

A PILOT STUDY ON THE EFFECTS OF A YEAST PROBIOTIC ON SOW AND PIGLET  
BEHAVIOR AND WELL-BEING

BY

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THESIS

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## ABSTRACT

In 2006, a ban on the use of antibiotics was implemented in Europe which has resulted in an increase interest in finding alternatives to therapeutic use of antibiotics in swine industry. Thus, we hypothesize that feeding sows probiotics during gestation and lactation could potentially improve health and well-being of not only sows but her piglets' as well. The objectives of this study were to: (a) evaluate the effects of feeding a bolus of yeast *Saccharomyces boulardii* (yeast probiotic) to sows during gestation and lactation on her behavior and well-being as well as her piglets' well-being, and (b) assess the effects of weaning stress on piglets from sows that were fed probiotics. At d 84 of gestation, 18 sows derived from Genetiporc maternal line across 3 blocks (6 sows/block) were randomly allotted to receive either a placebo bolus (control; **CON**) or probiotic bolus (treatment, **PRO**) once per day till the end of lactation. The probiotic bolus used was composed of a monogastric-specific yeast produced by LALLEMAND, known as LEVUCCELL SB® (*Saccharomyces boulardii* CNCMI-1079). Data were collected to assess behavioral, physiological, and performance traits of sows and piglets. Data were analyzed using the Mixed Models procedure of SAS. Sows fed PRO bolus had higher ( $P < 0.01$ ) white blood cell counts compared to sows fed CON bolus. On d115 of gestation, sows fed PRO bolus had higher ( $P < 0.05$ ) level of total white blood cell and neutrophils compared to sows fed CON bolus. On day of weaning (~d135 of experimental period), plasma cortisol was less ( $P < 0.05$ ) among sows fed PRO bolus compared to sows fed CON. At birth, those piglets from sows fed PRO bolus had less ( $P < 0.01$ ) total plasma cortisol compared to those piglets from sows fed CON bolus. Piglets from sows fed PRO bolus had higher ( $P < 0.05$ ) white blood cell counts overall during lactation compared to piglets from sows fed CON. On d7 of age, those piglets from sows fed PRO bolus had less ( $P < 0.05$ ) neutrophils (%) but more ( $P =$

0.057) lymphocytes (%) compared with those piglets from sows fed CON bolus. The results of this study indicate that probiotics may have beneficial effects on both sow and piglet behavior and well-being. Future research should continue to compare various aspects of yeast-like products that may have potential physiological effects on immune status, behavior and productivity of sows and their offspring, thus improving well-being.

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# **CHAPTER 1**

## **LITERATURE REVIEW**

### **1.1 Introduction**

There is mounting concern among the public and consumers on the use of prophylactic antibiotic use in food production not only on human health but food safety and animal well-being. In 2006, the European Union implemented restrictions of the therapeutic use of antibiotics in production animals and in 2012 the FDA proposed a voluntary phase out of the use of antimicrobials that are medically important in human medicine in animal production. Because of these new concerns and regulations, there has been an increased interest in the use of probiotics as a potential alternative to therapeutic use of antibiotics in food animals. Hundreds of clinical studies have shown beneficial health effects of consuming bacterial and yeast derived probiotic in humans. In 2007, Yoplait first introduced “Activia” a probiotic supplemented yogurt that if consumed could “help to regulate the digestive system” in humans. Hence, consumers are demanding more natural, holistic products. With this increase in demand it is important that we continue to research the possible advantages and disadvantages of probiotic use in our production animals.

### **1.2 What are Probiotics and How Do They Work**

According to the Webster dictionary, probiotics are defined as ‘pertaining to life’. More specifically, ANTIbiotics are a compound that kills an organism that has been encountered within the body, while PRObiotics is a substance that increases microbial organisms of the gut flora. Hence, instead of controlling the amount of bacteria in the gut, probiotics increase the

number of beneficial microbes so that once the infection occurs the body naturally fights off the pathogens without the assistance of antibiotics. In order for a probiotic supplement to be effective it must express certain characteristics—probiotics must survive the passage through the acidic gastrointestinal tract, be able to colonize and reproduce in the gut, and adhere to the intestinal epithelium while stabilizing the balance of the gut flora (Drisko et. al 2005).

### **1.3 Classes of Probiotics**

Probiotics can be either of bacterial or yeast origin. The most common species used to produce probiotics are *Acidophilus*, *Bacillus*, *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Saccharomyces* with the most studied probiotics being derived from *Lactobacillus* and *Bifidobacterium* (Lactic Acid Bacteria; LAB) and *Saccharomyces boulardii* (Weichselbaum, 2009). The LAB probiotic is a natural flora found in the gut while *S. boulardii* is a beneficial supplement. *Lactobacillus*, found in the small intestine, and *Bifidobacterium*, found in the large intestine, are known as lactic acid bacteria because they produce lactic acid as a byproduct. The LAB help maintain a healthy balance of intestinal flora by producing compounds such as; lactic acid, hydrogen peroxide, and acetic acid which all increase the acidity of the intestine and curb the reproduction of harmful bacteria. The human body is dependent upon these normal bacteria to help break down food, aid in nutrient absorption, and prevent the take-over of “bad” bacteria within the body. Moreover, these bacterial probiotics need support from other supplements, such as prebiotics, to survive the passage through the stomach and intestine. Despite the benefits of these various species they can also be detrimental if gut homeostasis is not maintained, especially during stress and illness.

*S. boulardii*, was first discovered by Henri Boulard in the year 1920 in Indochina. This yeast derived probiotic had different properties than a bacterial derived probiotic. Unlike the common “bad” yeast we usually encounter, *S. boulardii* commonly known as “baker’s yeast” is non-pathogenic but does not naturally occur in the gut like the bacterial probiotics. However, *Sacchromyces* is resistant to stomach acids, bile and pancreatic juices which makes it tolerant to varying pH levels and thermotolerant. Hence, these protective properties *Sacchromyces* increase survivability of this organism while moving through the gut. In contrast to other probiotics, *Sacchromyces* is resistant to antibiotics (Czerucka et al., 2007) and *S. boulardii* does not colonize in the gut and has been shown to be removed from the body and stool within 2 to 5 days after discontinuing use (Czerucka and Rampal, 2002).

#### **1.4 Possible Mechanisms**

Numerous studies have been conducted to determine the effects of various strains and combinations of bacterial and yeast probiotics on health and disease while the precise mechanism is largely unknown. Possible mechanisms by which probiotics may affect the host gastrointestinal system and overall immune functions have been proposed (see Figure 1). Servin and Coconnier (2003) found that *Lactobacillus* inhibits the adhesion of the *S. typhimurium* while supernatants from *L. johnsonii* has been shown to interfere with the growth and adhesion of the *H. pylori* in the gut. *S. boulardii*, has been shown to bind pathogens such as *C. difficile*, which deactivates the bacteria and inhibits attachment to the epithelium (Czerucka and Rampal, 2002). Giang et al., (2010b) found that *S. boulardii* can inhibit toxicity of *E.coli* extracellular endotoxins by restricting binding to enterocyte receptors but had no effect on actual *E.coli* counts. Also, *S.*



*boulardii* secretes a protease that can degrade Toxins A and B produced by *C. difficile*. Lactic acid, a substance produced by probiotics, has deleterious properties towards many microorganisms and can produce antibacterial properties (Marteau et al., 2004). *S. boulardii* has been shown to increase the concentration of short-chain fatty acids, lactic acid bacteria (LAB) population and lactic acid levels. An increase in VFA in the colon increases water and sodium absorption in the lumen and could decrease scouring during an *E.coli* infection (Giang et al., 2010a). The increased content of organic acids facilitated by *S. boulardii* would acidify the intestine and exert an antibacterial effect. Probiotics have the ability to enhance the defenses of the mucosal system. The GALT contains mucus gel which helps protect the intestinal mucosa from the luminal environment. Mack et al., (1999) found that *L. plantarum* and *L. rhamnosus* both can upregulate mucosal gel and inhibit the adherence of *E. coli* to the intestinal epithelial cells. The intestinal permeability is a key factor in pathogenesis of many mucosal diseases. Several trials have shown that probiotics may protect tight junctions from becoming leaky during infectious or inflammatory conditions. *L. brevis* and *L. plantarum* (Marteau and Boutron-Ruault, 2002) and *S. boulardii* (Czerucka et al., 2000) have all been shown to reduce the increased intestinal permeability during *E. coli* infection.

There is also some evidence that suggest probiotics can nonspecifically modulate the host immune system. The immune system is comprised of innate and adaptive immunity (Figure 2). The innate immune response primarily involves phagocytic cells, while the adaptive immune response involves T- (cell mediated immunity) and B- cells (humoral immunity) and appropriate cytokines need to be present to result in the appropriate immune response. More specifically, T-helper 1 (Th1) cells produce IL-2, interferon- $\gamma$  (IFN- $\gamma$ ) and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), whereas T-helper 2 (Th2) cells produce IL-4, IL-5, IL-6, IL-10 and IL-13 (Figure 3).

Probiotics may promote the nonspecific stimulation of the host immune system such as enhanced phagocytic activity since this is the first line of defense against bacterial challenges. In fact, bacterial probiotics such as *Lactobacillus* have repeatedly been shown to increase the rate of phagocytosis in the host (Klein et al., 2007; Olivares et al., 2006; Roessler et al., 2008). However, probiotics have been shown to have immunosuppressive properties by suppressing the inflammatory response most likely through disrupting levels of the pro- and anti-inflammatory cytokines. Specifically, *S. boulardii* has been shown to block the secretion of inflammatory cytokines such as IL-8, IL-6 and  $\text{TNF}_\alpha$  which are released in response to an infection (Dahan et al., 2003; Dalmaso et al., 2006a), whereas, *Bifidobacteria* and *Lactobacillus* have been shown to have no effect on  $\text{TNF}_\alpha$ . Olivares et al. (2007) reported an increase in plasma IL-12 in healthy adults when they received supplements with *L. gasseri* but Taylor et al., (2006) reported no effect when *L. acidophilus* supplements were used. Others have shown that the B-cell population in the duodenum increased after the administration of bacterial probiotic *L. reuteri* (Valeur et al., 2004), while intramucosal gut T-cells and IgA levels in the intestine have been shown to increase in healthy adults given *S. boulardii* derived supplements.

### **1.5 Bacterial Probiotic Effect on Humans**

The human body contains between  $10^{12}$  and  $10^{14}$  of bacteria with the majority of these colonizing in the gut starting at birth. These bacteria are vital for a healthy immune system to fight against the invasion of pathogenic strains, aid in digestion, and to assist in releasing vitamins and nutrients. Of these numerous strains, only a select few are culturable, which can potentially benefit the health of an individual by modulating the gut flora and aiding in these processes.

However, different strains, mixtures of strains, and delivery systems (i.e, yogurt, powder) can result in different benefits. The most commonly researched bacterial probiotic is *Lactobacillus rhamnosus GG* (LGG) and *Bifidobacterium*. Both, Szajewska et al., (2001) and D'Souza et al., (2002) found that LGG could reduce the number of cases of a Antibiotic-associated diarrhea (AAD) in children that were receiving Aminopenicillin therapy. Of course, specific strains do vary in effectiveness in treating disease. For example, researchers found that *L. rhamnosus* decreased symptoms associated with irritable bowel syndrome (IBS) (Gawrońska et al., 2007), whereas others found that *Lactobacillus johnsonii* had no effect on the occurrence rate of diarrhea in their patients (Marteau et al., 2006; Van Gossum et al., 2006). Moreover, Hickson et al., (2007) found that a combination of several bacteria (*L. casei*, *L. thermophilus*, and *L. bulgaricus*) mixed in yogurt could reduce the risk of *C. difficile* associated diarrhea and none of these patients developed AAD even though 9 out of 17 (52.0%) of these patients were positive for *C. difficile* toxin. It appears that a combination of different probiotics may be more beneficial over a single strain, several researchers have reported that combining 8 different bacterial strains (VSL#3) relieved the symptoms associated with Colitis and IBD (Kim et al., 2003; Venturi et al., 1999). In fact, Venturi et al. (1999) reported that 75% of these patients were still in remission 12 months post-treatment. Although the results vary from strain-to-strain these data provide support that there are health benefits associated with using probiotic supplements.

### **1.6 Bacterial Probiotic Effects on Piglets**

In 1970, Zimmerman et al., (1970) first studied the effects of challenging germ-free rats with microorganisms on the immune system. Today, researchers have broadened the scope to

include other species such as humans, dogs, livestock, and aquatic species (i.e., farm-raised fish). The public interest and demand for antibiotic-free products has led to an increase in studying the effects of using probiotic supplements in the livestock industry as a potential alternative to antibiotics as growth promotants. The most commonly used probiotics are bacterial derived species that come from either the genus of *Bacillus* or *Streptococcus*. Giang et al., (2010a) found that those piglets fed LAB had reduced incidence of diarrhea and improved average daily feed intake (ADFI), average daily gain (ADG) and lower feed conversion ratio (FCR) than did piglets in the control group. Moreover, a mixture of both *Bacillus* and *Streptococcus* tended to enhance ADG and FCR in piglets subjected to weaning and mixing stress (Taras et al., 2005). Following an *E. coli* challenge, those piglets fed a supplement of *Lactobacillus rhamnosus* GG (LGG) had a lower incidence rate of diarrhea as well as increased serum  $\text{TNF}_\alpha$  but decreased IL-6. Total intestinal IgA has been shown to be increased by *Bifidobacterium longum* (Vitini et al., 2000). During lactation, serum levels of IgG were not affected by *Enterococcus faecium*, but post-weaning serum levels of IgG were stabilized for piglets fed *E. faecium* compared to controls. Others have effects of LGG on the immune system of piglets. Collado et al., (2007) found that LGG could adhere to intestinal mucus in vitro and displace as well as inhibit *Salmonella*, *Colostridium*, and *E. coli*, while Zhang et al. (2010) found that LGG affected various pro- and anti-inflammatory cytokines but had no effect on total white blood cell count.

### **1.7 Bacterial Probiotics Effects on Sow and Offspring**

Bacterial probiotics fed to sows may potentially benefit not only the sow but her offspring too. Feed intake during lactation (5.2 vs. 4.2 kg/d) was increased in sows fed *Bacillus*

*cereus* var. *toyoi* probiotic and body weight loss was less post-lactation compared to control fed sows (Taras et al., 2005). Others have shown that feed intake was increased in sows fed *Enterococcus faecium* and body weight loss throughout lactation was less and these sows had increased litter size (Kreuzer and Zerhusen et. al., 1995; Bohmer, 2006). Also, fecal *E.coli* count and body temperature were lower amongst sows fed *E. faecium* compared to control sows (Bohmer, 2006). Bohmer attributes the effect on body temperature to the enhancement of the immune system when dealing with the stressors associated with farrowing. Also, feeding sows *B. cereus* led to an increase in fecal IgA during lactation (Scharek et al., 2005). Moreover, BioPlus2B, a probiotic which contains *Bacillus lincheniformis* and *Bacillus subtilis*, when fed to sows resulted in a decrease in preweaning mortality, diarrhea occurrence, and increase in percentage of fat in the milk during mid-suckling period (Alexopoulos et al., 2004). Moreover, sows fed BioPlus2B weaned more pigs and piglets from those sows fed BioPlus2B had greater body weight.

### **1.8 *Saccharomyces boulardii* Effect on Health**

Most of the research using *S. boulardii* as the primary probiotic has been conducted in humans and rodents. It has been shown that this specific strain can control diarrheal disease in rats and humans, but its precise mechanisms are not known (Buts, 2009). Moreover, others have shown that *S. boulardii* can reduce symptoms of IBD, such as Crohn's, colitis, and prevent or treat diarrheal diseases such as AAD, acute gastroenteritis, and chronic diarrhea in human immunodeficiency virus-infected patients.

### **1.9 *Saccharomyces boulardii* Effect on the Immune System**

In both humans and swine, yeast probiotics can cause the proliferation of LAB which has an effect on inflammation in the intestinal tract. The proliferation of LAB in the intestine leads to the secretion of immunosuppressive cytokines, such as interleukin-10 (IL-10) (De Moreno de LeBlanc et al., 2008). This LAB induced-secretion of IL-10 results in an “anti-TNF $\alpha$ ” effect in the intestine and IL-10 crosses the intestinal barrier to reach the local immune system under both normal and inflammatory conditions (Perdigon et al., 2002). Lactic acid bacteria enhances the non-specific and specific immune responses, helping to control intestinal infections, mainly through control of TNF $\alpha$ , (Dalmasso et al., 2006b), whereas, others have reported no effect of *S. boulardii* on the production of various cytokines such as IL-6, IL-10, IL-12p70, IL-23 and TNF $\alpha$  (Gad et al., 2011). *S. boulardii* has been shown to improve the condition of the intestinal barrier by decreasing lipopolysaccharide (Thomas et al., 2011), preventing the adherence of pathogens to the intestinal wall, and neutralizing bacterial endotoxins (Gad et al., 2011). These minor effects positively affect the intestinal system which in turn aids the immune response. These indirect effects are one reason why *S. boulardii* is such a good candidate against intestinal upsets.

### **1.10 *Sacchromyces boulardii* Effect on Swine**

One of the most common yeast probiotics investigated has been *S. boulardii* due to its positive effects in humans. Breves et al. (2000) found that supplementing a swine diet with *B. cereus* var. *toyoi* and *S. boulardii* increased sodium-dependent glucose absorption and trans-epithelial nutrient transport in the jejunum. Others have found that feeding *S. boulardii* led to an increase in the villous length in the small intestine of pigs (Kamm et al. (2004). Both,(Gad et al.,

2011; Perdigón et al., 2002) reported a reduction in pathogen-induced diarrhea amongst piglets fed a *S. boulardii* supplement compared to piglets without the supplement. Often weaning stress can result in the breakdown of the intestinal barrier function as detected by a decrease in villous height and nutrient absorption. Le Bon et al. (2010) found that feeding *S. boulardii* to piglets for 6 weeks then feeding *Pediococcus acidilactici* for the next 3 weeks led to an increase in the feed conversion ratio of piglets, most likely by decreasing the *E.coli* population, when compared to control pigs. Others have found that *S. boulardii* can inhibit toxicity of *E.coli* extracellular endotoxins by restricting the binding to enterocyte receptors but had no effect on actual *E. coli* count (Giang et al., 2010a). Supplementing swine weaning diets with *S. boulardii* may be extremely beneficial especially since the stress of weaning can cause the decrease in good bacteria while increasing the *E.coli* population which contributes to increased susceptibility to illness and health (Giang et al., 2010b).

### **1.11 Maternal Behavior and its Effect on Piglet Well-being**

Sow behavior during farrowing and lactation can be useful indicators of well-being. Pre-partum nest-building, post-partum duration in specific postural position and responsiveness to piglets expressed by the sow are very important for piglet survival throughout the lactation phase. Nest-building behavior is initiated around parturition due to the rise in prolactin and the drop of progesterone (Wischner et al., 2010). In confinement, nest building starts with sows digging or pawing with the front hooves (Algers and Uvnas-Moberg, 2007) but in the wild, sows dig in order to create a hole and will rip and gather branches and grass to fill the nest and distribute evenly. In confinement, often nest-building behaviors are displayed by sows biting and

pulling at the rails, pipes, waterer, and feeder with nodding head movements while rubbing their snout against the floor since these environments are devoid of actual physical nesting material (Lou and Hurnik, 1998). Sows that perform a greater amount of nest-building behaviors have been shown to respond better to stimuli expressed by piglets and overall have better performance (Algers, 1994). One of the leading causes of death in the first 72-h of life for a piglet is crushing. In the first few days of life piglets are unable to regulate their own body temperature. According to Hrupka et al. (1998), regardless of heat lamp location or air temperature piglets tend to lie next to the sow. The sow's pre-lying behavior and postural changes are very important for piglet safety due to their proximity to the sow. Research has shown that sows who sit first before lying down tend to crush more piglets (Wischner et al., 2009) while sows that tend to stay in one position for a longer period of time have improved maternal ability and increased piglet well-being (Cui et al., 2011). Staying in one position longer allows piglets to move closer to the udder for nutrients but also allows for a warm and safe environment. When Wischner et al. (2010) evaluated crushing rates of piglets by sows, they found those sows that displayed longer durations and more frequent bouts of standing and less sitting were less likely to crush their piglets.



## **CHAPTER 2**

### **A PILOT STUDY ON THE EFFECTS OF PROBIOTICS ON SOW AND PIGLET**

#### **BEHAVIOR AND WELL BEING**

##### **INTRODUCTION**

Antibiotics are routinely used in healthy production animals to improve feed efficiency and sustain healthy animals. However, the use of antibiotics in food animals has become a global concern due to the increased occurrence of antibiotic resistant bacteria. Hence, this has led to an increasing interest in finding an alternative to antibiotics, such as probiotics. Probiotics are microorganisms that, when ingested in adequate amounts, confer a health benefit on the host (Czerucka et al., 2007). Probiotics have been shown to improve intestinal microbial balance, thus inhibiting pathogens and toxin-producing bacteria. Recently it has been shown that feeding probiotics to piglets during lactation enhances animal well-being by reducing the incidence of pathogen-induced diarrhea post-weaning (Gad et al., 2011). If the benefits of probiotics are transferable from dam to piglets, the benefits of feeding the dam probiotics during gestation and lactation may outweigh the economic cost of the addition of the supplement to daily feed of wean-to-finish pigs. The goal of this research was to evaluate the effects of feeding a yeast probiotic, *Saccharomyces boulardii*, to sows during gestation and lactation on sow and piglet behavior and well-being and to evaluate how weaning stress may differentially affect the well-being of piglets from sows that were fed probiotics during gestation and lactation.

## **MATERIALS AND METHODS**

### **Animals, Housing, and Experimental Design**

Eighteen sows derived from the Gentiporc maternal line were kept at the University of Illinois Swine Research Center and housed in standard gestation stalls in a mechanically-ventilated insulated gestation building until ~d 112 of gestation. All diets were formulated to meet or exceed NRC requirements (NRC, 2012; Table 3.2). Sows were fed once daily during gestation based on body condition score. At d112 sows were moved to farrowing rooms and kept in individual farrowing stalls and maintained on a 10:14 h light:dark schedule with lights on at 0700 h and lights off at 1700 h. During lactation sows were fed ad libitum. On d 84 of gestation, sows were allocated to either a placebo bolus (CON) or probiotic bolus (PRO) based on body weight (BW) (n = 18 sows, 9 sows per treatment). The probiotic bolus was composed of monogastric specific yeast produced by LALLEMAND, known as LEVUCCELL SB® (*Saccharomyces boulardii* CNCMI-1079). The concentration of each bolus was  $2.0 \times 10^9$  (CFU/g). Levucell SB is non-toxic and harmless to humans and animals. It leaves no residues and has no withdrawal time.

Starting on d84 of gestation, sows were hand-fed 2 boluses every morning around 0600 h (feeding time) until the end of lactation (weaning ~ d 21 of lactation). The CON bolus was always fed first. Gloves were worn and discarded and hands were washed after the daily feeding of the probiotics bolus, to prevent the possible transfer of probiotics to the control-fed sows. The CON bolus was sugar based and was the same size and shape of the PRO bolus.

## **Physiological Measures**

Blood samples (~10 mL) were obtained from sows on d 84, 91, 98, 112 of gestation, 24-h post-farrowing and weaning via vena-puncture using vacutainers. Blood samples (~10 mL) were obtained from piglets on d 0 (immediately after birth), 1 (pre-process and post-process), 7, 14, and 21 of age (weaning), and then again at 24 h post-weaning and on d 7, and 14 post-weaning. Red blood cells were lysed with Zap-o-globin® (Beckman Coulter) and total white blood cell counts (**WBC**) were made electronically using a Coulter Z1 particle counter (Beckman Coulter) by adding 10µL of whole blood to Isoflow® (10 mL; Beckman Coulter, Miami, FL). Whole blood smears were made, fixed in methanol, stained with Hema-3® staining system (Fisher Scientific, Houston, TX) and viewed under a light microscope to determine leukocyte differential counts (Figure 1a).

For functional immune assays, whole blood was diluted with Roswell Park Memorial Institute medium (RPMI; Gibco, Carlsbad, CA) layered over Histopaque® -1077, (density = 1.077 g/mL; Sigma Aldrich, Saint Louis, MO) and -1119 (density = 1.119 g/mL; Sigma Aldrich) and centrifuged at 700g 30 min at 25°C. Lymphocytes were removed from the 1077 layer, washed twice in RPMI, resuspended, and counted. Neutrophils were removed from the 1119 layer, washed once, and then red blood cells were lysed from the neutrophil fraction, and washed again in RPMI (Figure 1b). Cell concentrations were adjusted with RPMI based on the specific immune assays' respective requirements.

## **Plasma Analysis**

Whole blood was collected and centrifuged at 700g for 30 min at 4°C to collect plasma for analysis. Using a validated commercial radioimmunoassay (Coat-A-Count®, Los Angeles,

CA) plasma cortisol concentrations were measured. Intra- and inter-assay CV were 4.5% and 5.6%, respectively. Using a Quantikine Porcine IL-12/IL-23 p40 Eliza kit (R&D Systems® Minneapolis, MN), sow and piglet plasma samples were measured for levels of IL-12.

### **Immune Assays**

Neutrophil Chemotaxis was measured using an assay previously described by (Salak et al., 1993). Neutrophils were used at a concentration of  $3 \times 10^6$  cells/mL to evaluate the ability of cells to migrate toward assay medium (control; random migration) or recombinant human complement-5a ( $10^{-7}$  M; Sigma Aldrich) and recombinant human IL-8 (100 µg/mL; Sigma Aldrich) (chemotaxis-directed migration).

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

A mitogen-induced lymphocyte proliferation assay was performed using a CellTiter 96® nonradioactive cell proliferation assay (Promega, Madison, WI) following the manufacturer's protocol with minor modification as previously described by (Sutherland et al., 2005). Briefly, porcine lymphocytes were used at a concentration of  $5 \times 10^6$  cells/mL and placed in triplicate into a sterile 96-well flat-bottom plate. Concanavalin A (CONA; Sigma Aldrich) and lipopolysaccharide (LPS; Sigma Aldrich) were used as mitogens (0, 0.2, 2.0 and 20 µg/mL) to stimulate T and B cells, respectively. Plates were incubated 68 h at 37°C in a 5% CO<sub>2</sub> humidified

incubator and 15  $\mu$ L Promega Dye was added to each well, and the plates were incubated 4 h. Promega Stop solution (100  $\mu$ L) was added, and the plates were incubated overnight at 37°C and then read using a microplate reader (BIO-TEK Instruments) at wavelength 550 nm with reference wavelength 690 nm. The results are expressed as a proliferation index (PI):

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

Natural killer (NK) cell cytotoxicity was measured using a commercially available nonradioactive cytotoxicity detection kit (Roche Diagnostics, Indianapolis, IN) as described previously by Sutherland et al. (2005). Briefly, porcine lymphocytes were used as effector cells and K-562 chronic human myelogenous leukemia cells (American Tissue Type Culture Collection, Manassas, VA) were used as target cells. Lymphocytes were adjusted to  $1 \times 10^7$  cells/mL and K-562 cells adjusted to a constant 10,000 cells per well. Samples were run in triplicate at effector (lymphocytes) to target-cell (K-562) ratios of 12.5:1, 25:1, 50:1, and 100:1, respectively. Plates were read using a microplate reader (BIO-TEK Instruments) at wavelength 490 nm and reference wavelength 690 nm after an 18 h incubation period. Percent cytotoxicity was calculated as described by (Lumpkin and McGlone, 1992) and an assay was considered valid if maximum release divided by spontaneous release was  $\leq 20\%$ .

### **Fecal Enumerations**

Fecal samples were collected on the same days as blood samples. Yeast enumerations were done by standard methods on Rose Bengal Chloramphenicol Agar (Oxoid). The first 10:1

dilution was made by adding 9 volumes Maximum recovery diluent (MRD) of the original sample weight. Further serial dilutions were made with MRD (100µl into 900µl) and then plated by spread plate method (100µl) onto duplicate agar plates in concentrations of  $1 \times 10^1$  to  $1 \times 10^9$ . The RBC agar plates were incubated aerobically for 3 days at 30 °C. After 3 days positive identification was based on the typical colony morphology of SCB control strain. Yeast colonies were successfully grown on RBC agar streaked with samples collected from only sows in the probiotic treatment. This was used to confirm that the control sows did not ingest a significant amount of the probiotic to maintain a colony in the GI tract. Sample pictures of SBC growth on RBC agar are shown (Figure 2.), provided by Dr. Ken Mellits at the University of Nottingham.

### **Behavioral Measures**

Behavior of sows was recorded in replicate 1 (n=4) using a Geovision GV-1240 video capture combo card and viewed using Window Media Player in real-time. Behavior was recorded continually for 24-h post-farrowing. Postural (sit, stand, lay), maintenance (eat, drink) and oral-nasal-facial (ONF) behaviors were observed and registered. Also, social interaction between sow and piglets were also registered, these included physical contact between sow and piglet and nursing bouts (Table 1). Behaviors were analyzed using continuous sampling.

### **Performance Measures**

Sow BW was recorded on d 84 of gestation and again at weaning. Piglet BW was recorded on d 1, 7, 14, and 21 days of age, and again at 24-h, d 7 and 14 post-weaning.

## **Statistical Analysis**

Data were analyzed using PROC MIXED with repeated measures SAS (SAS Inst. Inc., Cary, NC). All traits were tested for departures from a normal distribution. The model included fixed effects of treatment (control or probiotic) and day of gestation for sows and treatment and day of life for piglets. The random effect of parity was evaluated for sows. A preliminary analysis of data means and numerical trends was used to analyze sow behavior measurements. Behavioral observations for replicates 2 and 3 are still in progress, thus, the entire data set is not complete yet to do a valid statistical analysis. Estimates were obtained using the PROC MIXED of SAS. Significance was set at ( $P \leq 0.05$ ), and trends were discussed at ( $P \leq 0.10$ ).

## **RESULTS**

### **Sows -Gestation**

During gestation, there were no main effects of probiotics on sow immune or endocrine traits (Table 2), but there were main effects of day of gestation on immune traits (Figure 3 and 4). Whole white blood cells stayed consistent throughout gestation while neutrophil and lymphocyte populations changed (Figure 3). Neutrophil count increased between d 105 ( $3.6 \times 10^7$ ) and d 112 ( $4.4 \times 10^7$ ) while lymphocytes decreased between d 105 ( $2.3 \times 10^7$ ) and d 112 ( $2.0 \times 10^7$ ) (Figure 3). Percentage of lymphocytes decreased while neutrophils increased across days of gestation (Figure 4).

There was a day of gestation x treatment effect on cortisol (Figure 5). Sows fed the PRO bolus had greater ( $P < 0.05$ ) cortisol on d 98 of gestation than did those sows fed CON bolus.

### **Sows –Farrowing and Weaning Stress**

There were treatment effects on several immune and cortisol measures in response to farrowing stress (24-h post-farrowing) and weaning stress (Table 3). At 24-h post farrowing, sows fed PRO bolus had greater ( $P < 0.05$ ) total white blood cell and neutrophil counts but less ( $P < 0.05$ ) plasma cortisol compared to those sows fed CON bolus (Table 3 and Figure 6). At weaning sows fed the CON bolus still had greater ( $P < 0.05$ ) plasma cortisol compared to those sows fed the PRO bolus. Moreover, sows fed the PRO bolus had greater ( $P < 0.01$ ) LPS-induced lymphocyte proliferation than did those sows fed CON bolus. All others traits were similar between treatments at both 24-h post-farrowing and weaning (Table 3).



### **Sow Performance**

Sow body weight and litter weight were similar between sows fed CON and PRO boluses (Table 4).

### **Behavior**

No significant differences were seen between treatments on post farrow sow behavior (Table 5). This is probably in part to low numbers (n=6). Apart from this fact there could still be a potential that probiotics could affect sow behavior. The duration of maternal behaviors displayed, nursing bout and physical contact with piglets, did not vary by much between treatments. Sows fed the CON bolus spent almost double the amount of time drinking compared to sows fed the PRO bolus, but didn't differ in time spent eating. Sows fed the CON bolus displayed a greater amount of stereotypic oral-nasal-facial behavior compared to sows fed the PRO bolus. Sows fed the PRO bolus tended to display less lay and stand behavior compared to sows fed the CON bolus. Conversely, sows fed the CON bolus tended to display less sit behavior compared to sows fed the PRO bolus.

### **Maternal Treatment Effects on Offspring During Lactation**

In general there were dam treatment effects on her offspring (Table 6). Those piglets from dams receiving the PRO bolus had less ( $P < 0.05$ ) total plasma cortisol but greater ( $P < 0.01$ ) total white blood cell count and greater ( $P < 0.01$ ) Interleukin-12 than did piglets from sows fed CON bolus. All other traits were similar between piglets from dams fed either PRO or CON boluses.

There was a dam treatment x piglet day age effect on several traits (Table 7). At birth, piglets from dams fed PRO bolus had less ( $P < 0.001$ ) cortisol but greater total WBC ( $P = 0.32$ ) and percent segmented neutrophils ( $P < 0.05$ ) than did piglets from dams fed CON bolus. Piglets from dams fed PRO bolus tended to have a higher ( $P < 0.10$ ) percentage of monocytes on Preprocess, but had less ( $P < 0.05$ ) percentage lymphocytes on post process compared to offspring of CON sows (Table 8). At d 7 of age, piglets from sows fed PRO bolus had greater ( $P < 0.05$ ) total white blood cell and percentage of lymphocytes ( $P < 0.10$ ), but less ( $P < 0.05$ ) but less segmented neutrophils (Table 7). At d 14 of age, those piglets from sows fed PRO bolus had greater ( $P < 0.05$ ) neutrophil chemotaxis in response to both IL-8 and C5a and IL-12 compared to piglets from sows fed CON bolus (Table 7). Across all days of age, only IL-12 was affected by dam treatment, with those piglets at 14 d of age having greater ( $P < 0.05$ ) IL-12 than piglets from sows fed CON bolus (Table 7). At d21 of age, those piglets from sows fed PRO bolus had less ( $P < 0.05$ ) lymphocytes but greater ( $P < 0.10$ ) neutrophil phagocytosis compared to offspring of CON sows. Those piglets from sows fed CON bolus had greater ( $P < 0.05$ ) neutrophil chemotaxis in response to IL-8 compared to piglets from sows fed PRO bolus.

### **Maternal Treatment Effects on Piglets to Weaning Stress**

The change in measures of well-being between the 24hr period of weaning and post weaning had little effect on immune measures. In all measures that were effected, the stress of weaning seemed to have less of an impact on offspring from sows fed PRO bolus compared to those piglets from sows fed CON bolus (Table 9). Total white blood cell count ( $P < 0.001$ ) and percentage of banded neutrophils ( $P < 0.10$ ) were both less for piglets from sows fed PRO bolus compared with piglets from sows fed CON. Although not statistically relevant it is biologically relevant that during the weaning processes we saw a change in total lymphocyte count

5.16x10<sup>7</sup> in offspring of PRO sows compared to a change of 9.14 x10<sup>7</sup> in offspring of CON sows (Table 9).

### **Long-term Maternal Treatment Effects on Piglets (Post Wean)**

Maternal treatment effect on piglets immune traits post-weaning (Table 10). Total white blood cells, neutrophils, and lymphocytes counts were all lower ( $P < 0.05$ ) while percentages of lymphocytes were greater ( $P < 0.01$ ) and neutrophils less ( $P < 0.01$ ) in offspring from sows fed PRO bolus compared to offspring from sows fed CON (Table 10). Plasma cortisol was greater ( $P < 0.05$ ) and NK less ( $P < 0.10$ ) in the offspring from sows fed PRO compared to piglets from sows fed CON (Table 10).

There were maternal treatment effects on piglet immune traits at 24-h post-wean, and 7 and 14 d post-wean (Table 11). At 24-h post-wean, piglets from sows fed PRO bolus had less ( $P < 0.01$ ) total white blood cell count and neutrophil bands ( $P < 0.10$ ) but greater plasma cortisol ( $P < 0.001$ ) compared with piglets from sows fed CON bolus (Table 11). At d 7 post-wean, piglets from sows fed PRO bolus had greater ( $P < 0.01$ ) percentage of lymphocyte but less ( $P < 0.05$ ) NK cytotoxicity than did piglets from sows fed CON bolus. Percent of eosinophils were lower ( $P < 0.05$ ) in those piglets from sows fed CON bolus (0.45%) compared to piglets from sows fed PRO (0.93%) (Table 11). At d 14 post-wean, piglets from sows fed PRO bolus had lower total neutrophils ( $P < 0.001$ ) and lymphocytes ( $P < 0.10$ ) counts and less segmented neutrophil ( $P < 0.01$ ), a greater percentage of lymphocytes (0.01) and greater ( $P < 0.05$ ) neutrophil chemotaxis in response to both IL-8 than did piglets from sows fed CON bolus (Table 11).

Piglet weight and levels of Interleukin-12 did not vary between treatment throughout the whole trial (Figure 7 and 8).

## DISCUSSION

Feeding yeast-derived probiotic boluses to sows during gestation and lactation can affect the physiology of both dams and her piglets. An increase in total white blood cell count was found for sows that consumed probiotic bolus. Positive effects of probiotics were also found in plasma cortisol levels. During the weaning process sows fed CON tended to have a higher cortisol reading than sows fed PRO. Control sows were always bled first, and blood was not taken from the sow until her own litter had been removed from the pen. Sows were kept in one single room so there possibility remains that some sows were stressed out from the general commotion of the weaning process before her own litter was even removed. IF, the probiotic fed sows, always bled proceeding control fed sows, were affected by this commotion we would expect cortisol to already be elevated in the blood system and for levels to be skewed. If this was the case, we may have hindered the results from displaying even a bigger gap of cortisol levels between treatments.

Probiotics affected the physiology of both dams and her piglets. An increase in total white blood cell count was found for sows that consumed probiotic bolus and this resulted in their offspring also having higher total WBC count. Moreover, post-weaning, piglets from sows fed the CON bolus had lower total white blood cell count than piglets of sows fed the PRO bolus. The increase in the total number of white blood cells in the periphery may be indicative of an activated immune system in response to the effects of probiotics. On d7, cell populations were affected by probiotic supplementation with lymphocytes being higher and segmented

neutrophils were lower (matured banded neutrophils) in the blood of piglets of sows fed PRO compared to piglets of sows fed CON.

Cortisol levels of piglets at birth showed an as interesting picture. Offspring of sows fed the CB had a cortisol mean level of 203.55pg/mL compared to of those offspring of sows fed the PB, 129.77pg/mL. Sows were observed 24/7 around farrowing and when a piglet was born, they were immediately dried off and bled, preventing any outside influences on cortisol level.

It appears that farrowing is more of a humoral driven stress response and weaning is more of a “cell-mediated” driven stress response. This is explained by the day effect of IL-12 plasma concentrations tending to be at significantly higher levels on weaning compared to 24h post farrowing. Interleukin-12 is a cytokine produced by the TH1 T-cell subset. IL-12 produces cytokines that activate macrophages enabling them to destroy intracellular microorganisms as well as activate b-cells to produce strong antibodies. This is known as a cell-mediated response. Offspring of the sows fed PRO also had higher levels of IL-12 compared to their counter parts. This could potentially be attributed to their maternal transfer across the GI tract occurring through the dam’s milk allowing for the passage of IL-12 from dam to piglet. On the contrary, Gad et al found that *S. boulardii* was unable to stimulate cytokines like IL-12. The TH1 subset also contains the Interleukin, IL-10. This interleukin is produced by lactic acid bacteria (LAB). This LAB has the ability to secrete suppressive cytokines like IL-10. When probiotics increase the production of LAB, it would cause the decrease the dendritic cell activation (De Moreno de LeBlanc et al., 2008). A clear example of this effect can be seen on PreWean of the piglets of sows fed CON with a higher amount of migrated cells by the chemokine IL-8 than the off spring of sows fed PRO. The immune response of phagocytosis by neutrophils is also suppressed. Piglets of sow fed CON are less successful at engulfing the florescent beads compared to the

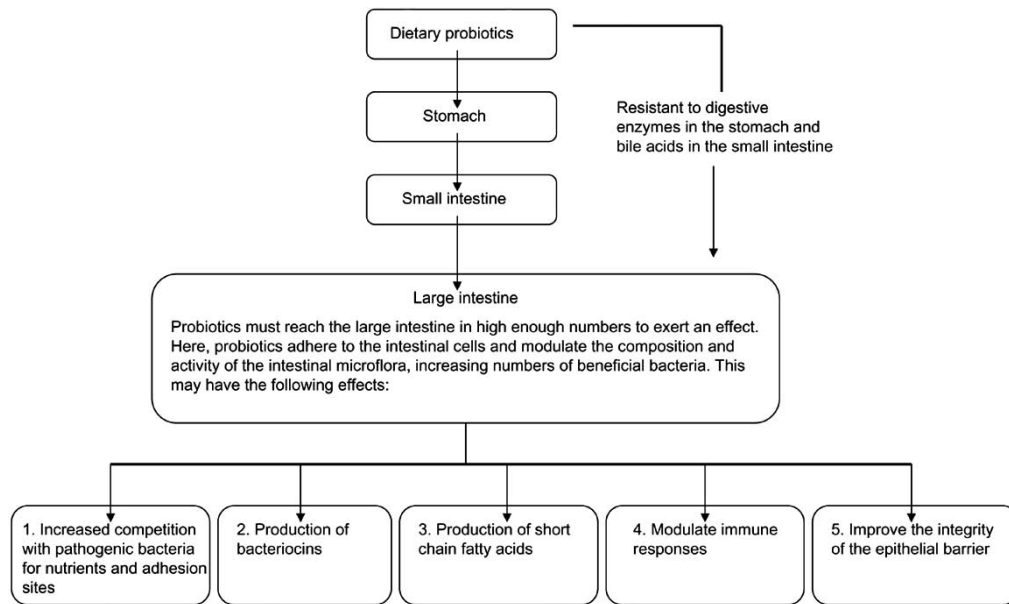
offspring of pigs fed PRO. Although this is a suppression of the immune system, it could be a positive benefit to a young piglet. Early in life a piglet's immune system is very naïve, the immune system is put into gear sometimes too quickly. Dalmaso et al, (2006) found that an over-stimulation of the inflammatory response can lead to chronic systems such as IBS. Thus this suppression allows the piglet's immune system to stay in a more stable state and not become potentially hypersensitive to stress.

## **IMPLICATIONS**

According to Bauer et al. (2006), it takes usually about seven weeks for the intestinal composition to be comparable to that of a mature animal. Probiotics enhanced the sow's immune system. From the data we assume that the difference in immune measures is related to the maternal transfer and the indirect effects of probiotics on the dam's immune system. In light of this, the immune system may have been statistically different between treatments for immune and endocrine measures but performance measures did not vary. If neither treatment of piglets are able to cope with the various stressors that they encounter in the first few weeks of life, then the response may not vary as much. Probiotics that are directly fed to piglets help assist in this process, by protecting the intestinal population of good bacteria/yeast by amping up the population numbers. Since the maternal transfer benefits, not the direct feeding of probiotics, was the focus of the study to offspring, we may not experience such an extreme degree of difference as hoped for. In order to get a more holistic picture of the effects of probiotics, more performance and productivity measures should be evaluated. The following are suggestions for further investigation; more frequent weight measurements of sows during gestation, feed intake of sows during farrowing, standardized litters, the evaluation of various interleukins found in dam's colostrum, and the administration of an immune challenge by the inoculation administration of E-Coli or LPS (lipopolysaccharide).

## FIGURES AND TABLES

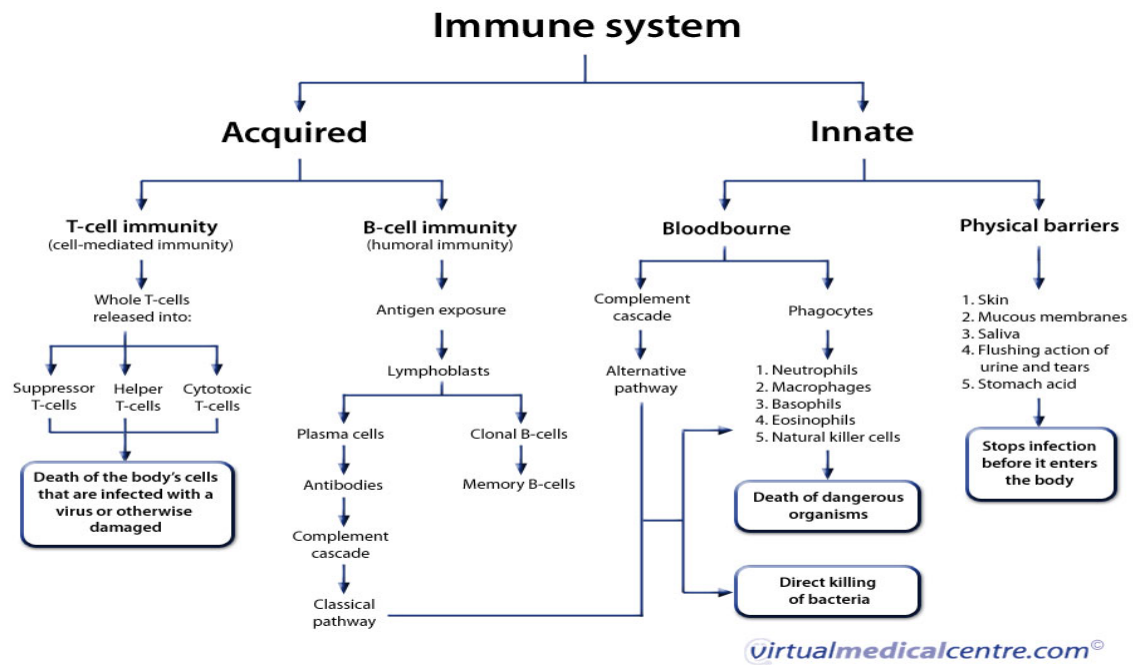
**Figure 1. Probiotic's Effect on the Gastrointestinal System**



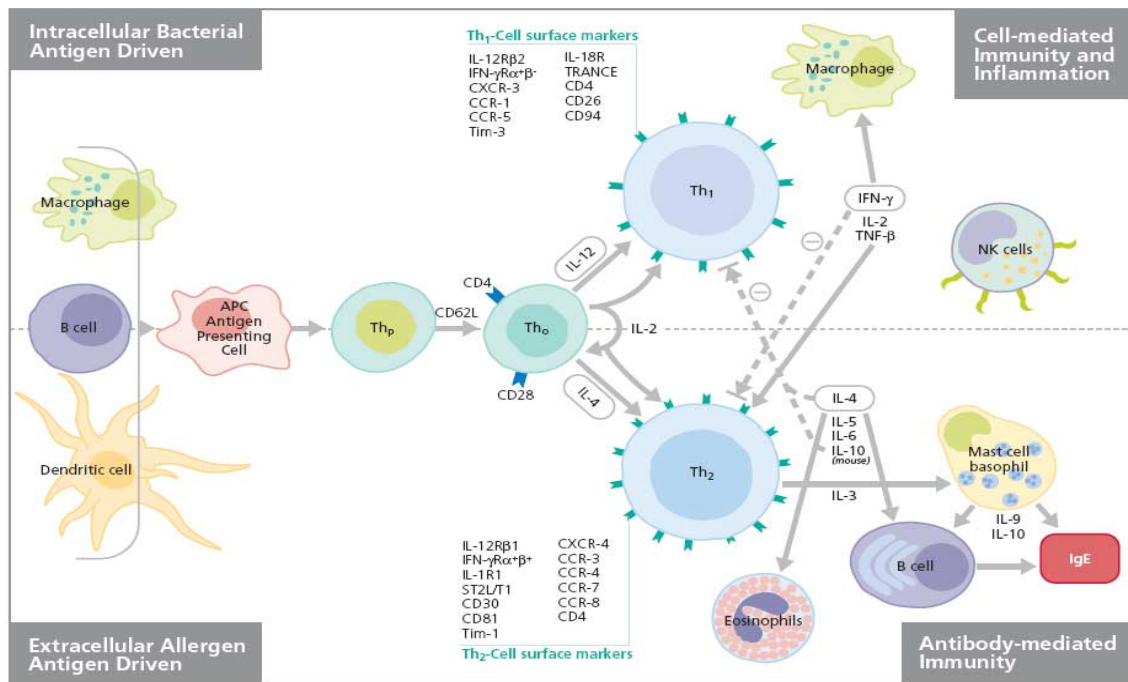
(Lomax et al., 2009) Bacteriocins are produced by bacteria to inhibit the growth of similar or closely related bacterial strain. Produced by *Lactobacillus* and other species which produce lactic acid.



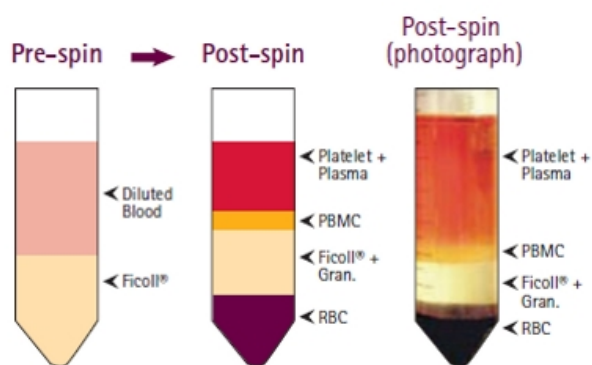
**Figure 2. A Breakdown of the Immune System: Acquired versus Innate**



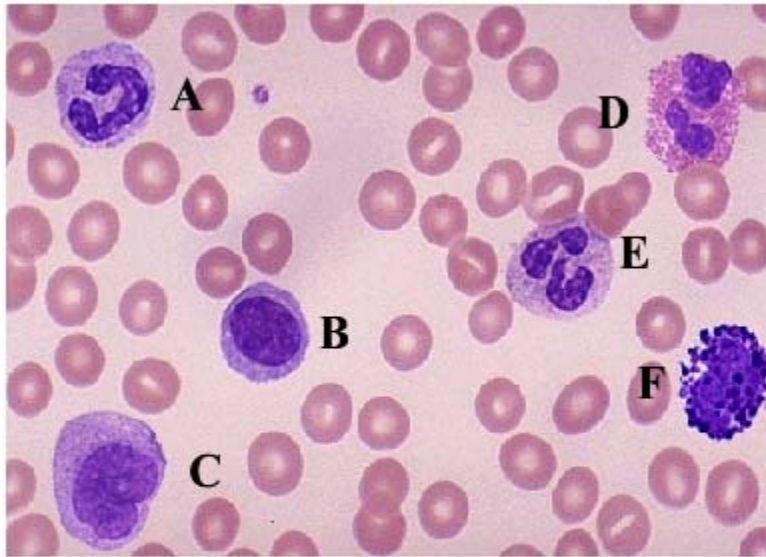
**Figure 3. TH<sub>1</sub> and TH<sub>2</sub> Driven Immunity**



**Figure 4a. Process of Lymphocyte/Neutrophil Separation from Whole Blood**

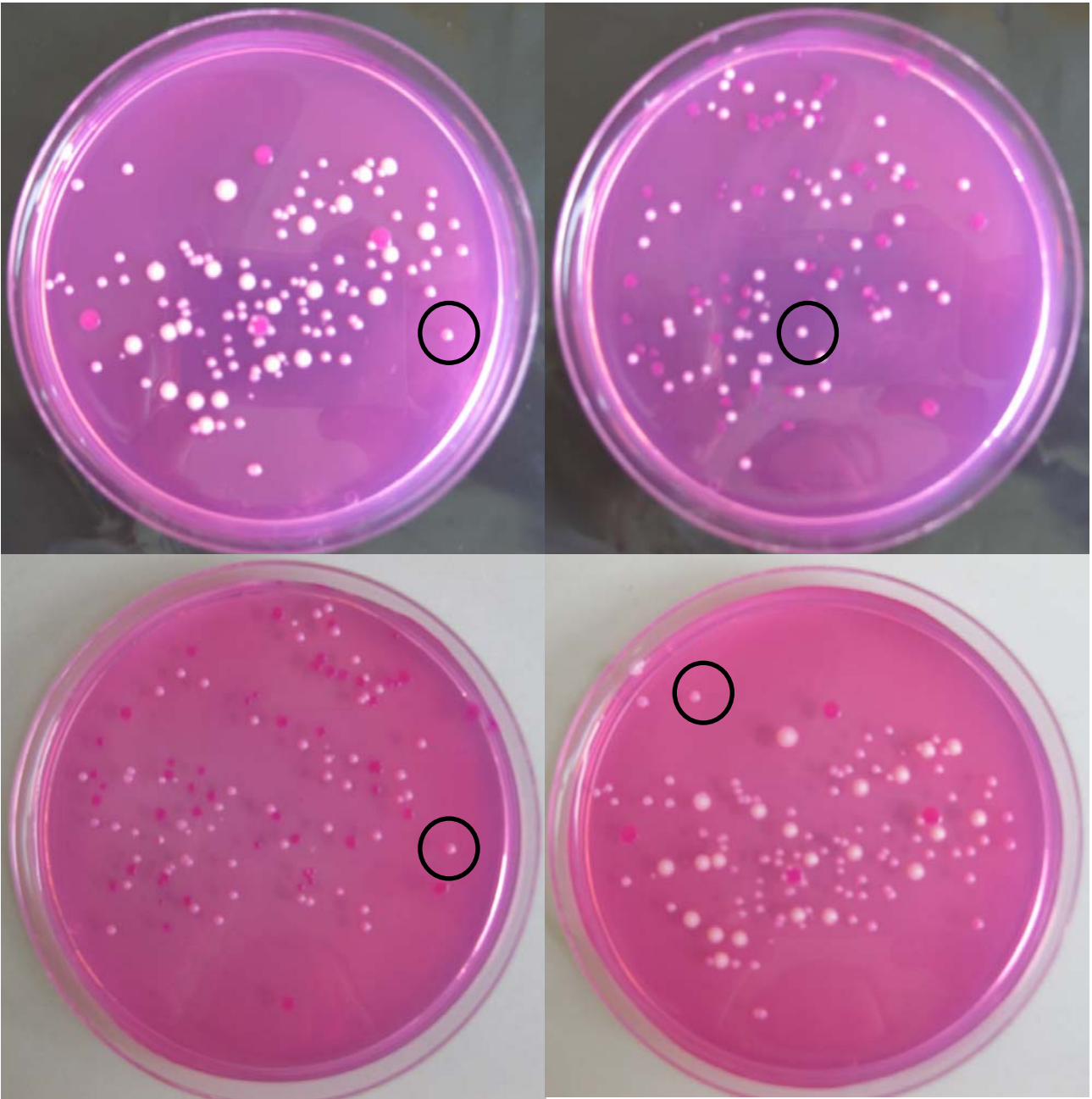


**Figure 4b. Diagram of Leukocyte Subpopulations in a Blood smear**

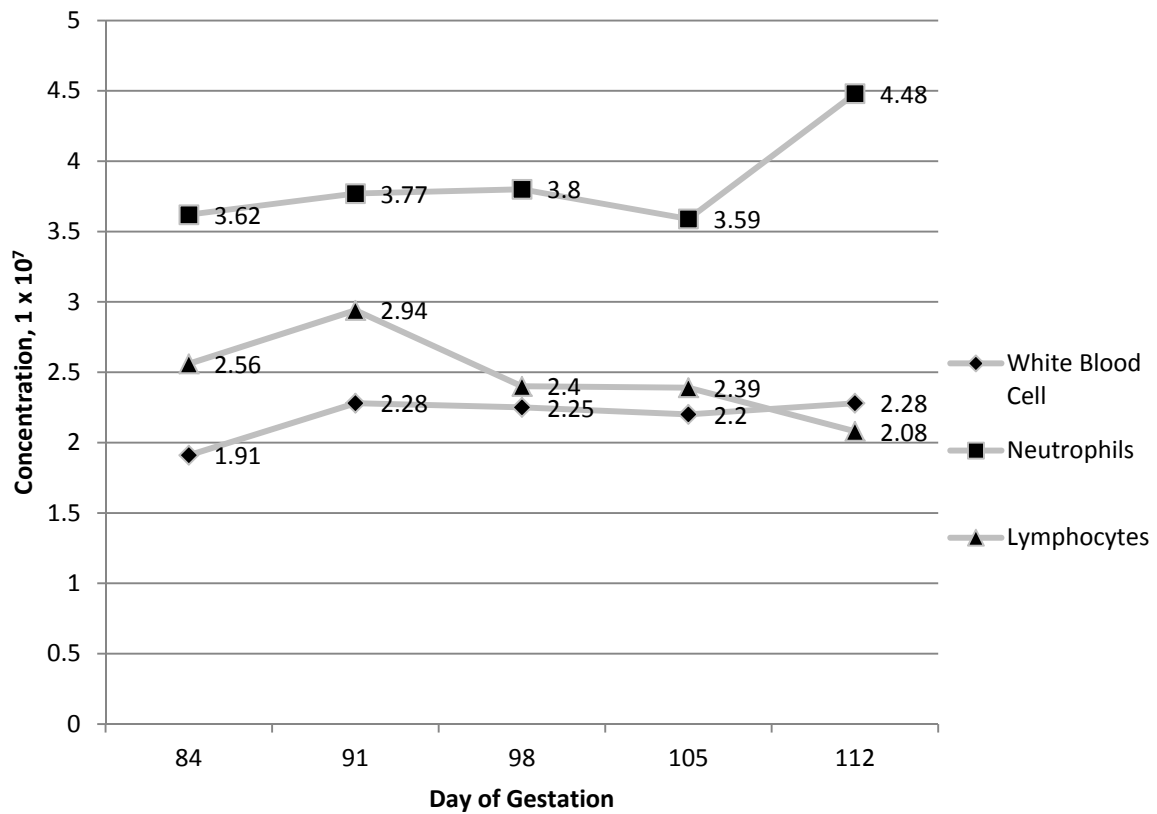


**A. Banded Neutrophil B. Lymphocyte C. Monocyte  
D. Eosinophil E. Segmented Neutrophil F. Basophil**

**Figure 5. *S. boulardii* Positive Colonies on Rose Bengal Chloramphenicol Agar**

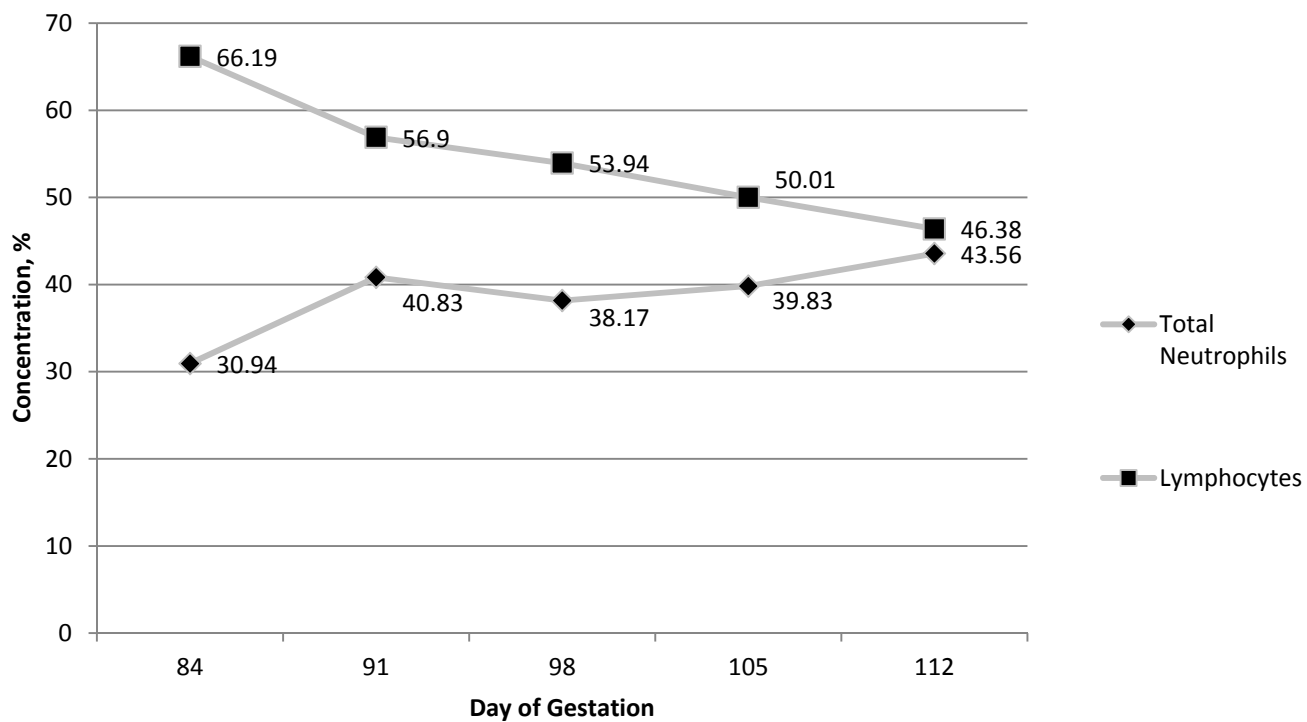


**Figure 6. Effect of day of gestation on sow total white blood cell, neutrophil and lymphocyte counts (least squares means  $\pm$  SE).**

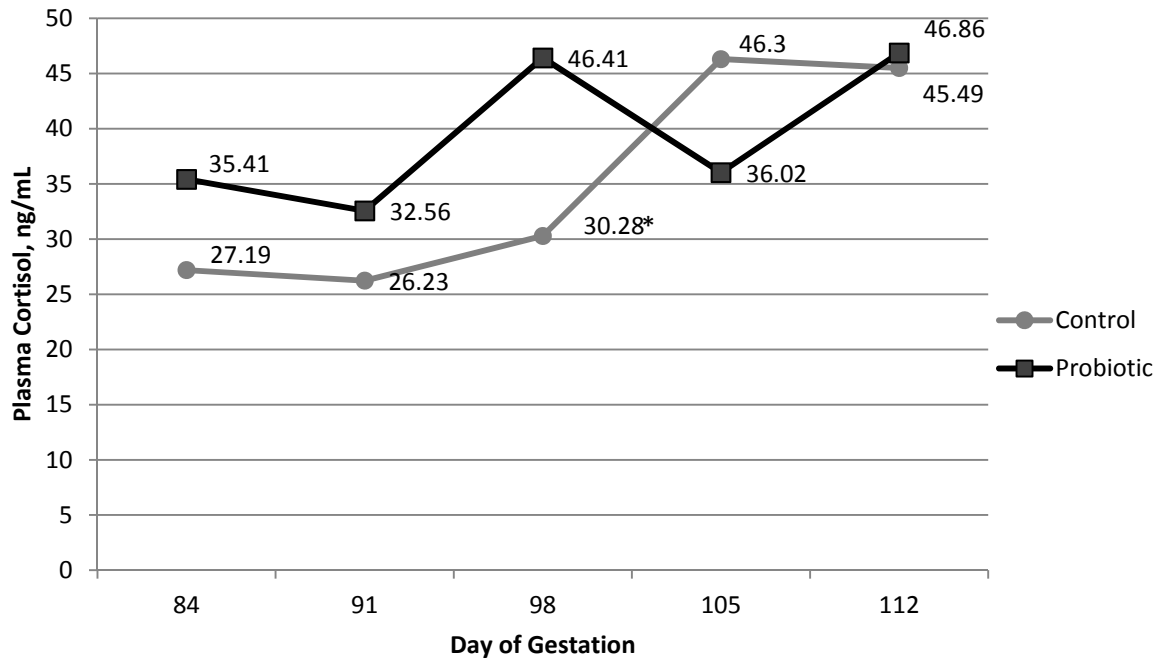


Least square means do not differ between days of gestation for total white blood cell count and neutrophil count. D91 and d112 for Lymphocytes differ ( $P < 0.05$ ), all other days do not differ.

**Figure 7. Effect of day of gestation on percentage of neutrophils and lymphocytes (least squares means)**



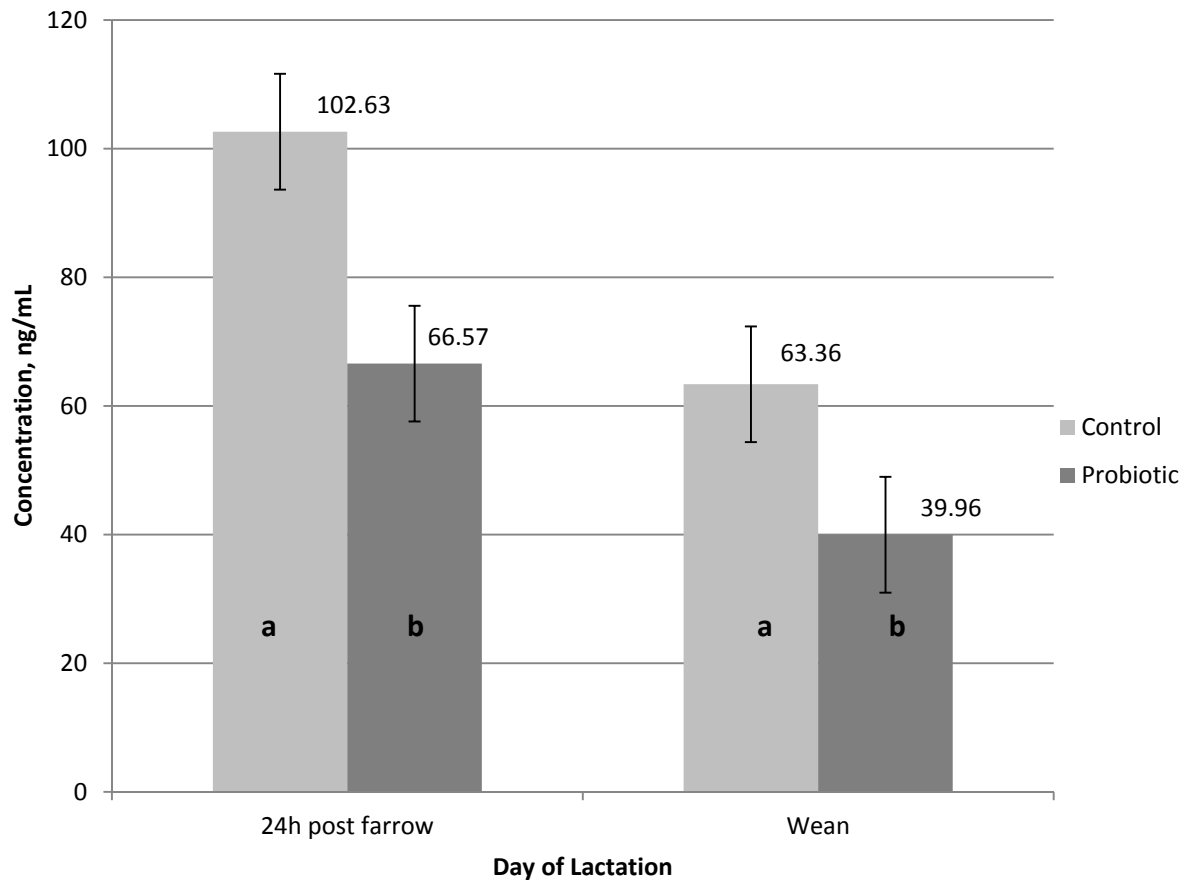
**Figure 8. Effect of treatment by day of gestation on plasma cortisol (least squares means  $\pm$  SE)**



\*within a day, means differ ( $P < 0.10$ )

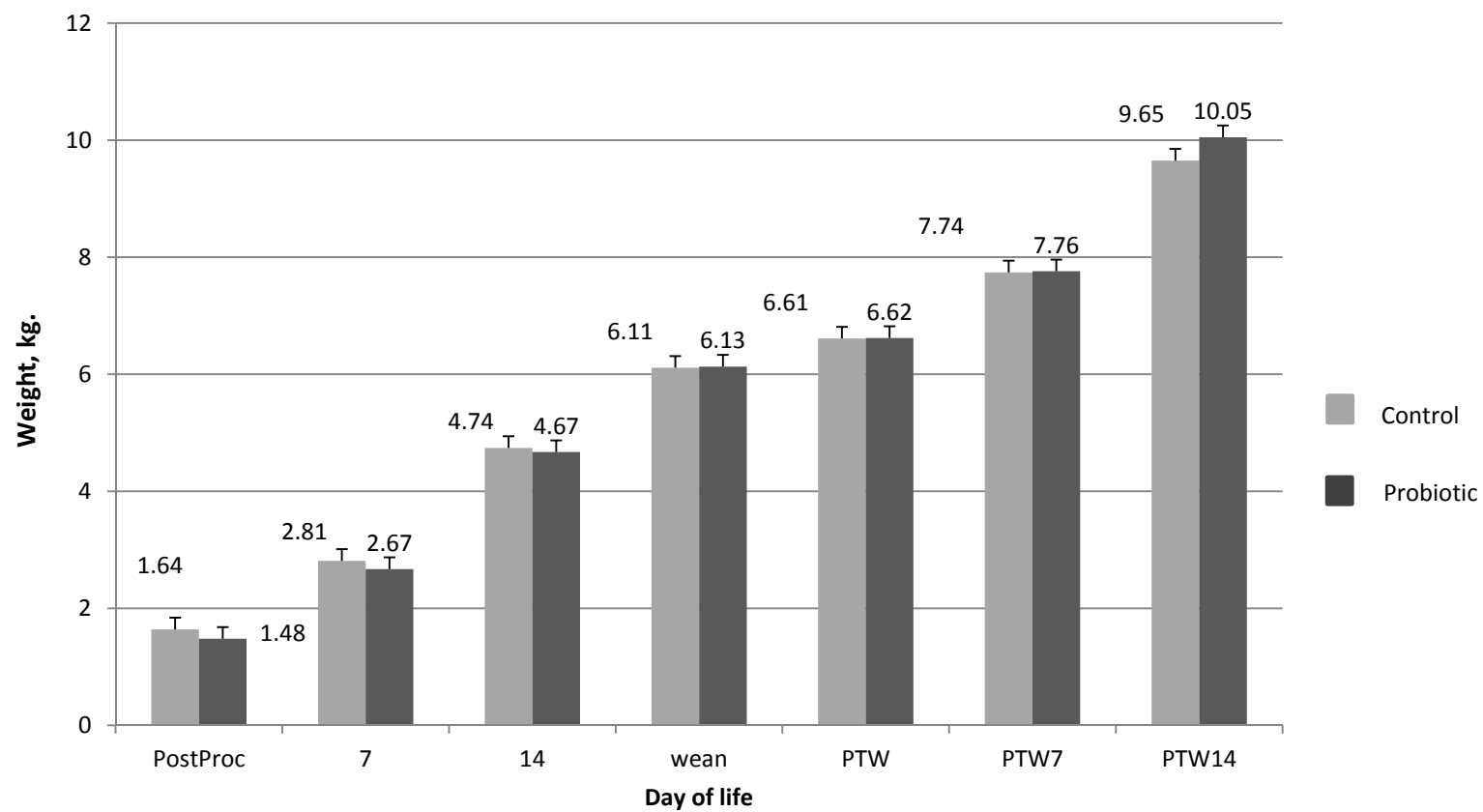


**Figure 9. Effects of treatment on sow plasma cortisol levels in response to farrowing stress (24-h post farrowing) and weaning stress (least squares means  $\pm$  SE)**

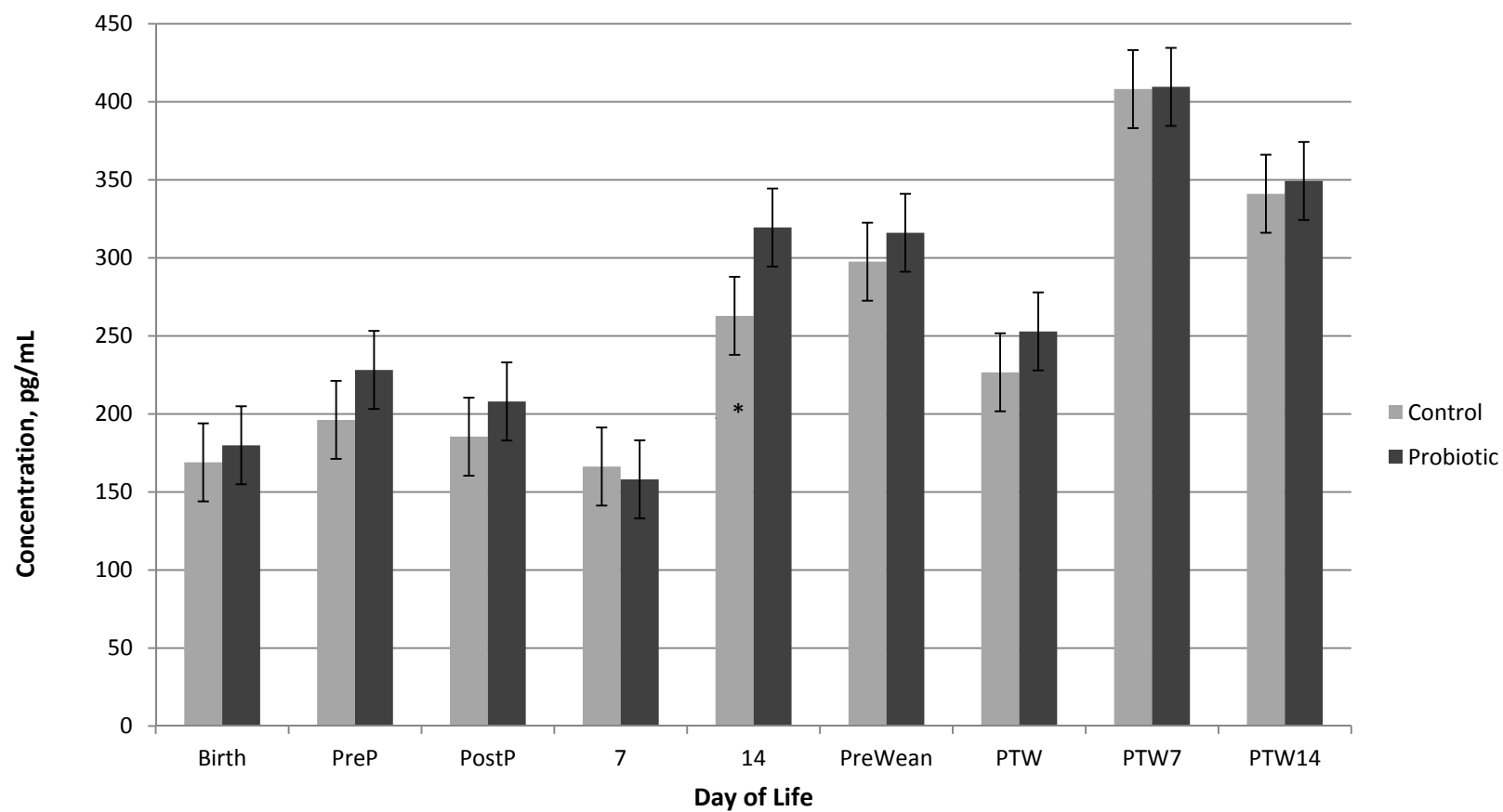


<sup>a,b</sup> within a day, means without a common superscript differ (P<0.05)

**Figure 10. Day of life and maternal diet treatment effects on piglet weight (least squares means  $\pm$  SE)**



**Figure 11. Maternal diet treatment effects by piglet day of age on plasma Interleukin-12 levels (least squares means  $\pm$  SE)**



\* within a day, means differ ( $P < 0.05$ )

Overall Treatment; CON piglets; 212.9pg/mL, PRO piglets; 235.0pg/mL ( $P = 0.08$ )

**Table 1. Definitions of Registered Behaviors**

<b>Behavior</b>	<b>Description</b>
Oral-nasal-facial	Snout/mouth in contact with any object other than feeder when feed is present
Sham-chew	Continuous chewing in absence of feed or substrate
Sit	Body supported primarily by rump and hind legs with front legs extended
Stand	Body positioned upright with all four limbs and hooves in contact with floor
Lay	Not supported by any limbs. Full contact with ground
Eat	Feed present and head is in or over feed trough
Drink	Stationary contact with snout/mouth to nipple waterer
Nest Building	Not a chewing action but pulling at the feeder or bars with mouth and/or pawing at the ground with hooves (ie, digging)
Physical Contact with piglet	Contact of the head of the sow to the head of a piglet or head of sow to body of piglet
Nursing Bout	Piglet's snouts and mouths in contact with sow's udder/teats while exhibiting rhythmic suckling; must be the majority of piglets of a litter (more than 75%)

**Table 2. Effects of treatment on sow immune and endocrine traits across gestation (least squares means  $\pm$  SE)**

Measure	Treatment		P-value
	Control	Probiotic	
Total WBC, $10^7$	2.8 $\pm$ 0.13	2.3 $\pm$ 0.13	0.268
Lymphocyte, $10^7$	2.5 $\pm$ 0.17	2.5 $\pm$ 0.17	0.457
Neutrophil, $10^7$	4.1 $\pm$ 0.50	3.6 $\pm$ 0.50	0.979
Lymphocytes, %	56.5 $\pm$ 2.0	52.9 $\pm$ 2.0	0.204
Eosinophils, %	5.4 $\pm$ 0.85	6.3 $\pm$ 0.85	0.963
Segmented neutrophils, %	32.4 $\pm$ 1.9	34.6 $\pm$ 1.9	0.426
Banded neutrophils, %	2.3 $\pm$ 0.82	2.2 $\pm$ 0.82	0.931
Phagocytosis, %	54.4 $\pm$ 2.3	55.3 $\pm$ 2.3	0.777
Chemotaxis, C5a	7.0 $\pm$ 3.3	11.4 $\pm$ 3.2	0.606
Chemotaxis, IL8	10.6 $\pm$ 9.7	18.0 $\pm$ 9.3	0.584
Cortisol, ng/mL	35.1 $\pm$ 3.5	39.5 $\pm$ 3.4	0.376
Interleukin-12 pg/mL	187.7 $\pm$ 14.8	195.1 $\pm$ 14.8	0.723

**Table 3. Effects of treatment on sow immune and endocrine traits in response to farrowing stress (24-h post farrowing) and weaning stress (least squares means  $\pm$  SE).**

Measure	24hr post-farrow		Wean	
	Control	Probiotic	Control	Probiotic
Total WBC, $10^7$	1.35 $\pm$ 0.3*	2.35 $\pm$ 0.3	2.0 $\pm$ 0.3	2.34 $\pm$ 0.3
Lymphocyte, $10^7$	1.9 $\pm$ 0.4	2.20 $\pm$ 0.3	2.30 $\pm$ 0.3	1.64 $\pm$ 0.3
Neutrophil, $10^7$	2.9 $\pm$ 0.4*	4.35 $\pm$ 0.4	3.54 $\pm$ 0.4	3.52 $\pm$ 0.4
Lymphocytes, %	41.8 $\pm$ 5.0	45.7 $\pm$ 5.0	44.20 $\pm$ 5.4	52.4 $\pm$ 5.0
Banded neutrophils, %	11.9 $\pm$ 3.8	12.0 $\pm$ 3.8	1.77 $\pm$ 4.2	2.18 $\pm$ 3.8
Segmented neutrophils, %	40.1 $\pm$ 4.6	33.7 $\pm$ 4.6	46.1 $\pm$ 5.0	37.0 $\pm$ 4.6
Interleukin-12 pg/mL	141.4 $\pm$ 21.7	137.6 $\pm$ 21.0	204.9 $\pm$ 20.0	221.3 $\pm$ 20.0
Cortisol ng/mL	102.6 $\pm$ 9.01*	63.4 $\pm$ 8.28	66.6 $\pm$ 8.3*	40.0 $\pm$ 8.7
LPS-induced proliferation 02	1.45 $\pm$ 0.25	1.81 $\pm$ 0.21	1.62 $\pm$ 0.09*	1.0 $\pm$ 0.10
ConA-induced proliferation 02	1.05 $\pm$ 0.06	1.15 $\pm$ 0.05	1.10 $\pm$ 0.38	1.64 $\pm$ 0.38
NK cytotoxicity, % 12.5:1	.	.	78.23 $\pm$ 16.8	85.2 $\pm$ 16.8
Phagocytosis, %	60.41 $\pm$ 6.0	58.60 $\pm$ 5.8	54.10 $\pm$ 5.8	53.40 $\pm$ 5.5

\*within a day, means differ ( $P < 0.05$ ).

**Table 4. Effects of diet on sow performance traits (least squares means  $\pm$  SE)**

Measure	Dietary Treatment		P-value
	Control	Probiotic	
Starting sow body weight	225.2 $\pm$ 7.7	224.4 $\pm$ 7.7	0.993
Ending sow body weight	219.5 $\pm$ 7.7	215.7 $\pm$ 7.7	0.676
Piglets born alive	11.89 $\pm$ 0.9	12.56 $\pm$ 0.9	0.612
Piglets weaned	10.44 $\pm$ 0.9	10.56 $\pm$ 0.9	0.932

**Table 5. Effect of treatment on Post-farrow sow behavior (least squares means  $\pm$  SE)**

Behavior (min)	Control	Probiotic	P- Value
Lay	1342.2 $\pm$ 16.3	1324.1 $\pm$ 20.0	0.533
Stand	64.4 $\pm$ 10.5	41.5 $\pm$ 12.8	0.26
Sit	28.5 $\pm$ 25.6	69.1 $\pm$ 31.4	0.39
Drink	13.5 $\pm$ 5.7	7.10 $\pm$ 7.0	0.53
Eat	10.9 $\pm$ 6.7	10.3 $\pm$ 8.14	0.96
ONF	19.0 $\pm$ 4.4	7.1 $\pm$ 5.4	0.18
Physical Contact	31.0 $\pm$ 6.4	24.9 $\pm$ 7.9	0.59
Nursing Bout	41.7 $\pm$ 16.9	48.8 $\pm$ 20.6	0.81



**Table 6. Dam treatment effect on her offsprings' immune response during lactation (least squares means  $\pm$  SE)**

Measure	Control	Probiotic	P-value
Total WBC, $10^7$	$3.0 \pm 0.1^a$	$3.5 \pm 0.1^b$	0.02
Lymphocyte, $10^7$	$6.0 \pm 0.8$	$4.6 \pm 0.8$	0.24
Neutrophil, $10^7$	$3.0 \pm 0.2$	$2.7 \pm 0.2$	0.78
Lymphocytes, %	$47.3 \pm 1.0$	$45.8 \pm 1.1$	0.32
Segmented neutrophils, %	$49.1 \pm 1.0$	$50.3 \pm 1.0$	0.36
Cortisol ng/mL	$71.2 \pm 2.9^a$	$62.8 \pm 2.9^b$	0.04
Interleukin-12 pg/mL	$212.9 \pm 9.0^c$	$235.0 \pm 8.8^d$	0.079

**Table 7. Effects of sow treatment and age of piglet on piglet immune and endocrine traits (least squares means  $\pm$  SE)**

	Birth		D7		D14		D21	
Measure	Control	Probiotic	Control	Probiotic			Control	Probiotic
Total WBC, $10^7$	$1.6 \pm 0.5$	$2.3 \pm 0.4$	$6.7 \pm 0.4^a$	$8.0 \pm 0.4^b$	$3.3 \pm 0.3$	$3.1 \pm 0.3$	$1.9 \pm 0.3$	$2.5 \pm 0.3$
Lymphocyte, $10^7$	.	.	$4.2 \pm 1.4$	$3.4 \pm 1.5$	$2.8 \pm 1.4$	$4.0 \pm 1.4$	$10.9 \pm 1.4^a$	$6.4 \pm 1.4^b$
Neutrophil, $10^7$	.	.	$4.3 \pm 0.4$	$3.7 \pm 0.4$	$2.3 \pm 0.4$	$2.6 \pm 0.4$	$2.4 \pm 0.4$	$2.0 \pm 0.4$
Lymphocytes, %	$44.3 \pm 3.5$	$39.4 \pm 3.5$	$46.1 \pm 2.1^c$	$52.1 \pm 2.3^d$	$60.9 \pm 2.2$	$64.2 \pm 2.2$	$66.4 \pm 2.2$	$64.4 \pm 2.2$
Seg. Neutrophils, %	$49.2 \pm 3.3^a$	$58.7 \pm 3.3^b$	$50.4 \pm 2.0^a$	$42.5 \pm 2.2^b$	$37.0 \pm 2.1$	$32.7 \pm 2.1$	$31.9 \pm 2.1$	$33.0 \pm 2.1$
Cortisol ng/mL	$203.6 \pm 10.0^a$	$129.8 \pm 8.2^b$	$42.6 \pm 6.3$	$42.2 \pm 6.5$	$40.2 \pm 6.5$	$50.5 \pm 6.6$	$45.4 \pm 6.6$	$48.4 \pm 6.5$
Chemotaxis, C5a	.	.	$11.8 \pm 5.3$	$13.9 \pm 6.5$	$18.06 \pm 4.7^a$	$32.3 \pm 4.5^b$	$27.8 \pm 6.3$	$28.6 \pm 5.8$
Chemotaxis, IL8	.	.	$13.9 \pm 8.0$	$11.7 \pm 9.1$	$25.1 \pm 6.3^a$	$42.7 \pm 5.7^b$	$36.9 \pm 7.4^c$	$18.2 \pm 7.8^d$
Phagocytosis, %	.	.	$66.4 \pm 2.1$	$66.4 \pm 2.5$	$65.1 \pm 2.1$	$61.5 \pm 2.2$	$55.7 \pm 2.2^c$	$61.2 \pm 2.2^d$
Interleukin-12pg/mL	$169.0 \pm 31.6$	$180.0 \pm 26.6$	$166.3 \pm 18.8$	$158.1 \pm 19.9$	$262.9 \pm 18.6^a$	$319.5 \pm 19.4^b$	$297.6 \pm 19.9$	$316.1 \pm 19.4$

<sup>a,b</sup> if treatments vary between days  $p < 0.05$ , <sup>c,d</sup> if they vary  $P < 0.10$

**Table 8. Effects of sow treatment on piglet immune response on Pre and Post Process (least squares means  $\pm$  SE)**

Measure	Pre Process		Post Process	
	Control	Probiotic	Control	Probiotic
Total WBC, $10^7$	$2.3 \pm 0.3$	$2.7 \pm 0.3$	$2.0 \pm 0.3$	$2.4 \pm 0.3$
Lymphocytes, %	$28.4 \pm 2.4$	$24.9 \pm 2.7$	$37.4 \pm 2.2^a$	$29.8 \pm 2.6^b$
Banded neutrophils, %	$2.9 \pm 0.6$	$2.3 \pm 0.7$	$1.4 \pm 0.6^c$	$3.0 \pm 0.7^d$
Segmented neutrophils, %	$66.6 \pm 2.3$	$69.6 \pm 2.6$	$59.3 \pm 2.1^c$	$65.6 \pm 2.5^d$
Monocytes, %	$1.5 \pm 0.3^c$	$2.4 \pm 0.4^d$	$1.5 \pm 0.3$	$1.4 \pm 0.4$
Eosinophils, %	$0.72 \pm 0.1^a$	$0.36 \pm 0.1^b$	$0.07 \pm 0.1$	$0.24 \pm 0.1$
Interleukin-12 pg/mL	$196.2 \pm 20.3$	$228.2 \pm 22.0$	$185.5 \pm 19.9$	$208.0 \pm 20.6$
Cortisol ng/mL	$35.7 \pm 6.6$	$37.5 \pm 7.1$	$59.9 \pm 6.4$	$68.3 \pm 6.7$

<sup>a,b</sup> if treatments vary between days  $p < 0.05$ , <sup>c,d</sup> if they vary  $P < 0.10$

**Table 9. Change of piglet immune and endocrine traits during weaning (least squares means  $\pm$  SE)**

Measure	PreWean		Post Wean		$\Delta$ in measures within TRT	
	Control	Probiotic	Control	Probiotic	Control	Probiotic
Total WBC, $10^7$	2.0 $\pm$ 0.3	2.5 $\pm$ 0.3	2.1 $\pm$ 0.2	1.3 $\pm$ 0.2	1.8 $\pm$ 0.1 <sup>a</sup>	0.9 $\pm$ 0.1 <sup>b</sup>
Lymphocyte, $10^7$	10.9 $\pm$ 1.4	6.4 $\pm$ 1.4	2.2 $\pm$ 0.3	1.5 $\pm$ 0.3	9.1 $\pm$ 2.8	5.2 $\pm$ 2.2
Neutrophil, $10^7$	2.4 $\pm$ 0.4	2.0 $\pm$ 0.4	1.56 $\pm$ 0.4	2.03 $\pm$ 0.4	1.8 $\pm$ 0.7	1.8 $\pm$ 0.6
Lymphocytes, %	66.4 $\pm$ 2.1	64.4 $\pm$ 2.2	55.4 $\pm$ 2.0	56.4 $\pm$ 2.1	15.0 $\pm$ 1.8	14.9 $\pm$ 1.8
Segmented neutrophils, %	49.2 $\pm$ 3.3	58.7 $\pm$ 3.3	41.4 $\pm$ 2.0	40.6 $\pm$ 2.0	13.7 $\pm$ 1.7	14.4 $\pm$ 1.8
Banded neutrophils, %	0.15 $\pm$ 0.6	0.38 $\pm$ 0.6	0.63 $\pm$ 0.1	0.33 $\pm$ 0.1	0.77 $\pm$ 0.1 <sup>c</sup>	0.36 $\pm$ 0.2 <sup>d</sup>
Phagocytosis, %	55.7 $\pm$ 2.2	61.2 $\pm$ 2.2	68.3 $\pm$ 2.2	63.6 $\pm$ 2.3	25.9 $\pm$ 3.6	18.9 $\pm$ 3.6
Cortisol ng/mL	71.2 $\pm$ 3.0	62.8 $\pm$ 2.8	36.0 $\pm$ 3.2	52.0 $\pm$ 3.2	19.4 $\pm$ 3.6	23.4 $\pm$ 3.4
Interleukin-12 pg/mL	297.6 $\pm$ 19.9	316.1 $\pm$ 19.6	226.7 $\pm$ 23.5	252.9 $\pm$ 24.0	97.43 $\pm$ 19.5	121.7 $\pm$ 19.2

<sup>a,b</sup> if  $\Delta$  in least squared means differ between treatments ( $P < 0.05$ ), <sup>c,d</sup> if they differ ( $P < 0.10$ )

**Table 10. Overall Maternal diet treatment on piglet immune and endocrine response post weaning (24-h post wean to PTW14) (least squares means  $\pm$  SE)**

Measure	Control	Probiotic	P-value
Total WBC, $10^7$	$2.5 \pm 0.2$	$1.3 \pm 0.2$	0.042
Lymphocyte, $10^7$	$3.2 \pm 0.2$	$2.5 \pm 0.2$	0.007
Neutrophil, $10^7$	$3.9 \pm 0.3$	$3.0 \pm 0.2$	0.014
Lymphocytes, %	$53.9 \pm 1.3$	$58.6 \pm 1.3$	0.009
Segmented neutrophils, %	$42.8 \pm 1.3$	$37.8 \pm 1.3$	0.005
Cortisol ng/mL	$29.7 \pm 2.2$	$36.3 \pm 2.1$	0.032
NK cytotoxicity, % 25:1	$95.6 \pm 4.3$	$85.2 \pm 4.3$	0.087

**Table 11. Maternal treatment and age of piglet effects on piglet immune and endocrine traits post-weaning (least squares means  $\pm$  SE)**

	24h Post Wean		PTW7		PTW14	
Measure	Control	Probiotic	Control	Probiotic	Control	Probiotic
Total WBC, $10^7$	2.1 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.2 <sup>b</sup>	3.4 $\pm$ 0.2	3.4 $\pm$ 0.2	2.2 $\pm$ 0.2	2.10 $\pm$ 0.2
Lymphocyte, $10^7$	2.2 $\pm$ 0.3	1.5 $\pm$ 0.3	4.5 $\pm$ 0.4	3.6 $\pm$ 0.4	3.1 $\pm$ 0.3 <sup>c</sup>	2.5 $\pm$ 0.3 <sup>d</sup>
Neutrophil, $10^7$	1.6 $\pm$ 0.4	2.0 $\pm$ 0.4	7.7 $\pm$ 0.5	4.8 $\pm$ 0.5	2.3 $\pm$ 0.4 <sup>a</sup>	2.1 $\pm$ 0.4 <sup>b</sup>
Lymphocytes, %	55.4 $\pm$ 2.0	56.4 $\pm$ 2.1	58.8 $\pm$ 2.0 <sup>a</sup>	60.9 $\pm$ 2.0 <sup>b</sup>	47.5 $\pm$ 2.6 <sup>a</sup>	58.6 $\pm$ 2.6 <sup>b</sup>
Segmented neutrophils, %	41.4 $\pm$ 2.0	40.6 $\pm$ 2.0	49.9 $\pm$ 2.5	38.6 $\pm$ 2.5	37.3 $\pm$ 2.0 <sup>a</sup>	34.2 $\pm$ 2.0 <sup>b</sup>
Banded neutrophils, %	0.63 $\pm$ 0.1 <sup>c</sup>	0.33 $\pm$ 0.1 <sup>d</sup>	0.03 $\pm$ 0.2	0.34 $\pm$ 0.2	0.30 $\pm$ 0.1	0.30 $\pm$ 0.1
Eosinophils, %	0.35 $\pm$ 0.1	0.24 $\pm$ 0.1	0.47 $\pm$ 0.1 <sup>a</sup>	0.94 $\pm$ 0.1 <sup>b</sup>	0.9 $\pm$ 0.2	0.83 $\pm$ 0.2
Chemotaxis, IL8	28.5 $\pm$ 4.4	21.8 $\pm$ 5.7	29.9 $\pm$ 5.4	16.1 $\pm$ 6.2	14.1 $\pm$ 4.4 <sup>c</sup>	17.0 $\pm$ 3.2 <sup>d</sup>
NK cytotoxicity, % 50:1	92.5 $\pm$ 10.6	89.1 $\pm$ 11.0	.	.	.	.
NK cytotoxicity, % 25:1	67.4 $\pm$ 9.0	60.4 $\pm$ 9.3	86.4 $\pm$ 9.9 <sup>c</sup>	76.3 $\pm$ 6.8 <sup>d</sup>	.	.
NK cytotoxicity, % 12.5:1	43.2 $\pm$ 6.8	41.2 $\pm$ 7.0	49.3 $\pm$ 5.2 <sup>a</sup>	44.4 $\pm$ 5.1 <sup>b</sup>	86.6 $\pm$ 4.6	71.7 $\pm$ 4.9
Cortisol ng/mL	36.0 $\pm$ 3.2 <sup>a</sup>	52.0 $\pm$ 3.2 <sup>b</sup>	26.7 $\pm$ 4.3	24.8 $\pm$ 4.3	26.5 $\pm$ 3.8	32.2 $\pm$ 3.5

Within a day, means differ (P<0.05). <sup>A,b</sup> if treatments vary between days p<0.05, <sup>c,d</sup> if they vary P<0.10

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