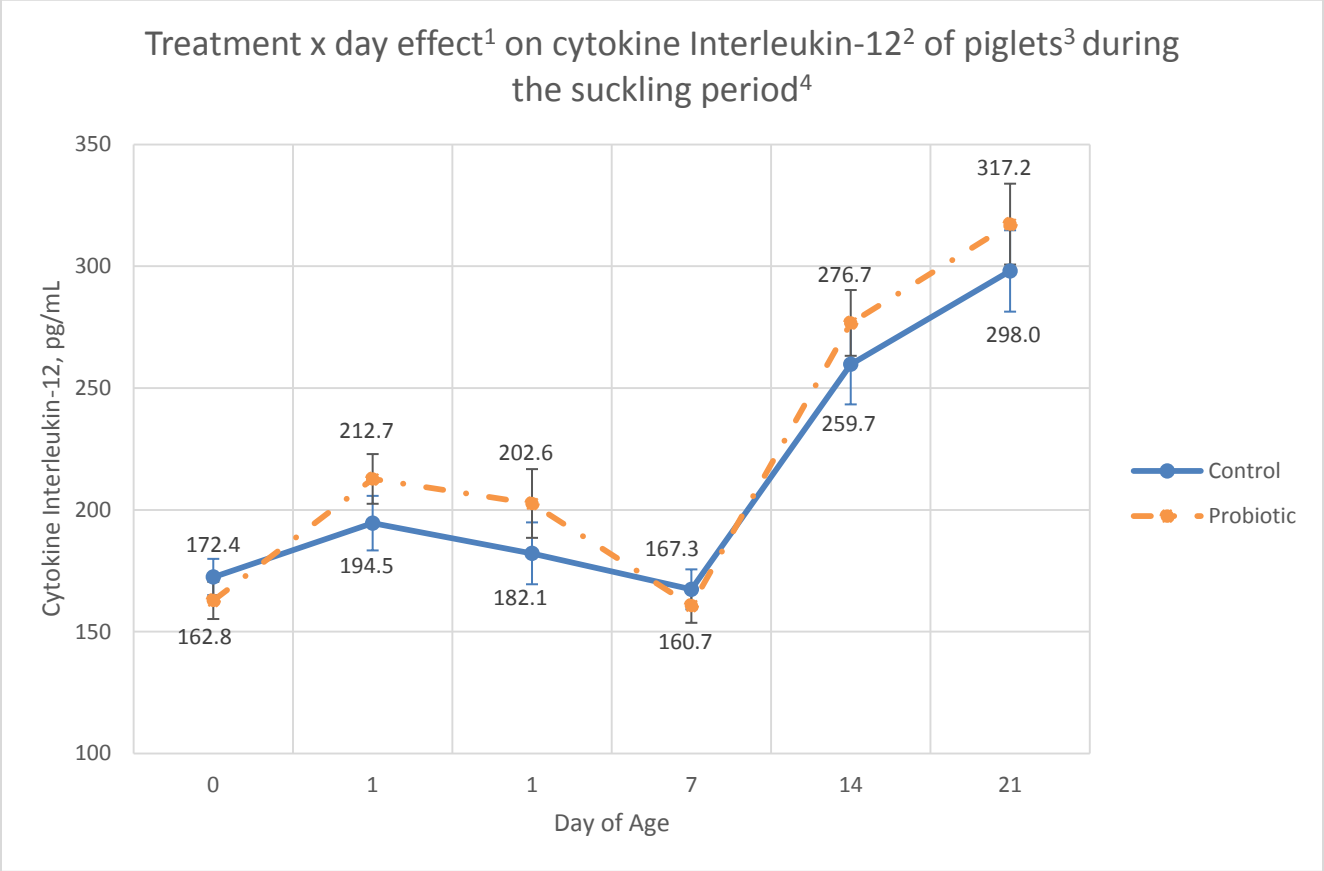
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.011
[2] Neutrophil phagocytosis, %
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$)

Figure 3.8 Treatment x day effect[1] on natural killer cell cytotoxicity[2] of piglets[3] during the suckling period[4]



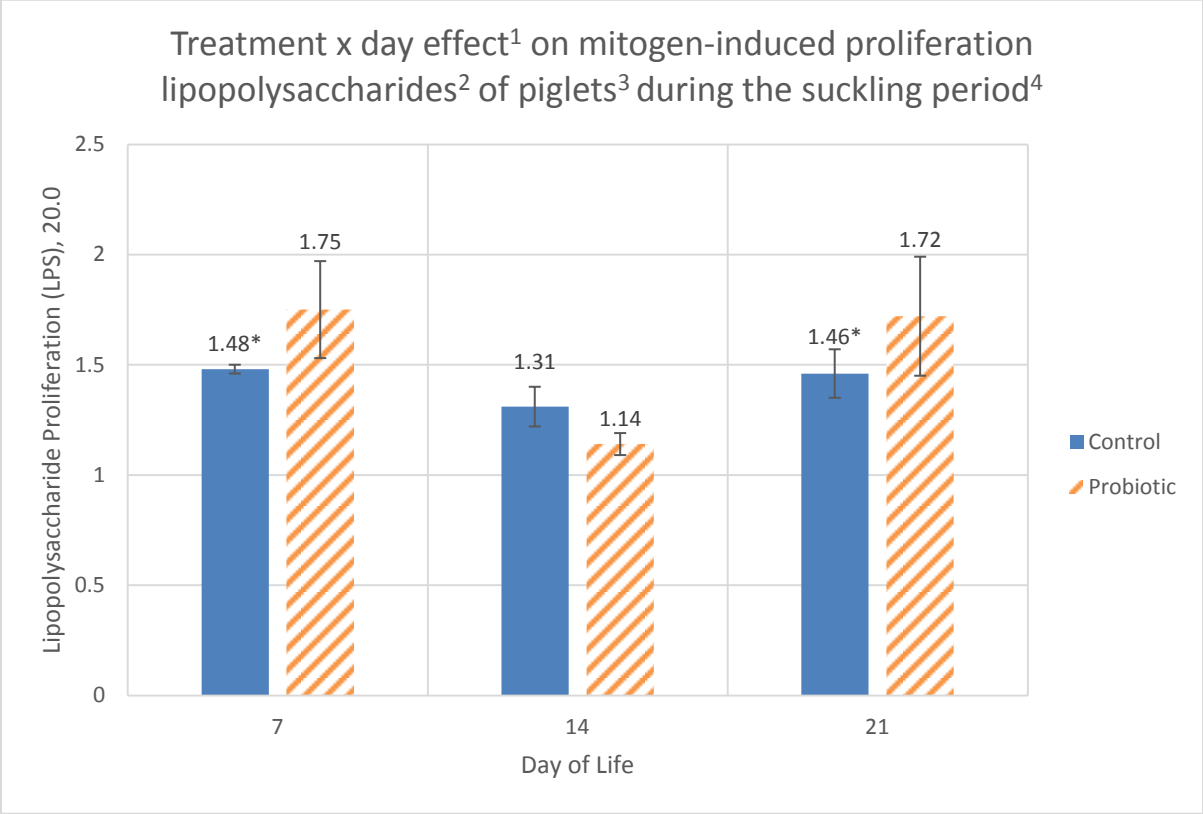
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.03
[2] Cytotoxicity %, 12.5:1 effector to target
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$)

Figure 3.9 Treatment x day effect[1] on cytokine Interleukin-12[2] of piglets[3] during the suckling period[4]



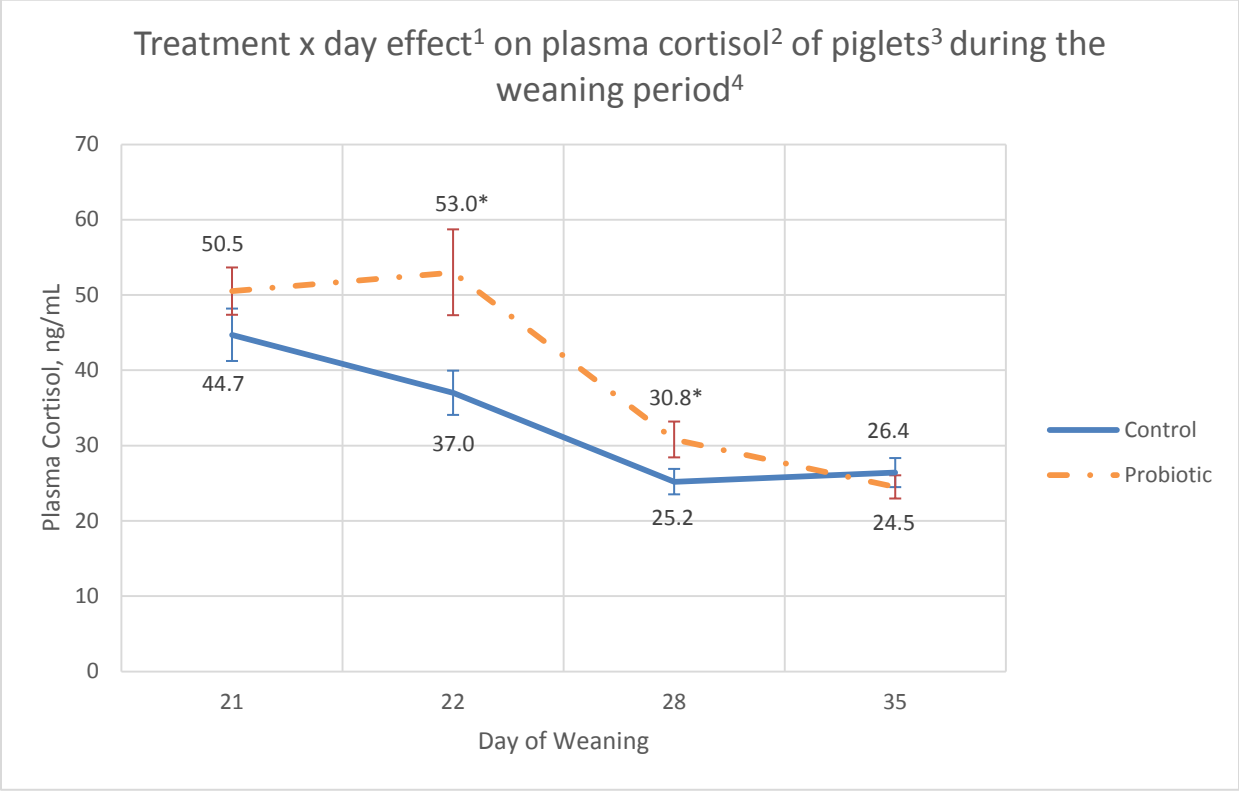
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.001
[2] Interleukin-12 (IL-12), pg/mL
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 0 (day of birth), d 1A (pre-processing), d 1B (post-processing), d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$)

Figure 3.10 Treatment x day effect[1] on mitogen-induced proliferation lipopolysaccharides[2] of piglets[3] during the suckling period[4]



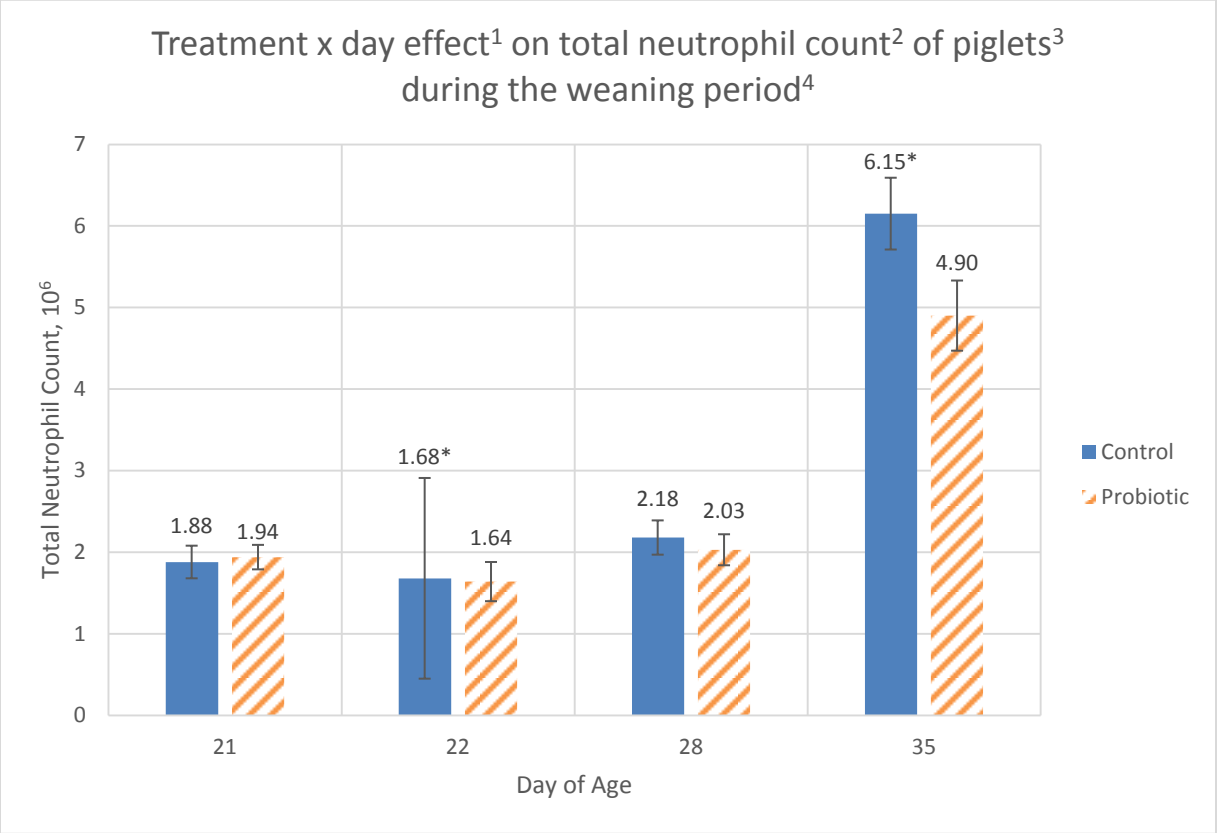
[1] Least squares means ± SE; Treatment x day effect across suckling phase; p = 0.05
[2] Mitogen-induced proliferation, Lipopolysaccharide Proliferation (20 LPS), 20.0
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of suckling are equivalent to days of age: d 7, d 14, and d 21 (at weaning) of age.
*Significant ($p \leq 0.05$) at d 7 and d 21 of age.

Figure 3.11 Treatment x day effect[1] on plasma cortisol[2] of piglets[3] during the weaning period[4]



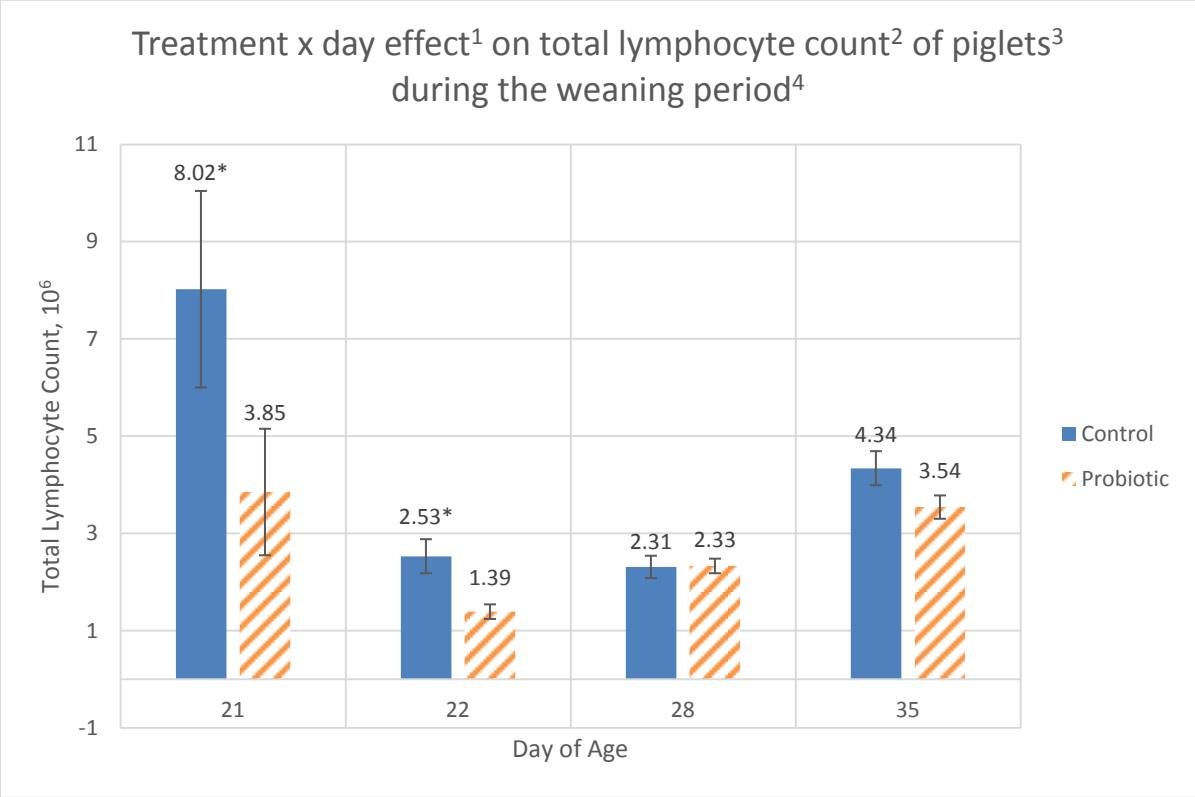
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.026
[2] Plasma cortisol, ng/mL
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 22 and d 28 of age.

Figure 3.12 Treatment x day effect[1] on total neutrophil count[2] of piglets[3] during the weaning period[4]



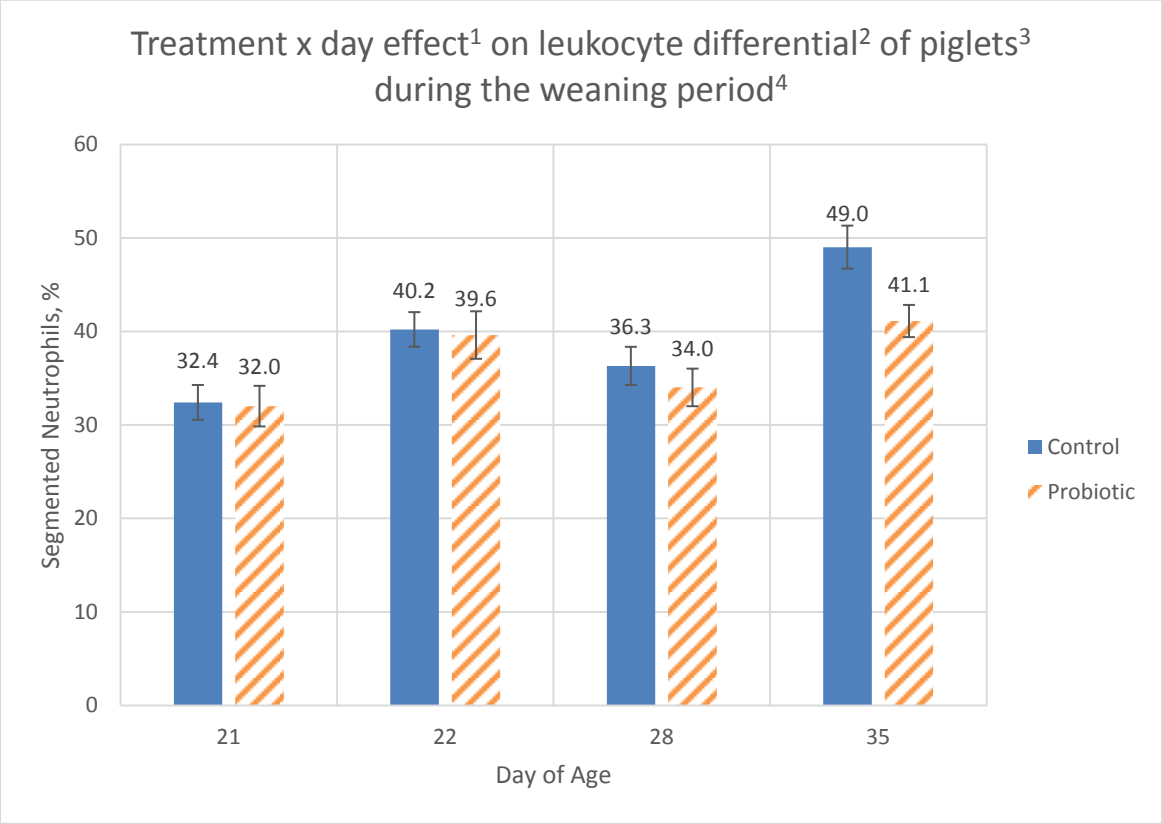
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.03
[2] Total Neutrophil Count, 10^6
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 22 and d 35 of age.

Figure 3.13 Treatment x day effect[1] on total lymphocyte count[2] of piglets[3] during the weaning period[4]



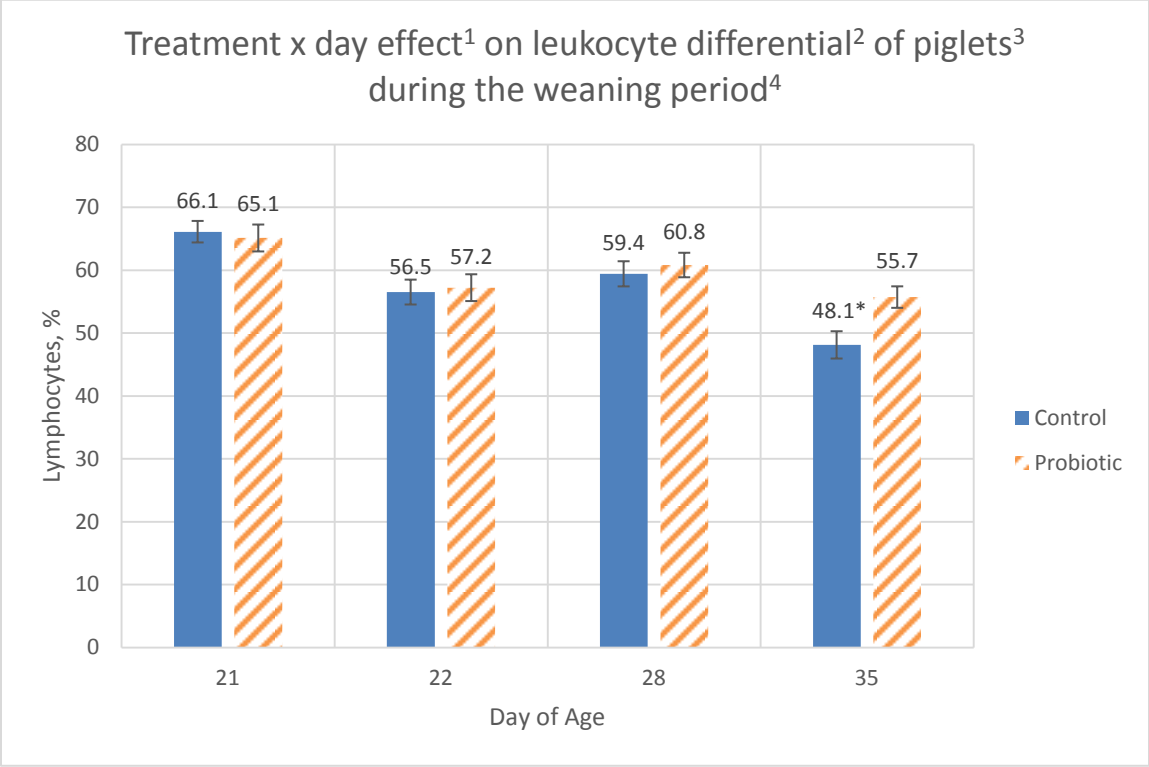
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.02
[2] Total Lymphocyte Count, 10^6
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 21 and d 22 of age.

Figure 3.14 Treatment x day effect[1] on leukocyte differential[2] of piglets[3] during the weaning period[4]



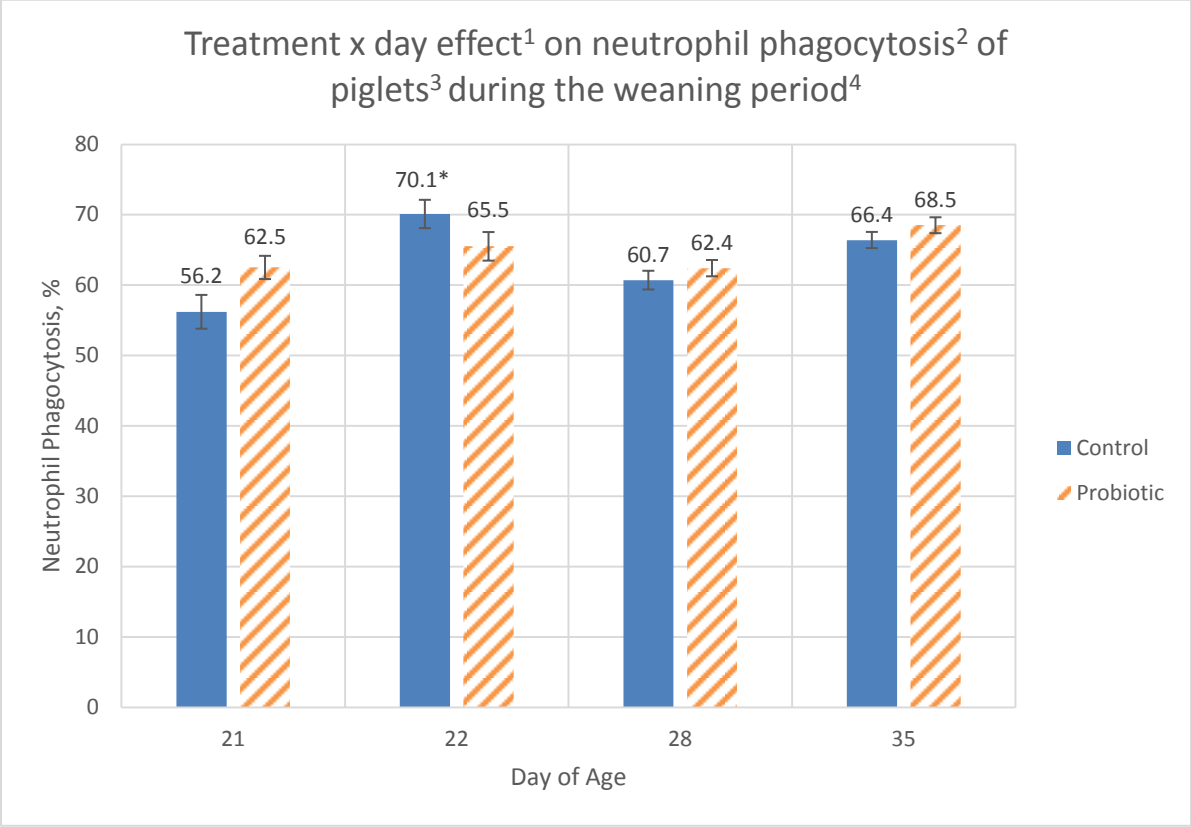
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.019
[2] Leukocyte Differential-Segmented Neutrophils, %
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$)

Figure 3.15 Treatment x day effect[1] on leukocyte differential[2] of piglets[3] during the weaning period[4]



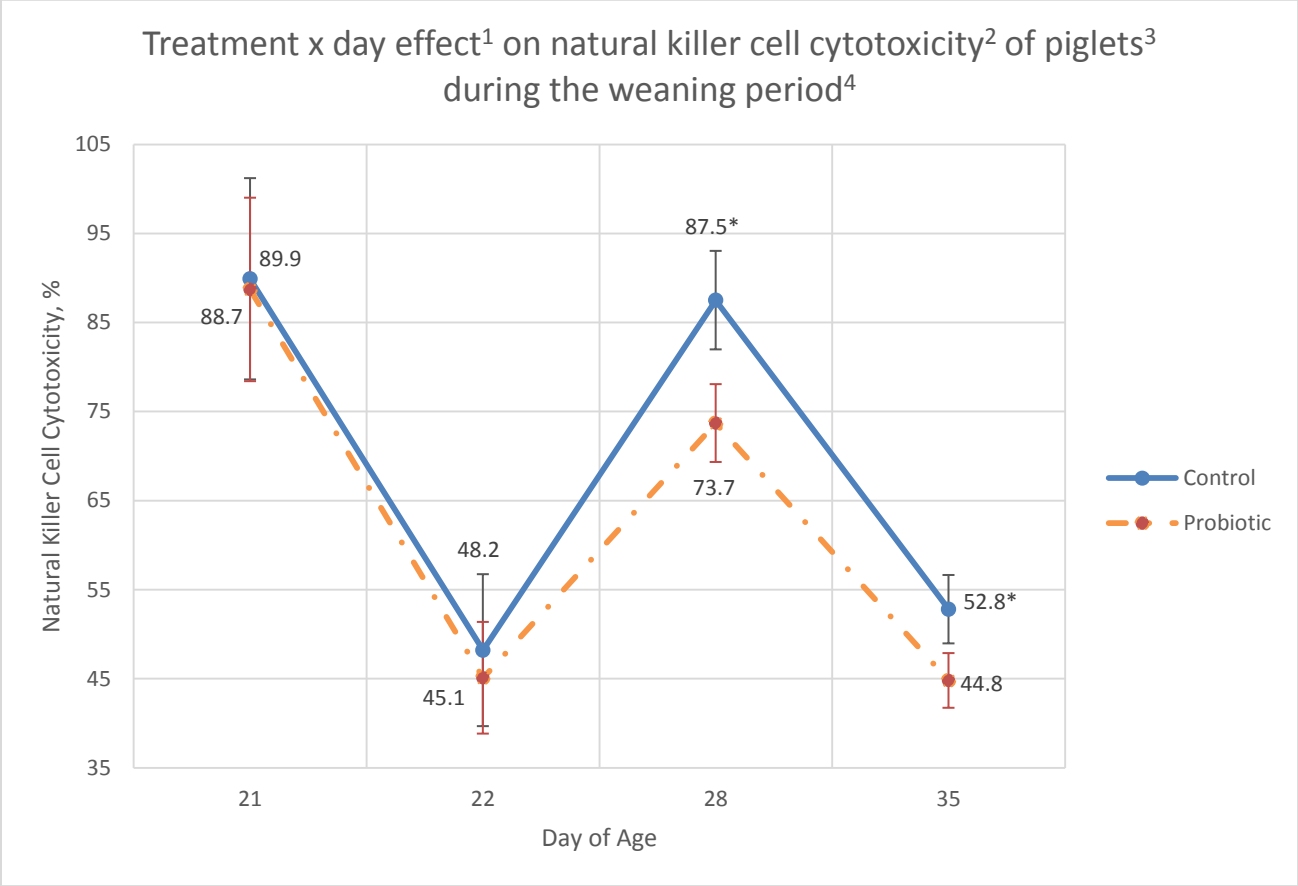
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.04
[2] Leukocyte Differential-Lymphocytes, %
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 35 of age.

Figure 3.16 Treatment x day effect[1] on neutrophil phagocytosis[2] of piglets[3] during the weaning period[4]



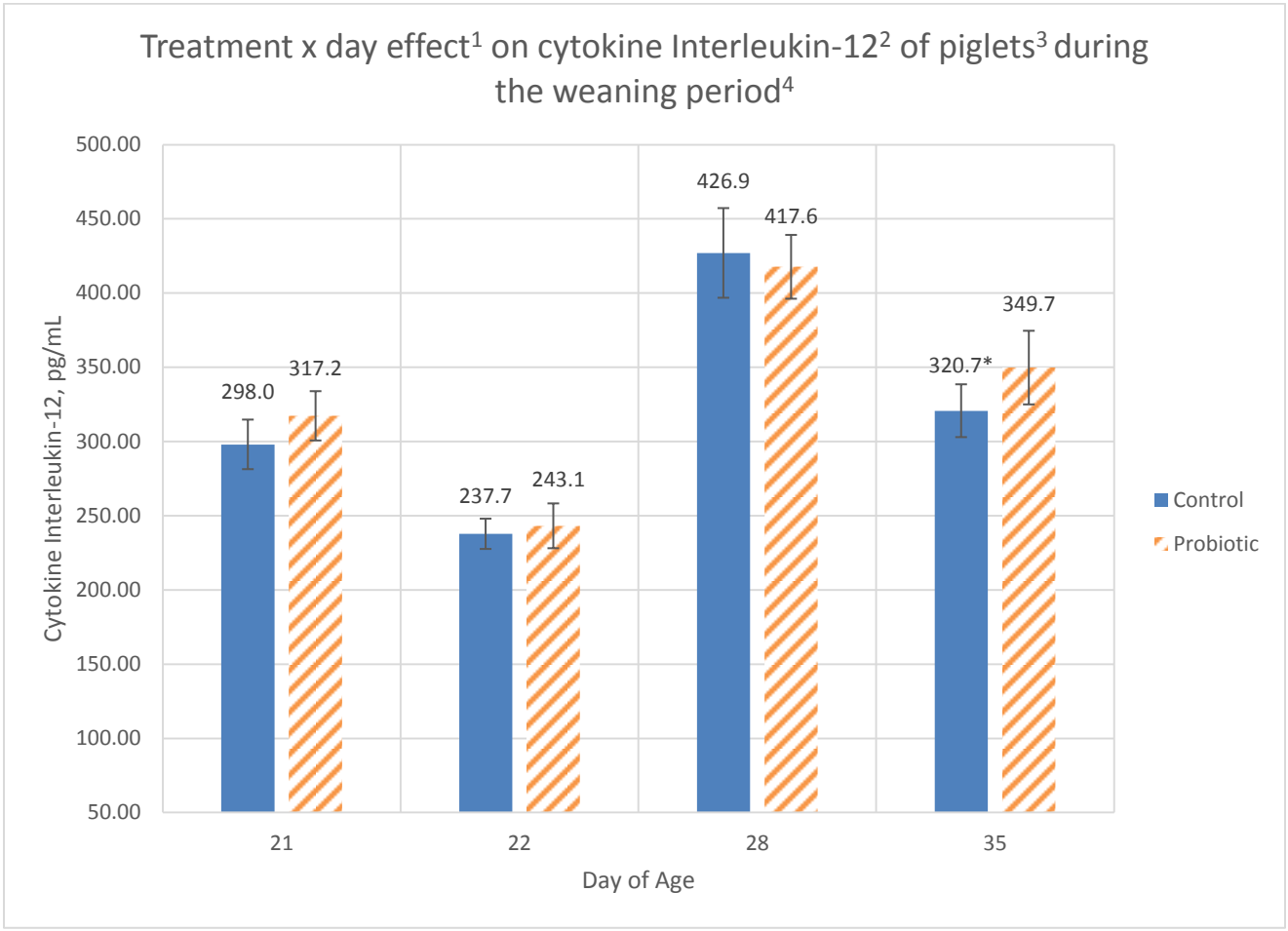
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.012
[2] Neutrophil phagocytosis, %
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 22 of age.

Figure 3.17 Treatment x day effect[1] on natural killer cell cytotoxicity[2] of piglets[3] during the weaning period[4]



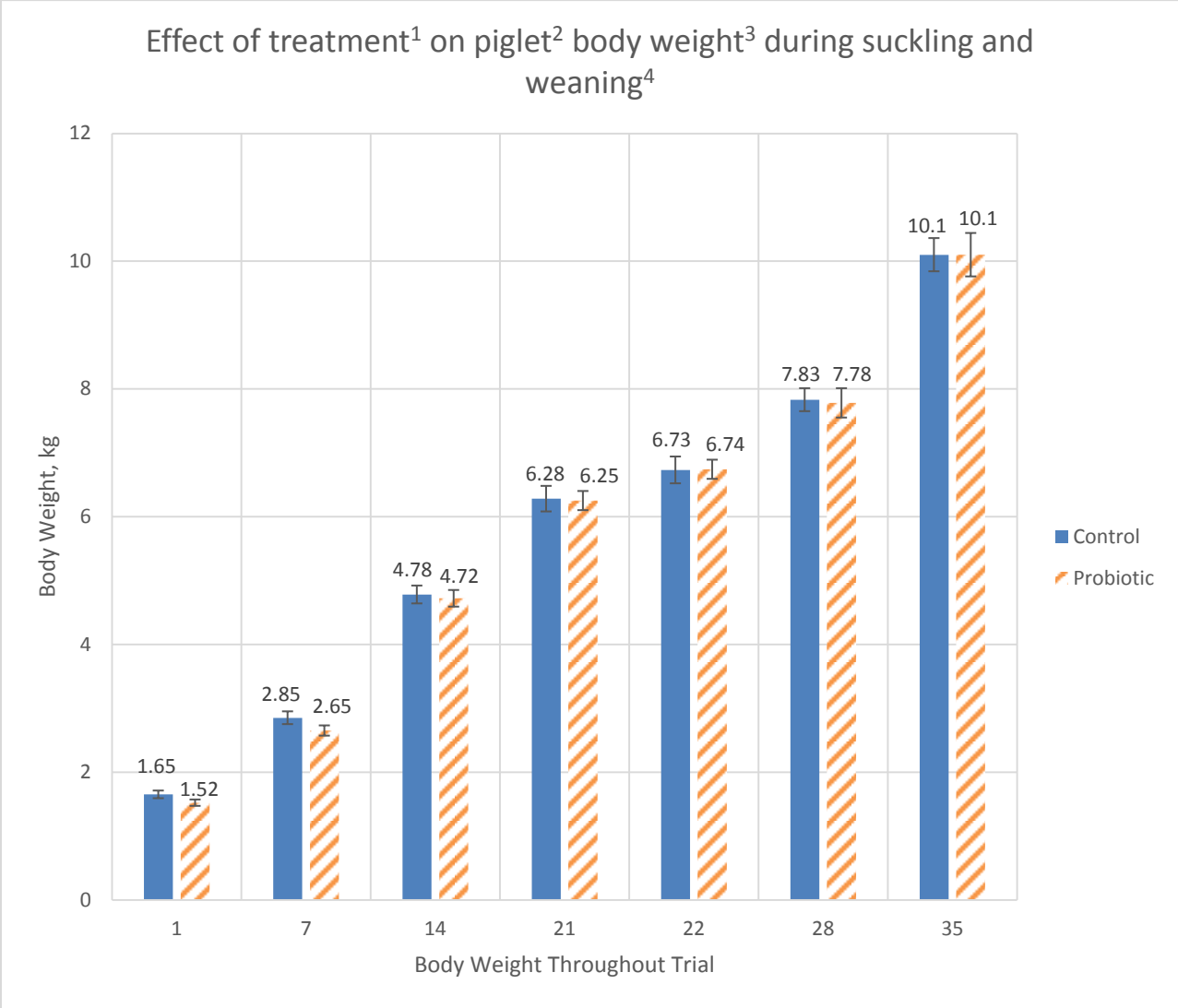
[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.014
[2] Cytotoxicity %, 12.5:1 effector to target
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 28 and d 35 of age.

Figure 3.18 Treatment x day effect[1] on cytokine Interleukin-12[2] of piglets[3] during the weaning period[4]



[1] Least squares means ± SE; Treatment x day effect across weaning phase; p = 0.001
[2] Interleukin-12 (IL-12), pg/mL
[3] Control (CON) vs. Probiotic (PRO) piglets
[4] Days of post-weaning are equivalent to days of age: d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.
*Significant ($p \leq 0.05$) at d 35 of age.

Figure 3.19 Effect of treatment[1] on piglet[2] body weight[3] during suckling and weaning[4]



[1] Least squares means ± SE
[2] Control vs. Probiotic piglets
[3] Body Weight, kg
[4] Days of suckling and weaning are equivalent to days of age: d 1B (post-processing), d 7, d 14, d 21 (at weaning), d 22 (24-hours post-weaning), d 28 (7 days post-weaning), and d 35 (14 days post-weaning) of age.

Piglet body weight (kg), for CON and PRO piglets was recorded during the suckling and weaning periods. During suckling, days 1B (post-process), 7, 14, and 21 (at weaning) of age were reported. During the weaning period, days 21 (at weaning), 22, 28, and 35 of age were reported. Throughout the study, piglet body weight was similar between the CON and PRO piglets. Therefore, for each time point taken, piglet body weight was similar, regardless of whether they were offspring from dams fed the control (CON) or probiotic (PRO) treatments (Figure 19).

CONCLUSION

The transfer of glucocorticoids from mother to fetus plays an important role in fetal programing of the HPA axis of offspring, as it has a direct effect on the brain (Nuriel-Ohayon, 2016; Wu et al., 1998). Direct maternal-fetal effects occur via the HPA axis, limbic system, and hippocampus, while indirect effects occur in the fetal organ system. In addition, glucocorticoids are important for normal development of the fetus, via growth and maturation of organ systems. The surge in glucocorticoids results in a developmental switch that results in changes in gene regulation in the organs/brain of the offspring, and these changes affect postnatal life. Maternal stress exposures to glucocorticoids results in long-term programming of HPA function and behaviors. This is because synthetic glucocorticoids can cross the placenta and enter the fetal brain, which in turn downregulates and has an effect on brain development (Nuriel-Ohayon, 2016; Wu et al., 1998). When presented with a challenge, such as farrowing and weaning stressors in sows or farrowing, processing, and weaning stressors in piglets, the yeast probiotic, *Saccharomyces cerevisiae boulardii* (*Scb*) was shown to have varying results for treatment x day interactions on both stress responsiveness and immune status of pigs. These data imply that the increased immune status and decreased stress response of the sows and piglets are interrelated in some cases, which may be altered by feeding *Scb* to sows during gestation and lactation.

In general, in the sow study, there were fewer treatment x day interactions than were reported for the effects that maternal treatment had on the piglets. This may suggest that the probiotic treatment of the sow affects the physiological responses of her offspring at farrowing and post-weaning. Results of the sow study, indicated that yeast probiotics fed from d 84 to d 112 of gestation and through lactation in response to stressors may be differentially affected by the immune and cortisol profile of the gestating sow. In general, plasma cortisol, innate immune measures (NK and chemotaxis), IL-12, and leukocyte subpopulations differed, with most immune measures being stimulated and cortisol being reduced. Moreover, feeding probiotics to sows during both gestation and lactation had an effect on her offspring. The piglets' profile found that total WBC count, total neutrophil and lymphocyte counts, innate immune measures (NK and phagocytosis), adaptive immune measures (LPS), IL-12, and leukocyte subpopulations differed, with most immune and adaptive measures being stimulated and cortisol being reduced.

Feeding S*cb* to sows was hypothesized to have an overall improved innate and adaptive immune status and cortisol levels, which would result in a greater responsiveness to acute

farrowing and weaning stress when compared to the CON-sows. Overall, PRO-sows were found to have greater innate immune responses, as well as lower plasma cortisol concentrations than did CON-sows. At d 31 (24-hours post-farrowing) post-treatment, PRO-sows had greater cytokine IL-12, and leukocyte differential (percentage of eosinophils, banded neutrophils, and lymphocytes), as well as lower plasma cortisol, in response to farrowing stress. However, CON-sows had greater natural killer cell cytotoxicity, neutrophil chemotaxis C5a, and leukocyte differential (percentage of segmented neutrophils and monocytes) at 24-hours post-farrowing. At d 51 (at weaning) post-treatment, PRO-sows had greater natural killer cell cytotoxicity, cytokine IL-12, and leukocyte differential (percentage of eosinophils, banded neutrophils, and lymphocytes), as well as lower plasma cortisol, in response to weaning stress. CON-sows, on the other hand, only had greater leukocyte differential (percentage of segmented neutrophils and monocytes) at weaning. These results showed that while CON and PRO sows both elicited differing immune responses to these stressors, the PRO-sows did have an overall greater response, especially in terms of a lower plasma cortisol concentration and a greater subset of the leukocyte differential and cytokine IL-12 at both lactation treatment days.

Previous research has indicated that plasma cortisol secretion in sows increases at the time of farrowing and weaning (Anil et al., 2005; Tsuma et al., 1995). However, results in this study showed both PRO-sows and piglets having lower plasma cortisol at farrowing than did CON-sows and piglets. This part of the cortisol response could be reflective of the sows' maternal-fetal influence, which occurred when the PRO-sows were fed *Scb* during the gestation period. In addition, PRO-sows had lower plasma cortisol at weaning than did CON-sows. However, CON-piglets had lower plasma cortisol concentration at weaning than did PRO-piglets. The PRO-sows greater responsiveness to farrowing and weaning stressors did not show any detrimental effects, as body weight remained unaffected by these changes, which is also true for the piglets at farrowing.

In the piglet study, it was hypothesized that sows fed the supplemented yeast probiotic diet, *Scb,* during the periods of late gestation until weaning could potentially transfer beneficial immune and stress responsiveness to her offspring. Therefore, piglets born to the probiotic-treated sows (PRO-piglets) were hypothesized as having an enhanced responsiveness to farrowing and processing stressors when compared to the piglets born to the control sows (CON-piglets). The maternal-fetal interaction that occurred *in utero* would likely be due to the *Scb*-

supplemented diet fed to the PRO-sows during gestation, resulting in immune status and stress responsiveness effects on the offspring within 24-hours post-birth. Overall, PRO-piglets were found to have greater innate and adaptive immune status than did CON-piglets during periods of farrowing and processing stress. At d 0 of age (birth), PRO-piglets had lower plasma cortisol and greater leukocyte differential (percentage of segmented neutrophils) in response to farrowing stress. However, CON-piglets had greater total WBC count, leukocyte differential (percentage of lymphocytes), and cytokine IL-12 at farrowing. At d 1A and d 1B (pre- and post-process) of age, PRO-piglets had greater total WBC count, leukocyte differential (percentage of segmented neutrophils), and cytokine IL-12 in response to processing stressors. CON-piglets, on the other hand, had lower plasma cortisol, as well as greater leukocyte differential (percentage of lymphocytes) before and after processing. These results showed that while CON and PRO piglets both elicited differing immune responses to these stressors, the PRO-piglets did have an overall greater response at farrowing (d 0 of age), especially in terms of a lower plasma cortisol concentration and a greater subset of the neutrophil leukocyte differential. However, during processing (d 1A and d 1B of age), both CON and PRO piglets had a similar response.

The first weeks of life constitute the most critical period for piglets, since they can be exposed to conditions that negatively influence neonatal immune responses, thereby compromising their ability to resist and combat pathogenic challenges. Factors such as environmental stressors, husbandry practices, and antigenic exposures of sows each may negatively affect the early development of the piglet's immune system, with a probable increase in susceptibility to infection or disease and a reduction in growth and performance. Prenatal maternal stress during late-gestation can impair the development and reactivity of the immune system of a sow's offspring and impacts the frequency of disease and mortality (Tuchscherer et al., 2002). During the weaning period, it was also hypothesized that piglets from PRO-sows will have a greater responsiveness to both the short- and long-term effects of acute stress compared to the CON-piglets. At d 21 (weaning) of age, PRO-piglets had greater total WBC count, neutrophil phagocytosis, cytokine IL-12, and LPS-induced mitogen proliferation index in response to acute farrowing stress. However, CON-piglets had lower plasma cortisol and greater total lymphocyte count at weaning. Both CON and PRO piglets had similar responses to weaning (d 21 of age) in regards to the leukocyte differential (percentage of segmented neutrophils and lymphocytes) and natural killer cell cytotoxicity. The fewest effects on stress responsiveness or immunity were

seen during the post-weaning period (i.e., long-term). This suggests that the probiotic treatment that the piglets had first experienced *in utero* may have diminished over time in the bodies of the PRO-piglets, leading to less of an impact during the weaning period.

There are numerous benefits to the research that was conducted within this thesis. The sustainability of the swine industry, which includes the health and welfare of the swine themselves, includes optimizing the parameters of animal well-being (e.g., behavioral, physiological, immunological, and performance measures). Probiotics will expectantly lessen the impact of animal-health issues and other unavoidable stressors that often challenge food animals. Moreover, this feed additive may reduce the incidence of infectious disease outbreaks, thereby optimizing health so that the need for sub-therapeutic antibiotics declines (Cheng et al., 2014; Cho et al., 2011). Probiotics can therefore be used as an alternative feed supplement to combating the negative impacts of antibiotics on the gut microbiota and overall health of the animal. New methods of promoting feed efficiency and health are especially important in situations where farm animal populations are more susceptible to new challenges. These methods can also be aimed at implementing new alternatives to weaning, so that separation of the dam and her piglets may be carried out in a manner that utilizes low stress level methods. Therefore, the goal of utilizing probiotics as an alternative feed additive to antibiotics in swine production includes the long term sustainability of animal husbandry.

Because of inconsistency in results, it is possible that altering the experimental design of this study could result in greater stress responsiveness and immune effects in the probiotic-treated animals. This may include increasing the dosage of the probiotic given to the sows, using a different strain of probiotic as the main supplemented feed additive, or measuring additional innate and adaptive immune metrics in order to further analyze the effects of the probiotic. Even though effects were not consistent, based on the general lack of changes in the CON-sows, some aspects of innate immunity implied the PRO-sows' immune system was being affected by the *Scb* probiotic. Future research should focus on increasing the overall understanding of combining the use of antibiotics and probiotics as feed additives that would maintain a healthy agricultural economy, while also promoting human health. The positive effects that probiotics extend to animals have also been noted to be more effective and consistent when administered to weaned piglets versus growing finishing pigs (Falaye et al., 2016). Upon weaning, the intestinal microflora of piglets is altered due to dietary and environmental changes, allowing for probiotics

to have a greater and more influential role at this time. Therefore, future research may also consider directly feeding probiotics to piglets, particularly around the time of weaning to potentially reduce the negative impact of stressors.

It is possible that feeding PRO-sows the yeast probiotic supplementation during the periods of gestation and lactation, resulted in positive outcomes of both innate and adaptive immune responses. This was specifically seen in the treatment x day interactions noted previously; however, results varied between the control and probiotic-treated animals. These interactive effects may have also aided the PRO-sows in having a decreased stress response to farrowing and weaning stressors. This was seen in the cases where innate and adaptive immune measures were greater in PRO-sows when compared to CON-sows, in addition to PRO-sows having lower plasma cortisol during the lactation period (thereby decreasing responsiveness to farrowing and weaning stress). Feeding modified gestation diets to sows throughout pregnancy may reduce the incidence of infectious disease outbreaks, thereby optimizing health so that the need for sub-therapeutic antibiotics declines. Therefore, the supplementation of the yeast probiotic boluses, *Scb*, during the period of gestation to weaning did have an impact on the immune status and stress response of PRO-sows during gestation and lactation. Maternal diet, during pregnancy and throughout lactation, affects the microbiota by potentially changing the abundance and type of bacteria that can be transferred from mother to offspring during the periods of gestation, and even in the early life of offspring (Nuriel-Ohayon, 2016). A potential mechanism of great importance to this topic is the microbial transmission across the maternal-fetal interface within the placenta. Feeding *Scb* to PRO-sows during gestation and lactation may have had a maternal-fetal interaction, thereby transferring the immune status and stress responsiveness effects from the dam to her offspring. The use of probiotic-supplemented feeds may lessen the impact of animal-health issues and other unavoidable stressors that often challenge food animals.

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