SUPPLEMENTAL YEAST FERMENTATION PRODUCTS EFFECT ON SOW LACTATION PERFORMANCE AND POST-PARTUM RECOVERY BASED ON UTERINE FLUIDS AND BLOOD PARAMETERS

by

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ABSTRACT

The longevity of high productivity sows in the herd has become a challenge in pig production. Several factors may contribute to increased mortality rates observed over the past few years as well as lower retention rates of young sows. Chronic inflammation and metabolic disorders are conditions that sows had evolved over the years together with the greater productivity. This dissertation underlines the immunomodulatory effects of using yeast fermentation product fed to lactating sows. In the interest of determining patterns of local and systemic immune response, a new methodology to access cytokine profiles in puerperium sows was developed. In Chapter 2, one hundred-forty sows were used to evaluate the effects of two different *Saccharomyces cerevisiae* fermentation product (SCFP), a liquid source (LIQ) and a dry source (XPC®; Diamond V), on sow and litter performance. Sows were fed a common gestation diet until d 112 of pregnancy and then allotted to one of four treatments: 1) Control diet (CON), 2) CON + 15 mL/d of LIQ from d 112 to weaning (LIQ), 3) CON + 0.20% of XPC from d 112 to weaning (DRY), and 4) DRY + 15 mL/d of LIQ from d 112 to d 7 post-farrowing (D+L). Colostrum immunoglobulin concentrations were estimated using Brix refractometer. Plasma of piglets (2/sow) was collected 24 h after birth for immunocrit ratio analysis and for determination of plasma IgA and IgG concentrations. Lactation water and feed intake (ADFI) were recorded daily. Post-weaning follicle growth was evaluated by transrectal ultrasonography. Sows had the same initial BW ($P > 0.13$) but those fed any SCFP were heavier at weaning ($P = 0.03$) while not affecting sow backfat and loin depth ($P>0.05$). Overall, sows fed SCFP had greater ADFI than CON fed pigs ($P < 0.01$) while water intake, reproductive performance (total born, stillborn, weaned) did not differ among treatments ($P > 0.05$). Sows fed LIQ had the greatest ADFI on weeks

1, 2, 3, and overall compared to CON ($P < 0.05$). Litter ADG from SCFP treatments tended to be greater than CON ($P = 0.10$) and litter weight variability was lower ($P = 0.10$). No treatment effects were observed in colostrum Brix values ($P > 0.77$), in the piglet plasma IgG and IgA, and serum immunocrit ratio ($P > 0.21$). The average daily post-weaning follicle growth was greater for SCFP treatments than CON ($P = 0.05$). The wean to estrus interval was shorter for sows fed LIQ than CON and DRY ($P < 0.01$).

In Chapter 3 a non-invasive methodology to assess cytokine profiles from post-partum uterine lavage is described. The uteri of fourteen second and third parity sows were flushed with sterile saline solution (0.9%) on days 2, 4, and 14 post-parturition. Uterine fluid collected was immediately centrifuged and the supernatant stored at -20°C. Samples were freeze-dried, re-suspended in sterile saline (2 mL), and stored at -80°C. Cytokine profiles of the uterine fluid were evaluated using a multiplex ELISA panel including interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Cytokine concentrations were calculated relative to protein content (pg/mg of protein). IFN-γ and TNF-α were lower than the limit of detection in most samples (5/38 and 1/38, respectively). IL-4 and IL-10 concentrations did not differ among days of collection ($P>0.14$). IL-8 was greater on day 4 than on days 2 or 14 ($P<0.05$). IL-1β and IL-6 were greater on days 2 and 4 than on day 14 ($P<0.05$).

The study presented in Chapter 4 refers to a subsample of sows (n=40) from the entire group of sows used in the study presented in Chapter 2. In this case, the methodology presented in Chapter 3 was used to evaluate SCFP effects on blood and uterine cytokine profiles in sows. A similar set of cytokines from Chapter 3 were evaluated on d 112 of gestation, d 2 and 6 post-farrowing in the plasma, and from uterine fluid collected on d 2, 4, and 6 post-farrowing. Serum C-Reactive protein (CRP) and haptoglobin concentrations were evaluated. No interactions

between treatments and day of collection were observed (P>0.13). LIQ and D+L sows had the greatest serum IL-10 concentration (P<0.001) and sows fed CON tended to have lower serum concentration of IL-8 (P<0.06) vs. other treatments. Serum CRP concentrations were greatest on d 2 (P<0.001), serum IL-10 (P<0.04) and IL-4 (P<0.07) linearly decreased while serum haptoglobin (P<0.02) and IFN-γ (P<0.001) linearly increased post-farrowing. In the uterine fluid, LIQ and D+L sows had greater IFN-γ (P=0.04) concentrations and CON tended to have the least concentration of TNF-α (P=0.08). Uterine fluid IL-1 tended to linearly increase (P<0.07) and IL-6 linearly decrease (P<0.01) post-farrowing. LIQ sows had the greatest daily feed intake and CON the least during the first week of lactation (P=0.04).

In conclusion, feeding SCFP to lactating sows improved feed intake and litter growth while not affecting milk yield and colostrum quality. Besides improvements on litter ADG, the uniformity was better for all sources of SCFP. The liquid sources had slightly better results over the other sources and CON, including the greatest feed intake, less body weight mobilization, and a reduction in WEI. The method proposed to evaluate cytokine profiles in the uterine fluids of sows after farrowing, accomplished the objective of being a non-invasive procedure to be applied in puerperium sows. This new procedure was applied to analyze the immunomodulatory effects of SCFP. The correlations observed between the uterine and serum cytokines lead to a refined description of immune response in puerperium sows. Feeding SCFP to lactating sows stimulates the immune system allowing sows to build a desirable immune responses. Thus, the quicker resolution of acute phase reaction as demonstrated by greater daily feed intake in the first week post-farrowing can be attributed to SCFP immunomodulatory effects, ensuring better lactation performance.

CHAPTER 1. LITERATURE REVIEW

Over the past twenty years the swine industry went through a lot of challenges while great advances in nutrition, management, and pig genetic selection took place. Disease outbreaks such as the porcine epidemic diarrhea in the United States in 2014 as well as the African swine fever in 2018, mainly in China, had strengthened advances in disease diagnosis, surveillance, and biosecurity procedures. In addition, pigs have been selected for traits related to resistance to diseases. The increase in food safety concerns around the globe had also impacted pork production. The pig industry moved to a much more conscious use of antibiotics in the feed which had stimulated researchers to look for more alternatives to keep up pig performance and health. Animal protein consumers became more aware of animal welfare which led the swine industry to make big changes in the production system such as group housing sows. As noticed, pig production is constantly evolving to meet consumer's needs. In this context, scientific research becomes one of the most important ways to leverage innovation and solutions for producers to succeed.

From many challenges the pig industry faces, maintaining high productivity healthy sows in the herd is one that had gained great attention in the last decade. Sow mortality rates have been increasing in many countries, the retention rate of young sows had decreased, and the sow efficiency to wean large litters decreased even though the number of pigs born per sow may not be a problem in most cases. The modern sow is more susceptible to metabolic diseases and chronic inflammation which often alters their voluntary feed intake and performance.

This dissertation highlights the effects of using yeast derivates fed to sows as well as new approaches and techniques to evaluate biomarkers of inflammation in different tissues of sows following parturition.

1.1 The gestating sow

Gestation in swine last around 114-116 days and may be divided in three main periods according to the physiological events, nutritional requirements, and fetus development. The early stage period is characterized by the establishment of pregnancy, which is ultimately a result of the successful interaction between embryos and the uterus determining the embryo survival. Therefore, it is a crucial stage to determine the litter size (Langendijk, 2014) as embryo mortality rates ranges around 30 to 50% in the first month of pregnancy (Geisert and Schmitt, 2002). As ovulatory rates had increased in commercial genetic lines, the uterine space have been experimentally proven to be a limiting factor to embryo survival (Town et al., 2004). The nutrients requirements in this stage are likely to be more important to the sow's maintenance and body condition recovery rather than embryo development. Yet, there are some clear evidence of the nutrition plane indirectly influence the luteal function and uterine conditions that are important to implantation and embryo survivability (Langendijk, 2014), and specific considerations must be applied to young sows as they are still growing and body reserves mobilization during lactation could negatively impact subsequent performance (Mallman et al., 2020).

In the second third of pregnancy, after day 35, uterine capacity starts to become a limiting factor for fetal growth (Bazer et al., 2014). The nutrient requirements are similar to the first third, basically for maintenance and weight gain through protein and lipid deposition (NRC, 2012).

The late gestation period is characterized by the exponential fetal growth (McPherson et al., 2004), mammary gland development (Farmer and Hurley, 2015), and greater nutrient requirements (NRC, 2012). The fetus growth from day 20 up to day 100 ranges from 0.06 to 1000 grams and it is highly correlated to placental length and placental total surface area (Jainudeen and Hafez, 2000). Many nutritional strategies have been done in late gestation aiming to improve birth weight of pigs. Indeed, increasing energy intake in late gestation can positively affect birth weight

but can also impact voluntary lactation feed intake (NRC. 2012). Lysine, calcium, and phosphorus requirements also increase in proportion with the fetal exponential growth in late gestation (NRC, 2012).

1.2 The transition period

The transition period encompasses all the physiological and metabolic changes during the last days of gestation and the first few days of lactation. In addition, at this time sows are moved from gestational housing to farrowing crates with different conditions and great dietary changes occurs, from restricted low energy diet to *ad libitum* high energy and protein lactation diet in most cases (Theil, 2014). As mentioned previously, fetal growth in late gestation is exponential. Indeed, the last 10 days of gestation, in the transition period, is when almost one third of all fetal weight gain occurs (Noblet et al., 1985).

Besides parturition, which will be detailed in the next section, the mammary gland development and the onset of lactation are two very important physiological events that take place in the transition period. The mammary gland growth in pregnant sows occurs from 10 d before to 10 days after parturition (Noblet et al., 1985). In young gilts, the mammary growth and development occurs from 90 days of age until first estrus. In both developmental cases, the nutrition levels, endocrine status, and different practices of management in the transition period can affect mammary development (Farmer and Hurley, 2014). Colostrum synthesis is known to start 7-10 days before parturition and last up to 30 hours after parturition with decreasing levels of its main components. The onset of milk production occurs as early as 23 up to 40 hours after parturition (Theil, 2014).

Sows in the transition period usually undergo a catabolic status due to the increased nutrient requirement to support fetal and reproductive tissues growth. This catabolic status persists in the

lactation phase leading to a significant increase in the production of free radicals and to oxidative stress (Shen et al., 2015). In addition, as result of multifactorial reasons, sows in the transition period are loaded with inflammatory mediators leading to the post-partum disorders such as low feed intake, fever, agalactia syndrome, vaginal discharge, mastitis, and metritis (Tummaruk and Sang-Gassane, 2013).

1.3 The parturition

Around day 114 of gestation a drastic reduction on corpus luteum weight results in decreasing levels of progesterone at the same time of estradiol and oxytocin peak. An orchestrated change in hormones profile, including increasing levels of corticosteroids, like fetal cortisol, results in the onset of parturition process (Jainudeen and Hafez, 2000). Relaxin and estrogen promote cervix dilation and mammary gland development (Eldridge-White et al., 1989).

The farrowing process can be divided in three main stages. The main event in the first stage is the cervix dilation: uterine contractions lead to alterations in fetuses position. Sows start to behave differently, increase respiration rates, and heartbeat. The second stage is the fetuses expulsion: stronger uterine and abdominal contractions take place, the cervix is completely dilated, and the amniotic membranes are broken leading to fetuses expulsion. The third stage is characterized by the placental expulsion: chorionic villous are detached, and uterine contraction slow down. Right before and after birth, piglets undergo many physiological changes such as lung maturation and cardiovascular alterations in response to high levels of cortisol (Jainudeen and Hafez, 2000).

1.3.1 Factors influencing farrowing duration

Modern commercial genetic lines have a substantial number of pigs born per litter compared to genetics from two decades ago. Greater litters have also increased the farrowing duration which is highly associated with stillborn percentage (Vallet et al., 2010). There is an estimation that sows given birth to 12 pigs had on average 2 h of duration (Madec and Leon, 1992) while sows given birth up to 19 pigs average more than 6 h (Yun et al., 2019). Many factors can affect farrowing duration. Van Djik et al. (2005) showed that from a variety of variables the one that seems to have a greater impact on farrowing duration was the sow`s breed. The number of stillborn had also a great effect on farrowing duration, however it was not clear to determine if that is a causative effect of longer farrowing duration or a result of it. Vallet et al., (2010) had also shown that longer the birth interval, greater is the stillbirth rates, as well as piglets born at the end of farrowing had both increased intervals and greater risk to stillbirth.

The environmental conditions around the sows at the time of parturition may also affect the farrowing duration. Oliviero et al. (2010) studied some of these factors and showed that reducing the constipation state and avoiding excessive backfat in late gestation as well as allowing the sow to move freely before farrowing reduced farrowing duration and stillbirth rate. As demonstrated above, larger is the litter prolonged is the farrowing duration. Indeed, the farrowing process demands a great amount of energy. Oliveira et al. (2020) found that supplementing sows before farrowing with an energy supplement decrease farrowing duration, stillbirth rate, and the need for assistance.

These mentioned above, are important factors to be considered as they are highly correlated to the occurrence of postpartum disorders in sows including agalactia syndrome, mastitis, metritis, and fever. First parity females are more susceptible to illness after farrowing and sows with

farrowing duration longer than four hours, significantly increase the risk of fever in the postpartum period (Tummaruk and Sang-Gassanee, 2013).

1.3.2 Post-farrowing recovery

The proper recovery after parturition is crucial to sows to return to homeostasis and to the subsequent reproductive performance. Starting at conception, sows go through several physiological and metabolism alterations as well as immunological adaptations to allow pregnancy to continue (Mor et al., 2017). Berchieri-Ronchi et al. (2011) showed that sows in mid and late gestation have a great level of oxidative stress indicated by greater concentrations of reactive oxygen species (ROS) while decreasing levels of antioxidants in the serum, and this condition is not fully recovered until weaning.

In the postpartum period several physiological and anatomical alterations occur in the uterus and ovaries. The period after parturition until the reproductive tract returns to its normal condition and functionality is called puerperium. In the puerperium, the most important event is the uterine involution, that is the uterus return to its non-pregnant size and functions. This process depends on myometrial contractions, pathogens elimination, and endometrial regeneration in response to hormonal changes and immunological response in the mucosa (Anderson, 2000; Jainudeen and Hafez, 2000).

As other reproductive events such as menstruation and ovulation, the farrowing process is an inflammatory event. Sows have the capacity to resolve this inflammatory process quickly and return to normal reproductive function (Jabbour et al, 2009). The sterile conditions in uterus during pregnancy is disrupted at the time of parturition, especially in commercial conditions where interventions are frequent, increasing the chances of infections. The proper uterine involution occurs depending on three processes: first, an efficient immunological response and defense

mechanisms including lymphocytes and macrophages migration to the mucosa and lumen, respectively; second, a massive secretion of prostaglandins leading to myometrium contraction and leftover tissues elimination; and third, the estrogen production by the ovaries. This process lasts about two to three weeks depending on the degree of postpartum contamination, placental retention, and estrogen secretion (Jainudeen and Hafez, 2000).

1.4 Lactating sows

1.4.1 Colostrum and passive immunity

Pig survivability, especially in the first days of life, is highly dependent on colostrum intake (Le Dividich et al., 2005) and piglet growth rate depends on milk intake (Quesnel et al., 2019). The importance of colostrum has been studied in a great variety of aspects, from its major nutritional and immunological functions to some epigenetics effects (Bagnel and Bartol et al., 2019). Colostrum is essential for thermoregulation as body energy reserves (hepatic and muscle glycogen) are rapidly used in the first few hours after birth (Theil et al., 2011). Immunoglobulins (Ig) present in colostrum, are essential to provide systemic (IgG) and mucosal protection (IgA) as immune system of newborn pigs are not fully developed until three to four weeks of age (Rooke and Bland, 2002).

Immunoglobulins G family are the most important line of humoral defense in response to pathogens and contain 70–75 immunoglobulins in the serum divided in four subclasses. The IgG1–3 immunoglobulins distinguish from other Ig because they can activate the complement cascade (Gocki and Bartuzi, 2016). Immunoglobulin A (IgA) is the major immunoglobulin in the blood and mucosa, its functions are related to its localization neutralizing and binding to antigens to prevent their adherence to the mucosa epithelium. Like other classes, IgA have two identical heavy

and light chains, for the recognition and interaction with various receptors and effector molecules (Woof and Ken, 2006). As mucosal protector, specializations of IgA include its transportation across epithelial cells, resistance to luminal enzymes, and binding to mucus. Functionally, IgA act preventing antigens and microorganisms from adhering to the epithelium as well as pumping antigens across the barrier (Bailey et al., 2005). Therefore, these major Ig present in colostrum providing systemic immunity and intestinal mucosa protection can prevent neonatal diarrhea as well as stimulate piglets immunological system maturation due to immunomodulatory factors present in colostrum and milk (Salmon et al., 2009).

The evaluation of immunoglobulins in the colostrum of sows have been done for research purpose only through ELISA and radial immunodiffusion methods, which are costly and non-feasible at large scale in the farm level. Recently, the Brix refractometer have been used in many species to evaluate passive immunity in neonatal animals. The methodology is based on measuring the total protein content in the sample through the refraction of light these solids components of the colostrum produce (Chavatte et al., 1998). This methodology has been successfully validated in swine colostrum samples (Balzani et al., 2016; Hasan et al. 2016).

1.4.2 Colostrum and milk yield

Like previously mentioned about uterine capacity becoming a limiting factor for fetal growth in the modern genetic lines, colostrum and milk availability per pig has decreased. Colostrum production, differently than milk, depend more on hormonal regulation (progesterone and prolactin) and parity rather than nutritional aspects (Quesnel et al., 2019). Yet, the colostrum quality can be affected by dietary changes, especially regarding its fatty acids profile (Zou et al., 2020) as well as due to sow vaccination protocols (Langel et al., 2016). On the other hand, milk yield can be influenced by several aspects: genetics, parity, environmental conditions,

management, feeding programs, dietary energy, amino acids supply, dietary fat composition, water availability, and the sow body composition. Another important difference between colostrum and milk yield is that differently than colostrum, milk yield can be drastically altered depending on litter size because of the suckling stimulus (Quesnel et al., 2019).

Few techniques are applied in research attempting to estimate milk yield. The simplest one is the weigh-suckle-weigh methodology. Litters are weighed repeatedly for consecutive suckling and milk yield is estimated by the pre- and post-suckling litter weights. The limitation of this technique is that it can underestimate milk yield because sows tend to decrease milk production as this procedure is stressful for them and piglet fecal and urine losses as litters wait for the next suckle must be accounted (Quesnel et al., 2019). More precise than weigh-suckle-weigh method, is deuterated water (DO) dilution technique. It basically measures the water intake from colostrum/milk by evaluating the dilution of DO by calculating the differences between pre- and post-suckling concentration of DO in the blood. This method is time consuming and expensive. Milk yield can also be estimated using equations based on whole litter weights gain by calculating the efficiency of pigs on converting milk into body weight gain for a time period during suckling phase (Quesnel et al., 2019).

1.4.3 Lactating sow feed intake

The modern high prolific sows have increased milk production in response to greater litter sizes but have also increased energy and nutrients requirements (NRC, 2012; Noblet et al., 1990). There are several factors that can affect lactation feed intake. Many of them have been a target of studies in the last decade such as the body condition of sows in the perinatal period (Zhou et al., 2018; Lewis et al., 2011), the n-6:n-3 ratio in the diet (Papadopoulos et al., 2009), the fiber source (Liu et al., 2021; Agyekum et al., 2019), the feeding program in late gestation and early lactation

(Mallmann et al., 2019; Martí et al., 2019; Lei et al., 2018; Poulopoulou et al., 2018; Ren et al., 2017), the dietary fat source (Shen et al., 2015), environmental conditions (Vilas Boas Ribeiro et al., 2018; Chen et al., 2018; Bergsma and Hermesch, 2012), exogenous enzymes in the diet (Walsh et al., 2012), and other feed additives (Wang et al., 2021; Pereira et al., 2020).

Feed intake during lactation is very important. Problems to guarantee adequate feed intake of lactating sows have been pointed as one of the biggest issues affecting litter performance. Inadequate feed intake in lactation, leads to increased losses of body reserves, especially in young sows. Moreover, it has been demonstrated that excessive body mobilization in young sows is related to increased culling rates and lower retention rates (Mallman et al., 2020; Eissen et al., 2003).

1.4.4 Follicular development in rebreeding sows

During the lactation phase, due to great levels of prolactin secretion in response to piglets suckling, there is an important negative feedback on gonadotrophic hormones (LH, FSH) secretion, leading to absence of follicular waves and follicular atresia. The endometrium becomes thinner, and the number of glands significantly decrease. After the removal of the suckling stimulus (weaning) follicular waves develop. At this time, hypothalamus secretions of GnRH and concentrations of LH and FSH increases leading to estradiol gradually increasing followed by the onset of estrus (Jainudeen and Hafez, 2000).

The resumption of ovarian follicular development pre-weaning varies with individual sows and the effects of weaning randomly affect wean to estrus interval. For instance, sows before weaning can be found in different stages of the follicular development like follicles around 5 mm with non-ovulatory waves or sows with follicles smaller than 2 mm reflecting ovary inactivity. This complexity and individual variation may explain and determine part of rebreeding efficiency

in sows (Lucy et al., 2001). At weaning, sows usually have 2-5 mm follicles, and a rapid development occurs up to ovulation (Dyck, 1983) when they are steroidogenic reaching the maximum of 6-7 mm in the onset of estrus (Liu et al., 2000) and even larger at the time of ovulation (7-8 mm; Soede et al., 1998).

1.5 Immune system

The immune system is a complex comprising three major defense mechanisms: external physical (mucosal membranes) and chemical (defensive peptides and mucus) barriers; innate immune response; and adaptive immune responses. The immune response is primarily mediated through interactions of the innate and adaptive immune systems to recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs); to pursue immune effectors functions; to regulate the immune response; and to create immunological memory (Dembic, 2015; Moser and Leo, 2010).

The innate immune system is distinguished from the adaptive immune system because its properties allow an immediate and substantial attack on invading pathogens. The innate immune system's mechanism lacks memory and is essentially focused on a particular set of PAMPs shared by many pathogens. Adaptive immunological response works as a second line of defense, usually at a later stage of infection, which is activated upon pathogen contact and is relatively slow. This type of response is characterized by a very large set of effectors molecules and cells, able to recognize, eliminate any familiar pathogen resulting in the establishment of immunological memory. Memory is the stamp of the adaptive immunological response that can be stimulated by both natural infection and vaccination (Sompayrac, 2008; Moser and Leo, 2010; Kalinski, 2012; and Pluske et al., 2018).

The immune system consists of entire organs, lymph vessels, individual cells, and proteins. Lymphoid organs are classified as primary lymphoid organs such as the thymus and bone marrow. Secondary lymphoid organs such as lymph nodes, spleen, tonsils, mucosal-associated lymphoid tissues (MALT) is where expansion of lymphocytes after antigen exposure takes place, as well as the production of memory T cells and effectors B cells. Finally, the tertiary lymphoid tissues are aggregations of lymphoid cells in autoimmune diseases (Pabst, 2007).

1.5.1 Immune response in mucosal associated lymphoid tissues

The mucosal associated lymphoid tissues can be divided into two functional areas known as inductive or effector sites (Fig. 1.1). The inductive sites include the areas of antigen sampling at mucosal surfaces: appendix, mesenteric lymph nodes (MLNs), isolated lymphoid follicles, and Peyer's patches (PP). Whereas the effector sites are where lymphocytes differentiation occurs to defend the organism in an immune response: basically all the immune cells outside the PP in the lamina propria (Brandtzaeg and Pabst, 2004) and selected cells of the intestinal epithelium, such as intraepithelial lymphocytes, Goblet cells, Paneth cells, and enterocytes (Wershil et al., 2008).

The MALT is compartmentalized, and their functions are not dependent from the systemic immune system. These types of immunological tissues can be described and differentiated from others because of the need for a tolerance to non-antigenic. Disruption of this balance between immunity and tolerance can lead to the development of detrimental inflammatory responses (Cerovic, 2014).

1.5.1.1 Effector mechanisms of the innate immune response

The most important effector mechanism of the immune response in the MALT is phagocytosis, which can be executed by all cells of the innate immune system. In phagocytosis

process, antigens are engulfed, surrounded within an intracellular vesicle for destruction (Moser and Leo, 2010). Primary representants of phagocytes in the mucosa are dendritic cells and macrophages. Dendritic cells can migrate from the mucosa to the lymph nodes to initiate adaptive immune responses by presenting antigens to naïve T cells and polarize them toward effector fates to establish the adaptive response. On the other hand, macrophages are resident in the mucosal lamina propria where they engulf and clear bacteria, translate alert signals to other immune cells, secrete cytokines, and maintain intestinal homeostasis (Cerovic et al., 2014; Gross et al., 2015).

Dendritic cells present in the MALT are specialized in T-cell communication activating T-cell immunity in response to antigens and inhibiting autoreactivity by tolerogenic mechanisms. These cells are the most specialists in presenting antigens through major histocompatibility complex (MHC) context, co-stimulation and instructing cytokines that govern T cell polarization into effector cells (Gross et al., 2015) and can migrate among lymphoid tissues through activation of the chemokines receptors (Jang et al., 2006). Tolerogenic mechanisms are also an important feature of dendritic cells in the MALT. For instance, they can secrete retinoic acid and TGF-β, which promote and amplifies the generation of Foxp3+ Treg cells (Gross et al., 2015 and Bakdash et al., 2013).

Macrophages have several functions in mucosal immunity like phagocytosis and production of mediators that drive epithelial cell turnover. Macrophages are highly phagocytic cells that can clear bacteria, cellular debris, and nonmicrobial antigens, which is essential for the maintenance of mucosal homeostasis (Gross et al., 2015). Moreover, macrophages produce large amounts of interleukine-10, which prevents inflammation by blocking pro-inflammatory responses, and promotes the survival and functions of local T-cell regulatory Foxp3+ cells in the mucosa (Hadis et al., 2011).

Mast cells are mainly responsible to IgE-mediated immune responses and reside in the tissues. In the mucosa, these cells works to maintain homeostasis and are involved in the mucosal response to intestinal pathogens, helminths, and food allergies. Mast cells secrete a variety of immune mediators such as histamines, leukotrienes, and prostaglandins (Mair et al., 2014). Besides mast cells, other proinflammatory leukocytes, such as neutrophils, basophils, eosinophils, and natural killer cells (NK), are responsible to produce and stimulate hepatic acute phase proteins and cytokines to mediate an inflammatory response (Pluske et al., 2018). Another important type of cells present in the MALT are the Goblet cells, the major producers of the mucus and thus provides the first line of defense against bacteria in mucosa like the intestine (Johansson and Hansson, 2016) as well as surface of the endometrium and endocervix (Moore et al., 1998).

1.5.1.2 Effector mechanisms of the adaptive immune response

Adaptive immunological response is highly sophisticated and involves antibodies and T cell receptors as recognition systems that have evolved together with the great mutation rate of pathogens over time (Dembic, 2015). Two important properties of this type of immune response, as a second line of defense, are that it often occurs at a later stage of infection and is relatively slow compared to innate response. Adaptive immune response is characterized by having a large set of effectors molecules and cells to efficiently recognize, eliminate, and establishes an immunological memory in response to pathogens. Lymphocytes are the key cell population in the adaptive immune response in the MALT (Pluske et al., 2018).

T cells play an important role in MALT immune response. They can be divided into two main categories according to the type of different cell surface glycoproteins: T helper (Th) cells (CD4+ T cells) and cytotoxic T cells (Tc; CD8+ T cells). CD4+ T cells recognize antigens that are presented by MHC II and kill intracellular microorganisms by releasing cytokines. CD8+ T cells

exclusively recognize antigens that are presented by MHC I and directly destroy infecting tumor cells and viruses (Miceli and Parner, 1991). In addition, lymphocyte populations can be characterized by the distinct cytokines they produce. Thus, CD4+T cell are divided into the Th1 subset (IL-2, IFN-γ and TNF producing) or Th2 subset (IL-4, IL-5, IL-6, IL-10 and IL-13 producing) and the type of immune cells stimulated drives the inflammatory response (Oswald, 2001).

Differentiation of naïve CD4+ T cells into Th1, Th2, or Th17 cells is mostly controlled by cytokines produced in early stages of antigen recognition. Several of these cytokines are produced by dendritic cells and can directly develop naïve CD4+ cells into Th1 effector cells. In the same way, IL-6 appears to play an important role in Th17 differentiation. Th1 and Th2 subsets appear to both crossblock each other and to inhibit Th17 development, mostly, this regulation is a result of cytokines signalization (Dembic, 2015; Moser and Leo, 2010).

CD8+ cytotoxic T lymphocytes, also called killer cells, can induce the death of infected and damaged cells through MHC I recognition and secretion of perforin and subsequent activation of proteases into the target cell leading to apoptosis (Dembic, 2015). These cells also secrete cytokines (TNF-α or IFN-ϒ) which bind to infected cells and inhibit intracellular pathogen replication (Moser and Leo, 2010). On the other hand, CD4+ T cells interact with antigen MHC II complexes and play a dual role (effector and regulatory) during an immune response by secretion a variety of cytokines at the site of infection to inhibit pathogens (Bartee et al., 2008). These secreted cytokines have an important role and effects (either enhancing or inhibiting) the activity of other immune effectors such as innate immune cells, B lymphocytes or T cells (Moser and Leo, 2010).

Another important type of effector cell on the adaptative immunity are the B cells. They are activated by T cells on independent or dependent pathways (Dembic, 2015). In this case, antigens can directly stimulate B cell proliferation and induces antibody production by plasma cells or activated B cells can mature to memory B cells depending on the type of induction (Moser and Leo, 2010).

Antibodies are proteins with bifunctional features that can both recognize and eliminate a given antigen or pathogen. Antibodies are molecules with a Y-shape with two heavy and two light chains made up constant and variable regions (Dembic, 2015). These two different regions of an antibody can determine their functional properties and contribute to the antigen-binding site. The type of heavy chain determines the isotype as immunoglobulin (Ig)-A, IgG, IgD, IgE and IgM antibodies. The class of the immunoglobulin can determine their ability to reach sites of infection and to recruit the effector mechanisms according to the needs (Amzel and Poljak, 1979). The major serum immunoglobulin is IgA which is also the predominant antibody class in the MALT. Its localization in the mucosa allows it to bind to antigens and prevent adherence of pathogens. (Woof and Ken, 2006).

1.5.1.3 Immune response regulation

Regulatory T cells (Treg) can inhibit an immunological response by inhibiting the activity of effector, helper, and antigen presenting cells. These cells play an important role in the maintenance of tolerance because it blocks unsuitable immune reactions to non-diseases stimulus (Sakagushi et al, 2008). Regulatory cells are mostly under the control of TGF-β, which is an immunosuppressive cytokine, working to limit immune response, and resolving chronic inflammatory responses can cause great tissue damage if uncontrolled (Moser and Leo, 2010).

The maintenance of mucosal homeostasis is essential for the development and health of swine (Burkey et al., 2009) as highly activated immune system may not be ideal as a protective mechanism because it can have a substantial negative effect on the performance of animals (Pluske, 2018). Induction of responses, cell proliferation and differentiation as well as production and secretion of effector molecules require energy from the body. The effector mechanisms of immune responses frequently result in inflammation, but the pathogenicity of microorganisms varies from severe to low or absent. Therefore, the immune system must be able to modulate immune responses independent of the antigenic load, and in cases such as food antigens, ideally would results in complete absence of immune responses, or 'tolerance' (Bailey et al., 2005).

1.5.2 Biomarkers of inflammation

1.5.3 Cytokines

Like hormones, cytokines are cell-to-cell messengers with immunomodulator effects acting mostly in the microenvironment that it was secreted. Cytokines are non-structure molecules (polypeptides, glycoproteins, and proteins) with multiple biological (pleiotropism) functions in the organism very well described in immunology (Dembic, 2015). They participate in a great variety of biological process including response to antigen and infections, embryonic development, disease pathogenesis, cell differentiation, response to vaccines, changes in cognitive functions, and progression aging. Cytokines have been the center of many researchers in important areas of human medicine such as diagnostic, prognostic, and therapeutic agents in diseases (Dinarello, 2007).

There are different ways to group and classify the cytokines. In general, the physiological functions of cytokines are tissue homeostasis, cellular activation, relocations, and differentiation.

The most common is to classify them according to their specific functions such as acting as growth factors, pro-inflammatory or anti-inflammatory, and polarizing the immune response to antigens (Dinarello, 2007).

Dembic (2015) described the five major functional classes of cytokines. The first class are the interferons (INF) that acts against viruses and regulation of immunity via specific membrane receptors. The second class are the interleukins (IL) which main functions are related to inter-cellular communication between leukocytes regulating immunity but also having an important role in the development and growth of cells of lymphoid tissues. These cytokines work through specific cell-membrane bound receptors and encompass IL-1 to IL-38, with few exceptions. The third class of cytokines are the chemokines, which have chemotactic properties and an important role in the migration of cells. There are 50 chemokines to date, and their function depends on binding specific cell-membrane cysteines receptors (ion channels) with promiscuous properties (receptors can bind more than one ligand). The fourth class encompass several cytokines with tissue regeneration and homeostatic cell division functions acting in single cells (e.g., erythropoietin, stem cell factor, macrophage colony stimulating factor, leukemia inhibiting factor), tissues (e.g., hepatocyte growth factor, fibroblast growth factors, epidermal growth factors, insulin-like growth factors, vascular endothelial growth factors) or across the entire organism (e.g., TNF-α, TNF-β, and TGF-β). The fifth class are those with functions other than the cited above such as FGF-23 which regulates calcium excretion independently of PTH.

Cytokines are secreted by monocytes, B cells, natural-killer cells, and DC, which are involved in initiation and driving of cellular immune responses. Cytokines most often are multi-reactive, have selective functions (e.g., pro-inflammatory, chemotactic, anti-inflammatory, differentiating), and usually affect particular cell types or tissues (Dembic, 2015). They are integral

mediators for maintaining mucosal homeostasis, as well as prominent effector molecules during mucosal inflammation. They also regulate the function of the mucosal barrier at different levels resulting in changes in the barrier permeability (Bamias et al., 2014; Lawrence, 2010).

Differently than hormones, the action of one single cytokine is less relevant as there are several cytokines that have overlapping functions (redundancy) on cells. Therefore, the absence of one cytokine can be covered by the functions of others (e.g. function of IL-2 and IL-4) except for IFN-γ which has a unique function in the organism (Dembic, 2015).

Cytokines can also be divided in groups according to the type of cell and tissues that were secreted from. Innate cytokines exert both pro and anti-inflammatory functions during homeostatic and inflammatory pathways. Adaptive immune responses are also complex in the mucosa, while inflammation progresses, a different set of cytokines mediates the immune response. Most cytokines are plastic in their function and the outcome is not affected only by the specific molecules but also by the mucosal environment overall (Bamias et al., 2014). In the inflammation process, the recruitment of cells and humoral molecules to the site of infection in tissues is potentialized as the synthesis and secretion of cytokines increase. (Moser and Leo, 2010).

1.5.3.1 Interleukine-1

Interleukin-1 (IL-1) encompass a set of cytokines with a great range of biological functions, mainly involved in pro-inflammatory, immunoregulatory, and hematopoietic processes. Among the stimulus for its secretions those that can be highlighted are cell injury, IL-1 as autocrine effect, TNF-α, immunoglobulins linked to antigens, components from complement cascade, bacterial cell membranes products, zymosan, leukotrienes, and granulocyte-macrophage colony stimulating factor (GM-CSF). The major IL-1 source in organisms are activated macrophages (Bent et al., 2018; Dembic, 2015). Important pro-inflammatory effects of IL-1 are the increase in body

temperature, up-regulation on synthesis and secretion of acute phase proteins, and muscle proteolysis (cachexia) because of its stimulus on TNF-α secretion (Andrews et al., 2018). Additionally, IL-1 immunomodulatory effects during T cell activation can be characterized by promoting development of Th17 helper T cells together with IL-6, IL-21, IL-23, and TGF-β. The secretion of IL-1 by immune cells can be reduced by drugs like dexamethasone, cytokines like TGF-β and IL-10, and other compounds like retinoic acid (Dembic, 2015).

Principal members of these group of cytokines are IL-1α, IL-1β, and IL-1Ra (receptor antagonist) which basically differ from each other based on the primary protein structure (Boutet et al., 2019). For instance, IL-1α and IL-1β are 30% different from each other in their structure and IL-1β can be found in the serum while IL-1α is not detectable because is it only secreted by dendritic cells on the activation stimulus. Other names for IL-1β can be found in the literature such as catabolin, pre-interleukin-1β, and pro-interleukin-1β (Dembic, 2015).

IL-1β together with TNF and IFN-γ, display a role in many acute and chronic diseases such as Alzheimer, septic shock, arthritis, atherosclerosis, kidney diseases, migraine, endometriosis, and in oncological processes in the like liver, bladder, and stomach (Dembic, 2015). Moreover, some skin related inflammatory diseases like psoriasis and psoriatic arthritis have overexpression of IL-1β as a major trigger (Boutet et al., 2019) as well the progression of tumors (Bent et al., 2018).

1.5.3.2 Interleukine-4

IL-4 is a glycoprotein secreted mainly by activated T lymphocytes (CD4 helper Th2 type, and cytotoxic CD8 Tc2). IL-4 is also secreted by B cells present in tonsils and mucosa, mast cells, dendritic cells, and basophils (Junttila, 2018; Banchereau, 1990). Main functions of IL-4 involve being a stimulus to B lymphocytes growth and proliferation as well as shifts in the isotypes of

immunoglobulins produced by B cells (Hansen et al., 1991). IL-4 can be considered an anti-inflammatory cytokine (Andrews et al., 2018; Hansen et al., 1991) as IL-4 stimulates the development of Th2 type response regulating humoral immunity, eosinophilia, mastocytosis, and most important, display functions on deactivation of macrophages because of stimulus of the secretions of Th2 cytokines like IL-10 and TGF-β. IL-4 does not promote proliferation of hematological progenitor cells itself but modulates the growth-factor dependent proliferation of these cell (Hansen et al., 1991).

IL-4 functions are very similar (redundant) to IL-2, IL-25, IL-31, and IL-33. While stimulating Th2 response, it also inhibits Th1 and Th17 cells enhancing the anti-inflammatory effects. IL-4 directly suppress the secretion of IL-1β, IL-12, IL-10, and TNF-α by monocytes and IL-1β and IL-12 by macrophages. IL-4 can stimulate migration of myoblasts to the site of the muscle lesion and stimulating formation of myotubules, ultimately having an important function on muscle regeneration (Dembic, 2015; Banchereau, 1990; Hansen et al., 1991).

As a major product of Th 2 type cells, IL-4 (also IL-13) is crucial for IgE production and for the development of Th2 cells that produce cytokines responsible for inducing allergic reaction (Nagai et al., 1994). IL-4 and IL-13 shared the same receptors in the intestine, in this specific mucosal tissues, these cytokines can contribute to the progression of colon cancer by increasing the production of oxygen reactive species and cell proliferation (Liu et al., 2017).

1.5.3.3 Interleukine-6

IL-6 is a glycoprotein that pertains to a group of cytokines that signal into the cell via receptors. It is secreted by T and B lymphocytes, monocytes, fibroblasts, and macrophages after activation. IL-6 is one of the best representers of the pleiotropism feature of cytokines as it has several functions in organisms. It displays functions in a great variety of cells like neurons,

megakaryocytes, osteoclasts, keratinocytes, mesangial cells, and myelomas as well as in wound healing process (Dembic, 2015). These overlapping but also distinct biologic functions of IL-6 can regulate hepatic acute phase reaction, B cell stimulation, regulate the equilibrium between regulatory and effector T cells, regulate the metabolism and neural functions. (Rose-John, 2018).

The different functions of IL-6 are likely to be pro-inflammatory (Andrews et al., 2018), especially on adaptive immunity after the activation of T and B cells, like the development of peripheral B lymphocyte into plasma cells. The production of acute phase proteins by the liver is stimulated by IL-6. It is needed for the development of Th22 helper cells just like TNF-α and for Th17 phenotype of helper T cells development. When secreted by dendritic cells, it can inhibit the regulatory effects of Treg cells breaking the tolerance process during an immune response (Dembic, 2015).

In human diseases, IL-6 have been studied as a major player in the destruction of joints, gathering of leukocytes, apoptosis, and activation of T cells in arthritis process. IL-6 also has a role in Paneth cells proliferation in the colon and in the development of colitis (Andrews et al., 2018). Blocking IL-6 has been demonstrated to benefit autoimmune diseases but metabolic side effects like excess weight gain and hyperlipidemia (Rose-John, 2018). Another hallmark of this biomarker is that IL-6 is secreted due to radioactive gamma rays, a radio-protective function proven in Sertoli cells and considered as a measure of received radiation dosage. IL-6 display function on blood flow and in high levels can impact quality of sleep in humans. IL-6 is down-regulated by glucocorticoids and up-regulated by insulin and catecholamines in the adipose tissue and play an important role in the skeletal muscles responses to exercises (Dembic, 2015).

1.5.3.4 Interleukine-8

IL-8 is a chemokine that has a few different names like CXCL8, monocyte-derived neutrophil chemotactic factor, or neutrophil-activating factor. Chemokines encompass a great group of cytokines that stimulate the migration of immune cells through attraction to sites with greater concentration of ligands, this gradient difference is detected by the cells with receptors for chemokines (Remick, 2005). Therefore, chemokines play a very important role in the immunological response as they can orient cells like dendritic cells during the migration to lymph nodes to initiate adaptive immunological response and the traffic of lymphocytes in physiological conditions, during inflammation, or infection. IL-8 is secreted by monocytes, macrophages, and endothelial cells and needs to bind to its specific receptors (CXCR1 and CXCR2) to transfer the signal into the cell. IL-8 receptors can be found in neutrophils, monocytes, fibroblasts, and lymphocytes (Dembic, 2015).

Some features of IL-8 can be highlighted to understand its importance in the regulation of the acute inflammatory response. IL-8 can be quickly synthesized and secreted at sites of inflammation recruit and activate cells and is resistant to temperature, proteolysis, and acidic environments allowing them to maintain stable at a site of acute inflammation like abscess and mucosal lumen even for several days (Remick, 2005).

1.5.3.5 Interleukine-10

Anti-inflammatory effects are the hallmark of IL-10 previously called as cytokine synthesis inhibitory factor and T-cell growth inhibition factor (Wei et al., 2019; Andrews et al., 2018). IL-10 is a non-glycosylated protein secreted by a great variety of cells mainly from T cell populations inducible upon activation like Th2 (helper CD4+) and Tc2 (cytotoxic CD8+) cells. Examples of

other types of IL-10 secreting cells are B cells in the later stages of activation, monocytes, macrophages, and mast cells (Dembic, 2015).

Immunoregulation and anti-inflammatory effects of IL-10 are described by reduction of the Th1-type cytokines (IL-1α, IL-1β, IL-6, TNF-α), MHC class II molecules, and costimulatory molecules on macrophages. It also improves B cells life-span, proliferation, and antibody production as well as inhibits dendritic cells maturation. Together with IL-4, IL-10 affects the activation of naïve CD4 and CD8 T lymphocytes into Th2 subpopulations while inhibiting the development of Th1 T lymphocytes subpopulations. In summary, IL-10 is a potent anti-inflammatory molecule and can inhibit macrophage and Th1 lymphocytes proliferation as well as reduce the secretion of proinflammatory cytokines (Neumann et al., 2019; Dembic, 2015).

Because of that IL-10 has been studied for therapeutic purposes to control autoimmune diseases, chronic inflammations, and a few types of cancer. In murine models, Quiros et al. (2017) showed that innate immune cells, (macrophages) are a crucial source for IL-10 secretion to promote mucosal healing. Several studies have demonstrated the positive effects of IL-10 on the maintenance of intestinal epithelial integrity (Neumann et al., 2019; Kominsky at al., 2014). Moreover, IL-10 displays a function on maintaining homeostasis and tolerance in the intestine thorough epithelial toll like receptors-4 ligation and the subsequent autocrine action of epithelial derived IL-10 (Hyun et al., 2015).

1.5.3.6 Tumor necrosis factor-α

TNF-α belongs to the superfamily of tumor necrosis factors, which includes more than 20 members that are soluble homotrimers glycoproteins with the transmembrane regions. TNF-α is mainly secreted by activated macrophages undergoing nuclear stress during inability to repair DNA and have mostly proinflammatory functions along with IL-1β as mentioned before (Andrews

et al., 2018). Its mechanism involves the transmission of apoptotic signals inside cells that can be killed. It also inhibits the expression of toll like receptors-4 on monocytes (Dembic, 2015).

In human medicine, TNF- α has been described as an important driver in intestinal chronic inflammation and development of mucosal diseases like Crohn disease and the inflamed bowel disease (Andrews et al., 2018) as it is highly associated to the process of intestinal cell apoptosis (Grabinger et al., 2017). In the gut, TNF- α is well known to trigger the reduction of the expression of the tight junction proteins like claudin-1, occludin, and zonula occludens protein-1 (Watari et al., 2017).

1.5.3.7 Interferon-γ

Interferons are proteins that stimulates defenses mechanisms against viral infection. There are four types of interferons: interferon-α, -β, -γ, and -λ (IFN-α, IFN-β, IFN-γ, and IFN-λ) all having effects on non-infected neighboring cells to prevent the virus spread and replication. Interferons also play an important role in immune response regulation. Among interferon family, IFN-γ can be considered the most important as it is the major activator of macrophages as well as attract leukocytes to the site of inflammation (Dembic, 2015).

IFN-γ is a homodimer and belongs to the type II interferons which are secreted by Th1-type activated cells (induced by IL-12 and IL-23), cytotoxic T cells, and activated natural killer cells. Its actin is acts through a cell-surface receptor that is a heterotetramer. Besides its antiviral activity, IFN-γ has important immunomodulatory effects by promoting Th1, inhibiting Th2, and suppressing Th17 development. IFN-γ induces cell-surface expression of MHC-II molecules and enhances the expression of MHC-I on antigen presenting cells (Schroder et al, 2004; Dembic, 2015).

Influenced by IFN-γ effects, monocytes can differentiate into macrophages. As mentioned above, IFN-γ is the major macrophage activator resulting in one of the most important set of activated cells during inflammation (Schroder et al, 2004). Macrophages are phagocytic cells that can destroy intracellular bacteria through a great variety of mechanisms such as producing nitric oxide, oxidative radicals, and hydrogen peroxide, as well as inducing the expression of other cytokines and receptors that have pro-inflammatory effects and/or attract other leukocytes and lymphocytes (Schroder et al, 2004; Dembic, 2015).

In human medicine, IFN-γ is known to have a role on inflammatory bowel disease, together with TNF-α, can cause disruption of tight junctions in the gut increasing the permeability and entry of pathogens (Dembic, 2015) as well as reduce epithelial cell proliferation (Nava et al., 2010). In addition to cell proliferation reduction, IFN-γ also promotes epithelial cells apoptosis (Schuhmann et al., 2010).

1.5.4 Acute phase proteins

Over the past decades, acute phase proteins (APP) have been more widely applied as biomarkers of inflammation in human and veterinary medicine while their discovery as early biomarkers of infections were reports in 1900s (Cray, 2012). These biomarkers of inflammation change in plasma concentration during immune response but also due to infection, neoplasia, stress, and trauma. The APP are well described in the literature as part of acute phase response from innate immunity and encompass C-reactive protein (CRP), serum amyloid A, haptoglobin, alpha 1 acid glycoprotein, alpha 1 protease inhibitor, alpha 1 antichymotrypsin, ceruloplasmin, and fibrinogen (Engler, 1995). These are highly conserved plasma proteins secreted by the hepatocytes in response to signals including endotoxins, alarmins from injured cells, and cytokines (Schrödl et

al., 2016). IL6, IL-1β, and TNF-α are recognized to be important regulators of the majority APP genes (Engler, 1995).

Functions of APP during early immune response can be described as a group of reactions to tissue injury resulting from inflammation, infection, trauma, stress, and neoplasia through interaction with ligands forming complexes which will be cleared later by the liver. Therefore, as part of innate defense mechanisms, APP are synthesized and secreted to protect the host as well as to reestablish homeostasis and to promote healing. The APP response varies in magnitude and type of complexes formed among different animal species (Cray, 2012; Engler, 1995). In humans for instance, the major APP are the CRP and serum amyloid as plasma concentration significantly rise during inflammation (Ye et al., 2015) while in ruminants the major APP are haptoglobin and serum amyloid (Reczyńska et al., 2018). Once secreted, mechanistically, they promote protease inhibition, neutralization of toxic molecules like the superoxide anion, clearance of cell membranes and chromatin (Engler, 1995) producing effects like leukocytosis, fatigue, hypoglycemia, alterations of vascular permeability, anorexia, lipolysis, fever, muscle catabolism, and metabolic changes (Schrödl et al., 2016).

The application of APP on diagnosis of diseases in animals has gained attention even though the major type of AAP differs among species (Cray, 2012) and APP are known to be very sensitivity in early innate response but also have very low specificity (Schrödl et al., 2016).

In pigs, evaluation of haptoglobin and CRP in the serum have been used to assess the health status of swine as non-specific biomarkers for inflammation and stress (Hennig-Pauka et al., 2019). These biomarkers have been studied to differentiate stressors other than infectious diseases, tail biting (Heinonen et al., 2010), stress response to transportation (Salamano et al., 2008), post-

weaning stress (Segalés et al., 2004) as well as systemic effect on the offspring because of passage through the milk in the case of haptoglobin (Hiss-Pesch et al., 2011).

1.5.4.1 C-reactive protein

C-reactive protein was identified as an APP in humans infected with *Streptococcus pneumoniae*. The calcium-dependent reaction with pneumococcal cell wall C-polysaccharide from the pathogens, originated its name. In humans, CRP is used on diagnosis of homeostasis establishment in healthy patients as well as those with diabetes and coronary heart disease (Cray, 2012; Mold et al., 2002).

CRP promotes opsonization on bacteria, fungi, and parasites to activate complement cascade and phagocytosis (Mold et al., 2002). CRP has also been described on cytokines induction related to adaptive immune response as well as involvement in pathogenesis of several disturbances. Besides hepatocytes, CRP is also secreted by lymphocytes, monocytes, neurons, atherosclerotic plaques, respiratory epithelia, kidney glomerular and tubular cells, and adipocytes (Schrödl et al., 2016). CRP reacts in a calcium-dependent phosphatidylcholine manner to bind pathogens and cell debris. Then, these complexes interact with complement components and immunoglobulin receptors which ultimately results in pro- and anti-inflammatory effects in different types of cells like endothelial cells, monocyte-macrophages, and vascular smooth muscle cells. Oher features of CRP are the ability to bind to leptin and contributes to cardiovascular diseases because of leptin resistance as well as the ability to enhance nitric oxide production having effects on reactive oxygen species concentrations and vascular smooth muscle cell proliferation (Hribal et al., 2014; Jialal et al., 2004).

1.5.4.2 Haptoglobin

Haptoglobin is an APP formed by 2α and 2β subunits with few different subtypes according to species. The major function of haptoglobin is to bind and form a complex with free hemoglobin that is released during inflammation and infection to be further phagocytosed by macrophages through CD163 receptors. It is considered an early biomarker of viral infections and stress. Besides hepatocytes, haptoglobins are synthesized by salivary gland, muscle, lung, mammary gland, leukocytes, stomach, and skin (Schrödl et al., 2016).

Haptoglobin is the major APP in cattle. For instance, its concentration can increase in the mammary gland up to 80-fold during mastitis. In swine, haptoglobin can be found in the saliva (with circadian pattern) and into bronchial fluid during respiratory infections (Barbé et al., 2011; Boehmer, et al., 2011; Angen et al., 2009).

1.6 Eubiotics fed to pigs

Over the past 20 years, several feed additives have been studied in pig production, especially those with properties that modify the gut microbiota, compete to adhere to the mucosa, improve gut barrier function, and modulate the immune system. Many of them are originated from microorganisms such as bacteria and yeast (and their metabolites) directly fed to pigs in different forms as well as products that promote health through enhancement of favorable microorganisms present in the gut (Aguilar-Toalá et al., 2018). These products that benefit pigs by promoting microbiota equilibrium are called eubiotics, which can be divided into a few categories.

Probiotics can be defined as “live microorganisms which, when administered in adequate amounts, confer a good health benefit on the host” by improving its intestinal microbial balance (Kenny et al., 2011). Besides that, another scientific term, direct-fed microbials (DFM), is also used interchangeably with the term of probiotics, but in fact both terms do not mean the same thing.

Many probiotic products also contain enzymes and/or crude extracts in addition to live microorganisms. Therefore, DFM can be defined as feed "products that are purported to contain live bacteria and/or yeast" (Bajagai et al., 2016). Probiotic studies have demonstrated that they can affect the intestinal microbiome and have immunomodulatory effects. However, contrasting results can be observed in the literature as experimental designs are often different, diets vary, intestinal colonization confer different microbiome, and strains are different (Roselli et al., 2017).

Another major class of eubiotics are the prebiotics. They are non-digestible oligosaccharides defined as "non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). Non-digestible carbohydrates are the main substrate for gut microbiota. However, only a fraction can be considered as prebiotic, because not all indigestible carbohydrates impact the microbial population (Liu et al., 2018). Therefore, it is expected that prebiotics benefit the host in a manner like probiotics. In practice, *Bifidobacterium* and *Lactobacillus* populations have been almost exclusively the targets of prebiotics (de Lange et al., 2010). Most prebiotics are synthesized or isolated from plant and algae polysaccharides (Wu et al., 2017). Beneficial effects of prebiotics in diets fed to pigs have been related to increased fermentation, and the subsequent synthesis of short chain fatty acids, resulting in reduced intestinal pH. Inulin, fructoligosaccharides, galactoligosaccharides, lactulose, arabino-xylans, xyloglucans, β-glucans, mannooligosaccharides, and resistant starch are the most common carbohydrates that have been recognized as prebiotics (Liu et al., 2018; Gaggìa et al., 2010). There are criteria for substrates to be considered a prebiotic: it must not be hydrolyzed or absorbed in the stomach or small intestine; it must be selective for

beneficial commensal bacteria in the large intestine; and fermentation of the substrate should induce beneficial luminal/systemic effects within the host (Scantlebury and Gibson, 2004).

More recently, a new emerging class have gained attention in livestock production, the postbiotics. Because not all mechanisms are strictly related to the live bacteria, but to its compounds, bacteria viability may not be necessary to directly promote health benefit to the host (Aguilar-Toalá et al., 2018). Therefore, postbiotics are defined as products containing metabolic compounds secreted by live microorganisms after their lysis such as peptides, cell surface proteins, short chain fatty acids, vitamins, enzymes, teichoic acids, peptidoglycan-derived muropeptides, plasmalogens, and organic acids (Kostantinov et al., 2013; Tsilingiri & Rescigno, 2013). Collectively, expected benefits of postbiotics when fed to animals are antimicrobial, antioxidant, and immunomodulatory effects (Sharma & Shukla, 2016).

1.6.1 Yeast products fed to sows

Since the 70s, yeast derived products have been fed to pigs aiming improve both health and performance in the different forms like live cultures, dry products, cell wall, nucleotides, and their metabolites (Holden et al., 1975; Koch et al., 1975; Knabe et al., 1988; Veum et al., 1988; Kephart et al., 1988; Jurgens et al., 1991; Veum, et al. 1995).

Zanello et al. (2013) fed different live yeast strains (probiotic) to sows in late gestation and lactation to evaluate the immunomodulatory effects through immunoglobulins in the colostrum and milk. One of the strains tested successfully improved IgG and IgA in the colostrum and milk, respectively, resulting in lower diarrheas incidence in the offspring while not affecting the sow lactation performance. Likewise, Lu et al. (2019) fed live yeast to gestating and lactating sows as well as to their offspring. Although, this yeast did not affect lactation performance, offspring from

sows that were supplemented had better nursery growth rate as well as improved nutrient digestibility when it was fed direct to the weaned pigs.

Few studies have tested yeast products as postbiotics fed to sows. Shen et al. (2017) evaluated the effects of *Saccharomyces cerevisiae* fermentation product (SCFP) fed to sows and their offspring in a two by two factorial design following the offspring up to market. Feeding SCFP to sows did not influence the offspring performance and intestinal health parameters while feeding the SCFP to the offspring had improved performance and carcass traits regardless the maternal effects. Another factorial design with SCFP fed to sows and environmental temperatures from late gestation to lactation was studied by Chen et al. (2020). It observed important negatives impacts on sow lactation performance due to heat stress conditions while SCFP partially recovered these results, possibly by improving antioxidant status in the serum and milk of the sows. Likewise, weaned pigs submitted to heat stress conditions had the intestinal mucosa protected when fed either SCFP or *Lactobacillus acidophilus* fermentation product (Kumar et al., 2017). The weaning weights and protein digestibility (reduction of plasma urea nitrogen) were improved in sows fed SCFP in gestation and lactation while not affecting colostrum and milk composition (Shen et al., 2011). Kim et al. (2008) and Kim et al. (2010) had demonstrated the benefits of feeding SCFP to sows during gestation and lactation. They observed improvements on litter gain and a shorter wean-to-estrus interval in supplemented sows. Tsai et al. (2016) reported that sows fed SCFP from late gestation until weaning had greater lactation feed intake, greater birth, and day 7 weights, lower stillborn rates, and a significant reduction in fecal *Clostridium perfringens* for both sows and offspring.

The effects of SCFP have been studied in different life stages in pig production as well as in other species and animal models. Benefits such as increasing volatile fatty acid production and

lowering *Salmonella typhimurium* in a rabbit intestinal model was reported by Butler (2017). In young pigs, SCFP have used to improve performance and health parameters. Some of the mechanisms reported are the modulation of acute phase response in weaned pigs challenged with lipopolysaccharides (Carrol et al., 2015), modulation of serum cytokines after *Salmonella typhimurium* (Sanchez et al., 2018a) and lipopolysaccharide (Sanchez et al., 2018b) challenges in weaned pigs. Price et al. (2010) showed that pigs challenged with *Salmonella* at weaning had better growth when fed SCFP because of gastrointestinal microbiota modulation. Diarrhea incidence and bacterial attachment to the intestinal mucosa were ameliorate when weaned pigs orally challenged with *Escherichia coli* K88+ (ETEC) were fed SCFP in combination with antibiotics (Kiarie et al., 2011; Kiarie et al., 2012). Weedman et al. (2011) also showed that weaned pigs can benefit by the immunomodulatory effects of SCFP when subjected to stressors like transportation.

It is expected that the SCFP immunomodulatory effects are due to the individual and complementary properties of its several compounds such as mannan-oligosaccharides, β-glucans, and other yeast fermentation metabolites and derivates (Price et al., 2009).

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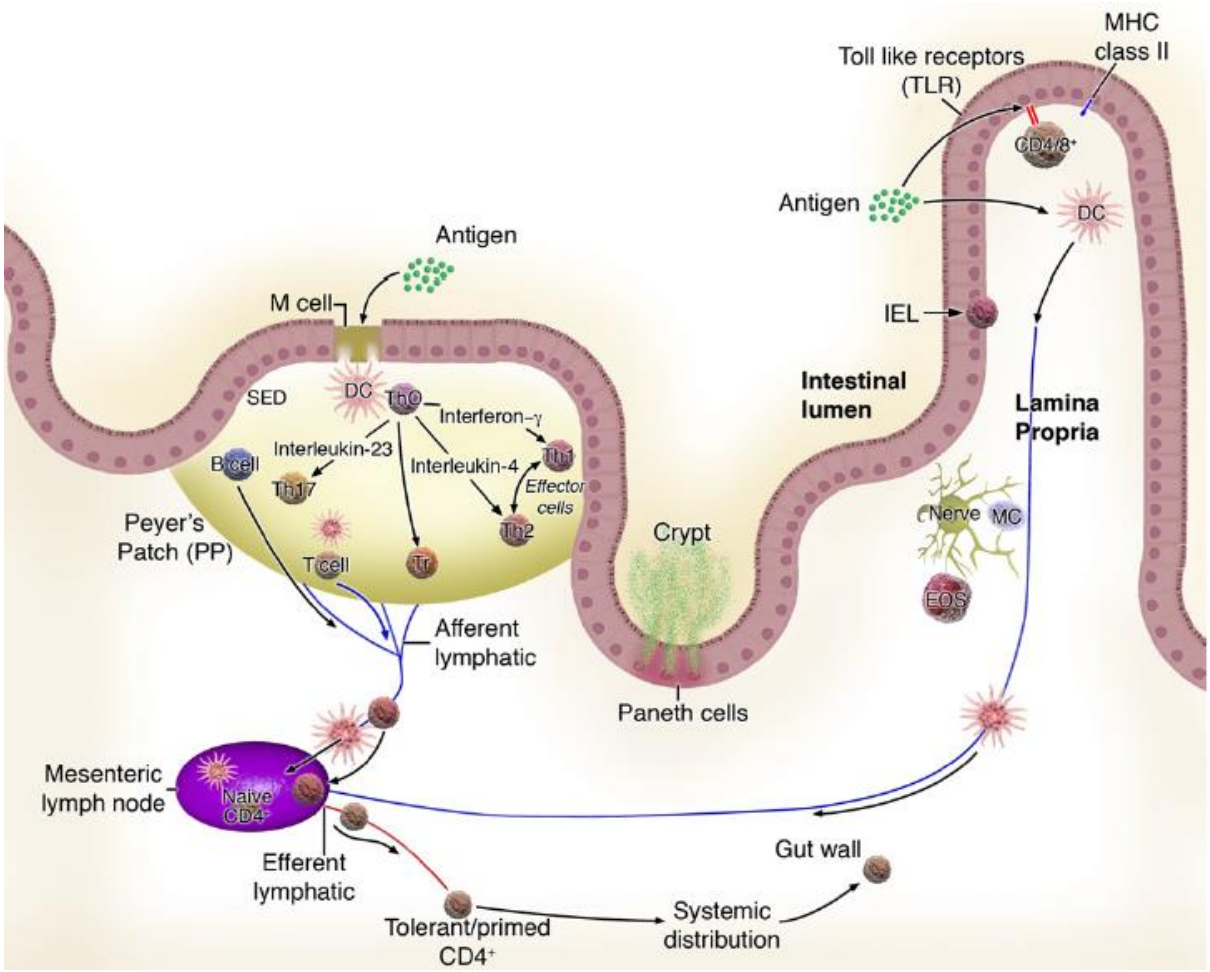

Figure 1.1 Mucosal associated lymphoid tissues inductive and effector sites.
Wershil et al, 2008.

CHAPTER 2. SUPPLEMENTAL YEAST FERMENTATION PRODUCTS EFFECTS ON SOW LACTATION AND OFFSPRING PERFORMANCE

2.1 Abstract

One hundred-forty lactating sows (Yorkshire x Landrace) were used to evaluate the effects of two different *Saccharomyces cerevisiae* fermentation product (SCFP), a liquid (LIQ) and a dry product (XPC®; Diamond V), on sow and litter performance. Sows were fed a common gestation diet until d 112 of pregnancy and then allotted to one of four treatments: 1) Control diet (CON), 2) CON + 15 mL/d of LIQ from d 112 to weaning (LIQ), 3) CON + 0.20% of XPC from d 112 to weaning (DRY), and 4) DRY +15 mL/d of LIQ from d 112 to d 7 post-farrowing (D+L). The liquid source of yeast products was given once daily using an oral dose gun and XPC was included in the feed. Immunoglobulin concentrations were estimated on colostrum samples using Brix refractometer. Plasma of piglets (2 per sow) was collected 24 h after birth for analysis of immunocrit ratio, IgA, and IgG concentrations (n=10/trt). Daily water and feed intake (DFI) from d 112 of gestation to d 7 post-farrowing and weekly average feed intake (ADFI) were recorded. Post-weaning follicle growth was evaluated by transrectal ultrasonography (n=10/trt). Sows had the same initial BW ($P > 0.13$) but those fed any SCFP were heavier at weaning ($P = 0.03$). There were no treatment effects on sow backfat and loin depth ($P>0.05$). LIQ sows had greater DFI than CON during first week of lactation ($P=0.01$). In weeks 2 and 3 as well as for the overall period, SCFP treatments had greater ADFI than CON ($P < 0.01$). Water intake, reproductive performance (total born, stillborn, weaned) did not differ among treatments ($P > 0.05$). Litter ADG from SCFP treatments tended to be greater than CON ($P = 0.10$). The proportion of pigs weaned lighter than 3.5 kg tended to be lower from sows fed SCFP treatments than CON ($P = 0.08$). The overall change

of BW coefficients of variation from d 1 until weaning of litters from sows fed SCFP treatments tended to be greater than CON ($P = 0.10$). No treatment effects were observed in colostrum Brix values ($P > 0.77$), in the pig plasma IgG and IgA, and serum immunocrit ratio ($P > 0.21$). The average daily post-weaning follicle growth was greater for SCFP treatments than CON ($P = 0.05$). The wean to estrus interval was shorter for sows fed LIQ than CON and DRY ($P < 0.01$). In conclusion, SCFP fed to sows improved litter performance and uniformity possibly through promoting a quicker recovery after farrowing and greater sow feed intake.

2.2 Introduction

Improvements in sow lactation performance through optimal colostrum and milk yield, adequate feed intake, and acceptable body reserves mobilization is an efficient way to enhance litter growth (Costermans et al., 2020; Paulicks et al., 2006; Eissen et al., 2003). Although colostrum and milk production are variable among sows, those with greater feed intake are likely to produce more milk (Strathe et al., 2017). In addition, an effective colostrum intake by the piglet is highly correlated to its chances to survive throughout the suckling period (Quesnel and Farmer; 2019). The substantial increase in the litter size due to genetic selection has increased the sow's nutritional and energy requirements to support litter growth (Noblet et al., 1990). Formulating a well-balanced diet is crucial to allow sows to express their genetic potential but voluntary feed intake of lactating sows can become a challenge in situations like heat stress (Bjerg et al. 2020; Silva et al., 2021), lameness (Heinonen et al., 2013), sickness, and over conditioned gestating sows (Eissen et al., 2000). Taken together, low feed intake and great body reserves mobilization during lactation, can negatively affect both litter growth and the sow subsequent reproductive performance (Stathe et al., 2017; Eissen et al., 2003).

Normally, in the mid and late gestation periods, hyperprolific sows have a pro-inflammatory status (Liu et al., 2021; Cheng et al., 2018) as well as increased systemic oxidative stress (Berchieri-Ronchi et al., 2011) because of many reproductive events such as the onset of farrowing processes (Jabbour et al., 2009). Among many factors that can affect feed intake, inflammatory mediators can have an important role not only lowering the appetite but also modifying the partitioning of nutrients (Sauber et al., 1999). Thus, inflammatory mediators may change the animal's maintenance requirements, reduce milk production and piglet growth, and impact reproductive performance. Furthermore, these mediators such as cytokines, chemokines, and the reactive species of oxygen can be transferred from sows to their offspring through colostrum and milk (Shen et al., 2015).

Yeast fermentation products, classified as postbiotics, contain soluble compounds from yeast cells after their lysis as well as the medium of fermentation (Aguilar-Toalá et al., 2018). Yeast products and derivates are known to have positive effects in swine intestinal health and growth performance acting as prebiotics (Kumar et al., 2017; Price et al., 2010) and producing immunomodulatory effects both *in vitro* (Jensen et al., 2008) and *in vivo* (Sanchez et al., 2018 (a); Sanchez et al., 2018(b); Kumar et al., 2017; Zanello et al., 2013). Therefore, we hypothesized that modulation of inflammatory status of sows post-farrowing may promote greater feed intake in the early stage of lactation phase and consequently mitigate the effects of inflammatory processes after parturition. The objective of this study was to evaluate the effects of two different sources of *Saccharomyces cerevisiae* fermentation product (SCFP) fed to sows pre- and post-farrowing on their lactation performance.

2.3 Materials and Methods

2.3.1 General

All experimental procedures and animal housing were reviewed and approved by the Purdue University Animal Care and Use Committee (protocol #1909001949). In general, animal care followed the Guide for Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Sciences Societies, 2010) and individual therapeutic medication treatments were given and recorded when sows or piglets exhibited clinical signs of illness following the Purdue Veterinary Care SOP. The experiment was performed in the Animal Sciences Research and Education Center (ASREC) at Purdue University, West Lafayette, IN 47906, U.S.A. The two SCFP tested were provided by Diamond V (Cedar rapids, IA, U.S.A) and contains a variety of compounds from the fermentation processes such as mannan-oligosaccharides, β-glucans, yeast metabolites, yeast cell wall fragments as well as the media utilized in the fermentation. One of the sources is a liquid SCFP prototype (LIQP) and the second is a commercially available SCFP dry source (XPC®; Diamond V).

2.3.2 Animals and treatments

A total of one hundred and forty pregnant sows (Landrace x Yorkshire; BW 254 ±10.4 kg; parity 3.1±0.35) were blocked by parity and body condition score on approximately day 109 of gestation and allotted to one of four treatments: 1) Control diet (corn-soybean meal based diet; CON), 2) CON + 15 mL/d of LIQP from d 112 to weaning (LIQ), 3) CON + 0.20% of XPC from d 112 to weaning (DRY), and 4) DRY + 15 mL/d of LIQP from d 112 to d 7 post-farrowing (L+D). The LIQP was given once daily using an oral dose gun (Fig.2.1; Allflex model 30EM-SDP, Kenilworth, NJ, U.S.A) and XPC was included in the feed. Sows were fed a common gestation diet until d 109 of pregnancy and then were moved to the farrowing barn and fed a common

lactation diet (CON) until day 112 of pregnancy when dietary treatments were fed (Table 2.1). Farrowing stalls were 0.5 x 2.1 m with one stainless steel feeder and stainless steel water drinker. The farrowing pens were 1.52 x 2.64 m and the creep space for the piglets was 0.46 m wide on each side of the sow stalls. All sows were allowed to eat 3.1 kg/day (in two even meals; 0700 and 1500 h) upon entry until farrowing and *ad libitum* after farrowing until weaning divided in three additions (0700, 1200, and 1600 h). Feed left over was weighed every morning before the first feeding to calculate daily feed intake in the first week and on days 8, 15, and at weaning to calculate periodic average daily feed intakes. Each crate was equipped with a water meter (Assured Automation, model WM-Pd-050, Roselle, NJ, U.S.A) and daily water intake (DWI) until d 7 of lactation and weekly average DWI (ADWI) were calculated until weaning.

2.3.3 Animals handling and measurements

Sows were weighed on lactation day 1, d 7, and on weaning day using a calibrated scale (IQ plus 355-2A; 227 g precision; Rice Lake, WI, U.S.A). Backfat (BF) and loin depth (LD) were evaluated on day 112 of gestation and on weaning day using ultrasound (Aloka SSD 500V, Aloka Co., Ltd., Tokyo, Japan). The BF and LD measurements were performed parallel to the spine from the last rib forward, 6.5 cm away from the vertebral column (P2 point) on each side to calculate the means. Sows farrowed either naturally or were induced on day 115 of gestation (2 cc prostaglandin; Lutalyse ®, Zoetis Inc., Parsippany-Troy Hills, NJ, U.S.A). Farrowing was monitored (0600 to 2400 h) and assistance were given when exceeded 40 min by palpation of the birth canal, extraction of piglets and/or use of oxytocin. The reproductive performance was evaluated as the number of total piglets born, piglets born alive, stillborn piglets, and mummified fetuses.

Pigs were weighed on day 1 (before cross-fostering) using a calibrated scale (RL-DBS-2; 5 g precision; Rice Lake, WI, U.S.A), on day 7 and weaning day using a calibrated scale (IQ plus 390-DC; 45 g precision; Rice Lake, WI, U.S.A) to calculate individual pig and litters ADG. After 24 h of birth and up to 48 h, litters were equalized within the same treatments according to the number of functional teats. Piglet processing was performed within the first 24 h which encompass receiving supplemental iron injection (1 cc, Uniferon 200, Pharmacosmos A/S, Holbaek, Denmark), ear notching, tail docking, teeth clipping, and castration for males. All pigs were weaned on the same day (18.1 ±0.30 days of age).

2.3.4 Milk production estimation

Milk production was estimated using a weigh-suckle-weigh method on days 3 and 14 of lactation. The litter was removed and kept separated from the sow for 1 h. Then, the litter was weighed using a calibrated scale (IQ plus 390-DC; 45 g precision; Rice Lake, WI, U.S.A) right before moved back to the sow and allowed to nurse for 30 min. After nursing, litters were weighed again. Milk production estimation on days 3 and 14 was calculated as the difference between pre-suckle litter weights and post-suckle litter weights.

2.3.5 Immunological parameters

Colostrum samples (50 mL) were collected from the front, middle, and back teats within 8 h of the first pig born to estimate immunoglobulin concentration using a Brix refractometer (Misco PA201, Misco, Solon, OH, U.S.A). The average of three readings were used to calculate the Brix value.

Blood samples (2 pigs/litter) were collected from a jugular vein 24 h after birth in EDTA tubes (1 mL/pig). The plasma obtained after centrifugation (2000 x g for 10 min; E8 Centrifuge,

LW Scientific Inc, Lawrenceville, GA, U.S.A) was aliquoted in three parts, two parts were stored at -80° C for immunoglobulins analysis, and the third part was used immediately for immunocrit ratio analysis. Immunoglobulins (IgG and IgA) were analyzed using a commercially available ELISA kit (Eagle Bioscience, Inc., Nashua, NH) according to the manufacturer's instructions. The immunocrit ratio was calculated by adding 50 µL of ammonium sulfate [40%; (NH4)2SO4] to 50 µL of plasma. The mixture was used to fill a hematocrit microcapillary tub that was centrifugated for 10 min using a micro-hematocrit centrifuge (14800 x g; LWS M24, LW Scientific Inc, Lawrenceville, GA, U.S.A). Then, the microcapillary tubes, in triplicates per pig, were placed in a microhematocrit reader (EZ Reader, LW Scientific Inc, Lawrenceville, GA, U.S.A) to get a direct reading of the ratio between the length of the mixture and the length of the precipitated immunoglobulins.

2.3.6 Wean to estrus interval and follicle growth dynamics

After weaning, sows were moved to individual gestation crates (0.6 x 2.3 m) and fed a common gestation diet (2.3 kg/d). Twice a day (0900 and 1500) sows were checked for estrus signs by back pressure test and exposure to a mature boar. Insemination was performed 12 h after onset of estrus and repeated at 12 h hours intervals until females showed no signs of estrus. The wean to estrus interval (WEI) was recorded. Sows were kept in individual crates up to day 30 of gestation and checked for pregnancy using ultrasound with a 5 mm convex transducer rC60xi/5-2 MHz probe (SonoSite Inc., Bothell, WA, U.S.A) before transferring to a group housing system.

A subsample of sows (n=40) were evaluated for follicle growth by transrectal ultrasonography (SonoSite MicroMaxx Ultrasound, SonoSite Inc., Bothell, WA, U.S.A) from day 2 after weaning until ovulation was confirmed. The linear transducer (5 mm, 5.2 MHz; SonoSite Inc., Bothell, WA, U.S.A) was attached to polyvinyl chloride tube curved-down in its end and

covered with lubrified disposable plastic gloves. Twice a day (0700 and 1900), after identifying one of the ovaries, the diameter of a minimum of three follicles were measured and recorded to calculate the average follicle size (Fig. 2.2). Ovulation was confirmed when no preovulatory follicles were identified (Fig. 2.3). The duration of wean to ovulation as well as estrus to ovulation were calculated.

2.3.7 Statistical Analysis

All variables measured were tested for normality using Shapiro-Wilk test before analysis, and any variable that failed to follow a normal distribution was transformed through the RANK procedure in SAS (9.4 SAS Inst. Inc., Cary, NC). The sow and its litter were considered the experimental unit for the lactation performance. Data were analyzed using the PROC MIXED procedure in SAS. Pre-planned orthogonal contrasts were used to compare the different sources of SCFP since one of them is a prototype. First contrast was used to compare all SCFP (LIQ, DRY, and D+L) sources to CON, the second to compare the LIQ to CON, and the third contrast to compare the DRY to LIQ. For the subsequent reproductive performance, the sow was considered the experimental unit. The daily water and feed intake in the first week of lactation as well as the follicular growth dynamics after weaning were analyzed as repeated measurements. Correlations between pig plasma immunoglobulins, immunocrit ratio, and the colostrum brix values were analyzed as Pearson correlation coefficients using the CORR procedure in SAS. All data are reported as least squares means with standard errors of the means. Means were considered significant different when $P \leq 0.05$ and trends were discussed when $0.05 < P \leq 0.10$.

2.4 Results

2.4.1 Sow body weight and tissue mobilization

Sows had similar BW ($P > 0.13$), BF ($P > 0.74$), and LD ($P > 0.40$) at the beginning of the experimental period (Table 2.2). On day 7 of lactation, sow BW did not differ among treatments ($P > 0.11$). Sow BW at weaning was 5% greater for SCFP treatments compared to CON ($P = 0.03$) and LIQ sows tended to be 4% heavier than CON ($P = 0.09$) but no difference between LIQ and DRY was detected ($P = 0.82$). Sows from LIQ group had less BW losses (4.9%) throughout the lactation period compared to CON (7.2%; $P = 0.05$) and to DRY (6.8%; $P = 0.09$). No difference among treatments was observed in the final backfat thickness and loin depth ($P > 0.19$) as well as in the overall change in both tissues ($P > 0.24$).

2.4.2 Sow feed and water intake

After farrowing, a linear effect of day ($P < 0.001$) during the first week of lactation was observed on feed intake (Figure 2.4). No interaction between treatments and day were detected ($P > 0.36$). The ADFI tended to be greater for SCFP treatments compared to CON ($P = 0.06$; Table 2.3) and LIQ sows had greater ADFI than CON ($P = 0.01$) and tended to have greater ADFI than DRY ($P = 0.09$) in the first week of lactation. In the second week of lactation, from d 15 until weaning as well as for the overall lactation period, SCFP treatments had greater ADFI than CON ($P < 0.01$), LIQ sows had greater ADFI than CON and DRY ($P \leq 0.04$). No treatment effects or treatment and day interactions were observed for DWI before farrowing until d 7 of lactation (Figure 2.5; $P > 0.22$). Regardless of the treatment, sows had the least water intake on the farrowing day and on days 1 and 2 of lactation and the greatest on d 7 of lactation ($P < 0.01$). Thereafter, weekly as well overall ADWI did not differ among treatments ($P > 0.17$; Table 2.4). The total

intake of SCFP on dry matter basis of sows fed LIQ was constantly 9.9 g/day. The total intake of SCFP on dry matter basis of sows fed DRY was 9.2 g/day on week 1, 12.1 g/day on week 2, 11.8 g/day on week 3, and 10.9 g/day for the overall lactation period. The total intake of SCFP on dry matter basis of sows fed L+D was 19.1 g/day on week 1, 22.3 g/day on week 2, 22.6 g/day on week 3, and 21.1 g/day for the overall lactation period.

2.4.3 Reproductive performance

The number of total born, born alive, stillborn, mummified fetus, the number of pigs sows were allowed to nurse after cross-fostering, and the number of pigs weaned per sow did not differ among treatments ($P > 0.15$; Table 2.5). Total litter weights on d 1, d 7, at weaning as well as the total litter gain did not differ among treatments ($P > 0.13$). Litter ADG in the first week of lactation was not affected by treatments ($P > 0.45$) but litters from SCFP treatments had greater ADG than CON from d 8 until weaning ($P = 0.04$) and tended to have greater ADG than CON in the overall lactation period ($P = 0.10$). Individual pig weights were not affected by treatments on d 1 or d 7 of life ($P > 0.13$). At weaning, pigs from LIQ sows tended to be 5.5% heavier than pigs weaned from CON sows ($P = 0.10$) but did not differ from DRY ($P = 0.32$). Pigs weaned from sows fed SCFP treatments had greater individual ADG than CON ($P = 0.05$) while pigs from LIQ sows tended to have greater ADG than CON ($P = 0.09$) and did not differ from pigs of DRY sows ($P = 0.61$). The proportion of pigs weaned lighter than 3.5 kg from sows fed SCFP treatments ($P = 0.08$) or sows fed LIQ ($P = 0.10$) tended to be lower than CON. The body weight coefficients of variation within the litters on d 1 or at weaning day were not influenced by treatments ($P > 0.20$) but the overall change in variation from d 1 until weaning of litters from sows fed SCFP treatments tended to be greater than CON ($P = 0.10$) and litters from LIQ sows tended to be less than DRY ($P = 0.09$). The

pre-weaning mortality rates in the first week as well as the overall lactation period were not affected by treatments ($P > 0.50$).

2.4.4 Milk production

Results from weigh-suckle-weigh procedure to estimate milk production are shown in Table 2.6. No effects of SCFP on milk production estimation were observed on day 3 ($P = 0.31$) or on day 14 ($P = 0.66$). Sows fed LIQ tended to have greater milk output on day 3 than sows fed DRY ($P = 0.09$) but similar to CON on day 3 ($P = 0.73$), and CON on day 14 ($P = 0.53$), and similar to DRY on day 14 ($P = 0.41$).

2.4.5 Immunological parameters

No effects of treatments were observed in the sow colostrum Brix values ($P > 0.77$; Table 2.7) nor in the pig plasma immunological parameters IgG, IgA, and immunocrit ratio ($P > 0.21$). Nevertheless, moderate correlations were observed between the immunoglobulins in the pig plasma with the immunocrit ratio or the colostrum Brix values ($P < 0.05$; Table 2.8). A moderate correlation was also detected between the colostrum Brix value and the immunocrit ratio ($P < 0.01$).

2.4.6 Follicle growth

The average follicle size during the follicular phase was not affected by treatment ($P > 0.32$; Table 2.9). The average daily follicle growth was greater for the SCFP treatments than CON ($P = 0.05$) and tended to be greater in sows fed LIQ than CON ($P = 0.09$) but did not differ between LIQ and DRY ($P = 0.99$). The wean to ovulation interval was not affected by SCFP treatments ($P = 0.130$) but LIQ sows tended to have 0.5 day shorter interval than CON ($P = 0.069$) and similar to DRY ($P = 0.233$). The estrus to ovulation interval was not affected by treatment ($P > 0.100$).

The wean to estrus interval was 0.5 day shorter in sows fed LIQ than CON sows ($P < 0.01$) and 0.9 day shorter than DRY sows ($P < 0.001$).

2.5 Discussion

In the present study, SCFP were fed to sows pre- and post-farrowing aiming to improve lactation performance. Less BW losses were observed only in sows fed LIQ even though all SCFP groups had greater BW at weaning. Lactation feed intake was improved (13%) by all SCFP and markedly greater (19%) in sows fed the liquid prototype source of yeast derivates. Shen et al. (2011) evaluated the same dried version of SCFP used in this study for gestating and lactating sows and found that litter BW gain was improved, possibly through increased milk production while not affecting colostrum and milk nutrient composition. Although, milk output estimation did not differ among treatments, our results showed that possibly due to the greater feed intake, sows had litter performance improved, as shown by the greater litter and individual pig ADG from sows fed any of the SCFP tested. These results corroborate those found by Tsai et al. (2018) feeding SCFP to gestating and lactating sows. The litter BW gain was improved in other studies although the voluntary feed intake was not affected by feeding yeast cultures to lactating sows (Shen et al., 2011; Kim et al., 2008; Kim et al., 2010). Indeed, it was observed that the litter ADG after the first week until weaning was significantly greater in those sows fed SCFP. It is possibly to infer that those sows fed SCFP had a quicker recover post-farrowing improving their capacity to eat and support litter growth. Tsai et al. (2018) had also observed greater pig BW at day 7 of life from sows fed SCFP which was explained by the greater ADFI in the first week of lactation. Moreover, the greatest effect of SCFP on feed intake observed in sows fed the liquid source allowed them to wean heavier pigs than CON sows. To our knowledge, this is the first SCFP in a liquid form tested in sows. We speculate that some of the benefits over the dried source is due to the fixed amount

given daily instead of variable amount observed by changes in ADFI when mixed in the feed, which ultimately varies according to the daily feed intake. In addition, the acceptability of the product was not a problem as no sows had refused to ingest the product. Similar, a liquid product containing *Lactobacillus* has been demonstrated to improve litter growth when fed to lactating sows (Yang et al., 2019). Additionally, liquid supplements for pigs are often easier to adjust the dosages and less vulnerable to fluctuations in daily intake as feed additives are (Escudero et al., 2018).

Several nutritional strategies to increase colostrum and milk quality and output have been reported in the literature. Adequate AA supply has been proven to support milk production as demonstrated for valine (Roth-Maier et al., 2004), methionine and cysteine (Kirchgessner et al., 1993), threonine (Paulicks et al., 1998), and tryptophan (Paulicks et al., 2006). Feed additives have also demonstrated a benefit on milk production and litter growth. Ho et al. (2020) found that chitosan oligosaccharide fed to gilts improved milk yield, litter growth, and the concentration of serum immunoglobulins in the offspring. In this study the colostrum quality, measured by the Brix value, was not affected by SCFP in the diet of sows. Several nutritional and endocrine factors may affect colostrum and milk yield and composition (Feyera et al., 2021; Quesnel, 2011) and the response to feed additives varies (Quesnel and Farmer, 2019). Hasan et al. (2018) showed that gestating sows fed yeast derivates had greater colostrum yield and fat content while not affecting the immunoglobulins levels. In this study, the lack of differences in colostrum Brix values, a good indicator of colostrum IgG concentration (Balzani et al., 2016; Hasan et al. 2016), had resulted in no difference in plasma IgG and IgA concentrations of pigs 24 h after birth as well as immunocrit ratio. Indeed, the moderate correlations found confirmed the applicability of the methods to evaluate colostrum quality. These results agree with a previous study showing that SCFP fed to

gestating and lactating sows did not affect IgG and IgA concentration in colostrum (Tsai et al., 2018) nor in the piglet`s blood, while improving maternal health status, as shown by a reduction in neutrophil cell count (Shen et al., 2011). On the other hand, feeding a specific strain of *Saccharomyces cerevisiae* to sows, during gestation and lactation period, had improved colostrum IgG and milk IgA concentrations, enhanced litter health status as diarrhea incidence reduced (Zanello et al., 2013). Tsai et al. (2018) found that SCFP fed to sows improved health status through reduction in *Clostridium perfringens* in the feces of sows and their offspring. These differences among studies may be explained by the duration of feeding the additive, the herd immunological status, feeding programs, and nutritional levels of the diets. Nevertheless, SCFP are expected to have immunomodulatory effects since they are rich in prebiotic compounds such as mannan-oligosaccharides, β-glucans, and other yeast fermentation metabolites and derivates (Price et al., 2009).

As expected, the reproductive performance was not affected by dietary treatments since SCFP were given only approximately three days before sows have farrowed. Likewise, Shen et al. (2011) did not find any difference in reproductive performance of sows fed SCFP during gestation period. Differently, when SCFP were fed to sows from late gestation (d 93) until weaning, a reduction in stillborn and improvements in birth weights was observed (Tsai et al., 2018). This study has not shown differences in pre-weaning mortality but litter uniformity at weaning was improved by SCFP fed to sows, as shown by the lower percentage of pigs lighter than 3.5 kg and the improvement of the BW coefficient of variation from birth to weaning. Reduction in BW variability at weaning is an important productive parameter to take into account when evaluating any strategies to improve lactating sows performance as it is related to efficiency and occupation

time of nursery and grow-finish facilities as well as to body weight of pigs at slaughter (Lopéz-Vergé et al., 2018).

In this study, no effects of SCFP were observed on daily water intake. Kruse et al. (2011) showed that lactating sows drink water 4.7 to 5.2 times the amount they eat, which represents approximately 26 to 32 L/day. Present results are consistent with mentioned above, the pattern of water intake had followed the pattern of feed intake with water-to-feed ratio around 5.4. That indicates that no water deprivation or change in water intake behavior has occurred and observed changes in feed intake are likely to be strictly related to dietary treatments.

The subsequent reproductive performance in sows, mainly WEI, is a good indicator of the degree of body reserves mobilization and energy balance during the previous lactation (Eissen et al., 2000; Mullan and Williams, 1989). Moreover, the onset of estrus is highly correlated to the follicle size (Cabezón et al., 2017). In this study, the average daily post-weaning follicle growth from sows fed SCFP was greater than CON. This fact might be related to the energy balance status of these sows during WEI as they consumed more feed pre-weaning, favoring follicle growth. In addition, sows fed LIQ had a reduction of 0.5 day in WEI and on wean to ovulation interval compared to CON which could be explained by the greatest feed intake and less BW change observed in this group of sows. Zak et al. (1997) found that the feed intake in late lactation is related not only to WEI but also to embryo survivability. Other studies found different results on subsequent reproductive performance feeding SCFP to sows. Kim et al. (2010) observed a reduction in WEI while Shen et al (2011) did not find improvements in WEI. The inconsistent results regarding subsequent reproductive performance might be related to body condition of the sows at farrowing and the extent of body reserves mobilization during lactation.

2.6 Conclusion

In summary, SCFP fed to lactating sows improved feed intake and litter growth while not affecting colostrum quality and immunoglobulins in the offspring. Pig weight at weaning was improved in sows given the liquid source of SCFP while pig ADG and litter uniformity at weaning were improved regardless of the source of SCFP. Sows given the liquid source of SCFP had the greatest feed intake, less body weight mobilization, and a reduction in WEI. In conclusion, SCFP improved sows lactation and litter performance possibly through promoting a quicker recovery after farrowing as demonstrated by greater DFI in the first week post-farrowing. Further investigations regarding the mode of action of SCFP around parturition is needed.

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Table 2.1 Lactation diet composition (as-fed basis)[1]

Item	CON	LIQ	DRY	L+D
Ingredient, %				
Corn	50.303	50.303	50.103	50.103
Soybean meal, 47.5% CP	32.600	32.600	32.600	32.600
Dried distillers grain with solubles, 7% fat	10.000	10.000	10.000	10.000
Swine grease	3.000	3.000	3.000	3.000
Limestone	1.430	1.430	1.430	1.430
Monocalcium phosphate	1.350	1.350	1.350	1.350
Vitamin premix[2]	0.250	0.250	0.250	0.250
Sow premix[3]	0.250	0.250	0.250	0.250
Trace mineral premix[4]	0.125	0.125	0.125	0.125
Se premix[5]	0.050	0.050	0.050	0.050
Phytase[6]	0.100	0.100	0.100	0.100
Salt	0.500	0.500	0.500	0.500
Availa Zn 120[7]	0.042	0.042	0.042	0.042
XPC[8]	0.000	0.000	0.200	0.200
Total	100.00	100.00	100.00	100.00
Calculated analysis				
ME, Kcal/kg	3353.30	3353.30	3353.30	3353.30
Crude Protein, %	22.33	22.33	22.33	22.33
Total Lysine, %	1.183	1.183	1.183	1.183
SID[9] Lys, %	1.001	1.001	1.001	1.001
SID Met, %	0.311	0.311	0.311	0.311
SID Met+Cys, %	0.615	0.615	0.615	0.615
SID Thr, %	0.693	0.693	0.693	0.693
SID Tryp, %	0.233	0.233	0.233	0.233
SID Iso, %	0.809	0.809	0.809	0.809
SID Val, %	0.889	0.889	0.889	0.889
Calcium, %	0.902	0.902	0.902	0.902
Total P., %	0.732	0.732	0.732	0.732
ATTD P, %	0.501	0.501	0.501	0.501
Analyzed composition[10]				
Ash, %	5.71	5.71	6.17	6.17
Crude Protein, %	20.52	20.52	21.07	21.07
Crude Fiber, %	2.84	2.84	2.85	2.85
Crude Fat, %	5.12	5.12	5.58	5.58
NDF, %	9.31	9.31	9.42	9.42
ADF, %	4.56	4.56	4.87	4.87
Calcium, %	1.03	1.03	1.19	1.19

(Table continues)

Total P, %	0.665	0.665	0.716	0.716
Lysine, %	1.10	1.10	1.11	1.11
Methionine, %	0.29	0.29	0.31	0.31
Cysteine, %	0.31	0.31	0.33	0.33
Threonine, %	0.74	0.74	0.74	0.74
Tryptophan, %	0.25	0.25	0.26	0.26
Isoleucine, %	0.93	0.93	0.94	0.94
Leucine, %	1.81	1.81	1.82	1.82
Valine, %	1.06	1.06	1.06	1.06

[1]Diets were fed from day 112 of gestation until weaning.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44.1 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.1 mg and B12 38.6 µg.
[3]Provided per kg of diet: biotin, 0.22 mg; folic acid, 1.65 mg; choline, 16 mg; pyridoxine, 4.96 mg; vitamin E, 22 IU; chromium, 0.20 mg; and carnitine, 49.6 mg.
[4]Provided per kg of diet: Fe, 121.3 mg; Zn, 121.2 mg; Mn, 15.0 mg; Cu, 11.3 mg; and I, 0.46 mg
[5]Provided 0.3 ppm Se.
[6]Phyzyme® (Danisco Animal Nutrition, Morlborough, UK) providing 600 phytase units (FTU)/kg.
[7]Provided per kg of diet: 50 ppm of zinc.
[8]Saccaromyches cerevisiae fermentation product (Diamond V, Cedar Rapids, IA, U.SA).
[9]Standardized ileal-digestible
[10]Analysis conducted by University of Missouri Experiment Station Chemical Laboratories.

Table 2.2 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n=140) body weight, backfat, and loin depth.

Performance	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
N	35	35	35	35	-	-	-	-
Parity	3.1	3.2	3.1	3.0	0.35	0.899	0.935	0.883
Lactation length, days	18.1	18.0	17.9	18.2	0.289	0.911	0.820	0.857
Body weight (BW)								
Initial BW (d 1), kg	247.5	252.0	259.0	256.0	10.36	0.130	0.492	0.282
BW (d 7), kg	245.9	251.7	256.5	253.9	10.67	0.114	0.355	0.439
Final BW (weaning), kg	230.2	240.3	241.7	240.5	10.48	0.034	0.090	0.818
Overall BW change, %	-7.2	-4.9	-6.8	-6.6	0.98	0.303	0.052	0.092
Backfat (BF)								
Initial BF (d 112), mm	21.0	20.9	21.1	20.7	0.98	0.782	0.744	0.960
Final BF (weaning), mm	16.5	17.5	16.9	16.8	0.79	0.391	0.275	0.699
BF change, %	-20.3	-16.5	-18.5	-18.6	2.06	0.305	0.190	0.486
Loin depth (LD)								
Initial LD (d 112), mm	55.7	55.2	56.4	58.2	1.37	0.403	0.971	0.527
Final LD (weaning), mm	53.9	53.0	54.1	53.8	1.34	0.837	0.568	0.488
LD change, %	-2.1	-4.3	-3.7	-7.5	2.05	0.237	0.560	0.981

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 2.3 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on sows (n=140) gestation and lactation feed intake.

Feed Intake, kg	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Gestation period								
d 112, kg	3.10	3.20	3.01	3.11	0.067	0.880	0.300	0.062
d 113, kg	3.18	3.14	3.17	3.13	0.088	0.754	0.661	0.657
d 114, kg	2.86	3.16	2.93	3.17	0.162	0.343	0.195	0.254
d 115 (farrowing day), kg	2.69	3.07	3.15	3.06	0.340	0.170	0.325	0.736
Lactation period								
d 1 – 7, kg	4.33	5.08	4.62	4.58	0.281	0.057	0.007	0.090
d 8 - 14, kg	5.60	6.65	6.03	6.21	0.358	0.005	<0.001	0.040
d 15 - weaning, kg	5.45	6.57	5.91	6.37	0.284	0.002	<0.001	0.034
Overall, kg	5.05	6.03	5.46	5.62	0.276	0.002	<0.001	0.025

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 2.4 Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n=140) average daily water intake.

Water intake, L/day	Treatments[1]				SEM	Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D		1	2	3
Gestation period								
d 112, L/day	20.24	20.30	18.81	16.65	2.651	0.687	0.957	0.736
d 113, L/day	25.89	19.93	22.15	19.43	2.836	0.171	0.182	0.551
d 114, L/day	24.02	20.80	20.40	20.51	2.821	0.358	0.402	0.929
d 115 (farrowing day), L/day	17.28	15.50	16.08	13.34	2.565	0.181	0.421	0.863
Lactation period								
d 1 - 7, L/day	22.25	23.80	22.84	20.58	1.876	0.955	0.654	0.654
d 8 - 14, L/day	32.73	31.79	33.62	29.07	2.315	0.539	0.630	0.398
d 15 - weaning, L/day	37.04	34.18	35.05	28.90	3.715	0.335	0.591	0.506
Overall, L/day	30.06	29.33	34.30	26.99	3.032	0.746	0.837	0.428

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 2.5 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n=140) and offspring performance.

Item	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Total born	13.1	12.6	13.3	12.4	0.59	0.813	0.695	0.452
Born alive	12.0	11.7	12.2	11.5	0.56	0.961	0.814	0.547
Still born, %	4.9	5.9	4.8	4.3	1.43	0.696	0.788	0.978
Mummies, %	3.3	1.3	3.2	2.7	1.48	0.607	0.145	0.065
Number after cross-fostering	11.9	11.7	11.9	11.8	0.24	0.684	0.477	0.496
Weaned, n	10.3	10.3	10.6	10.4	0.23	0.577	0.856	0.444
Litter weight d 1, kg	19.2	20.3	19.4	18.4	0.59	0.862	0.142	0.203
Litter weight d 7, kg	29.7	30.5	29.8	29.0	0.81	0.610	0.289	0.545
Litter weight at weaning, kg	60.1	63.2	62.6	63.3	1.94	0.199	0.260	0.633
Litter gain, kg	44.0	46.1	46.0	47.4	1.61	0.131	0.291	0.942
Litter ADG d 1 -7, kg	1.81	1.87	1.89	1.83	0.061	0.450	0.497	0.817
Litter ADG d 8 -weaning, kg	2.84	3.01	3.03	3.10	0.084	0.040	0.143	0.997
Litter ADG, kg	2.43	2.55	2.58	2.60	0.069	0.099	0.260	0.847
Pig weight d 1, kg	1.54	1.62	1.52	1.50	0.041	0.848	0.141	0.081
Pig weight d 7, kg	2.74	2.88	2.80	2.72	0.074	0.413	0.132	0.409
Pig weight at weaning, kg	5.82	6.14	5.95	6.11	0.157	0.120	0.098	0.318
Pig ADG, grams	238	251	247	253	6.3	0.047	0.087	0.610
PBW[3] at weaning <3.5 kg, %	5.4	2.0	2.5	2.9	1.91	0.077	0.104	0.791
PBW CV d 1, %	18.7	18.8	20.6	20.9	1.11	0.224	0.942	0.203
PBW CV at weaning, %	17.5	17.9	16.5	17.8	1.17	0.927	0.764	0.312
PBW CV change d 1-weaning, %	-0.9	-1.6	-4.5	-3.6	1.14	0.095	0.752	0.094
d 1 -7 mortality, %	9.8	9.8	9.6	9.7	1.80	0.856	0.944	0.979
d 1- weaning mortality, %	13.7	11.4	11.0	12.3	2.04	0.509	0.859	0.503

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
[3]PBW: pig body weight, CV: coefficient of variation.

Table 2.6 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n=40) milk output.

Item[3]	Treatments[1]				SEM	Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D		1	2	3
Day 3, g	124	141	55	60	36.0	0.3103	0.7293	0.0889
Day 14, g	284	247	298	246	45.4	0.6618	0.5291	0.4052

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
[3]Milk production was estimated using a weigh-suckle-weigh method. The litter was removed and kept separated from the sow for 1 h. Then, the litter was weighed right before moved back to the sow and allowed to nurse for 30 min. After nursing, litters were weighed again. Milk production estimation on days 3 and 14 was calculated as the difference between pre-suckle litter weights and post-suckle litter weights.

Table 2.7 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) sources for lactating sows (n=40) on colostrum quality and piglet immunoglobulins.

Item	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Plasma IgG, mg/mL	23.24	21.85	20.10	23.49	1.243	0.208	0.319	0.214
Plasma IgA, mg/mL	8.04	7.74	7.89	6.56	0.779	0.353	0.737	0.865
Immunocrit ratio[3]	0.20	0.19	0.19	0.19	0.014	0.561	0.490	0.720
Brix value[4]	25.98	26.32	26.09	25.79	0.473	0.960	0.774	0.836

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
[3]Serum obtained from two piglets/litter was analyzed for immunocrit ratio by adding 40% (NH4)2SO4 and measuring the length of the precipitate as an estimation of total immunoglobulins present in the serum.
[4]Colostrum immunoglobulin concentration was estimated (n=140) by using Brix refractometer between 1-8 h post-farrowing of the first pig of the litter.

Table 2.8 Pearson correlation coefficients for colostrum quality parameters of sows (n=40).

	IgG	Immunocrit ratio	Brix value[1]
IgA[2]	0.479*	0.512**	0.326*
IgG		0.587**	0.585**
Immunocrit ratio[3]			0.482**

[1]Colostrum immunoglobulin concentration was estimated by using Brix refractometer between 1-8 h post-farrowing of the first pig of the litter.
[2]Immunoglobulins (IgG and IgA) were analyzed using a commercially available ELISA kit (Eagle Bioscience, Inc., Nashua, NH)
[3] Serum obtained from two piglets/litter was analyzed for immunocrit ratio by adding 40% (NH4)2SO4 and measuring the length of the precipitate as an estimation of total immunoglobulins present in the serum.
*$P < 0.05$.
**$P < 0.01$.

Table 2.9 Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sows (n=40) subsequent follicle growth dynamic.

Item[3]	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Follicle size d 2, mm	4.9	4.5	4.6	4.8	0.28			
Follicle size d 3, mm	4.9	5.6	5.3	5.4	0.28			
Follicle size d 4, mm	6.1	6.3	6.2	6.0	0.28	0.321	0.353	0.986
Follicle size d 5, mm	6.7	7.0	7.0	6.7	0.28			
Follicle size pre ovulation, mm	6.8	7.0	7.2	7.2	0.28			
Average daily follicle growth, mm[4]	0.28	0.41	0.40	0.38	0.057	0.052	0.088	0.963
Wean to ovulation, days	6.6	6.1	6.4	6.2	0.23	0.130	0.069	0.233
Estrus to ovulation, hours	38.5	37.4	31.3	24.5	4.67	0.113	0.784	0.354
Wean to estrus interval, days	4.9	4.4	5.3	4.9	0.17	0.435	0.006	<0.001

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
[3]Follicle growth was evaluated by transrectal ultrasonography 2 after weaning until ovulation was confirmed. Twice a day (0700 and 1900) the diameter of a minimum of three follicles were measured and recorded to calculate the average size. Ovulation was confirmed when no preovulatory follicles were identified.
[4]Calculated as the difference between size prior to ovulation and size at d 2 divided by wean to ovulation days.

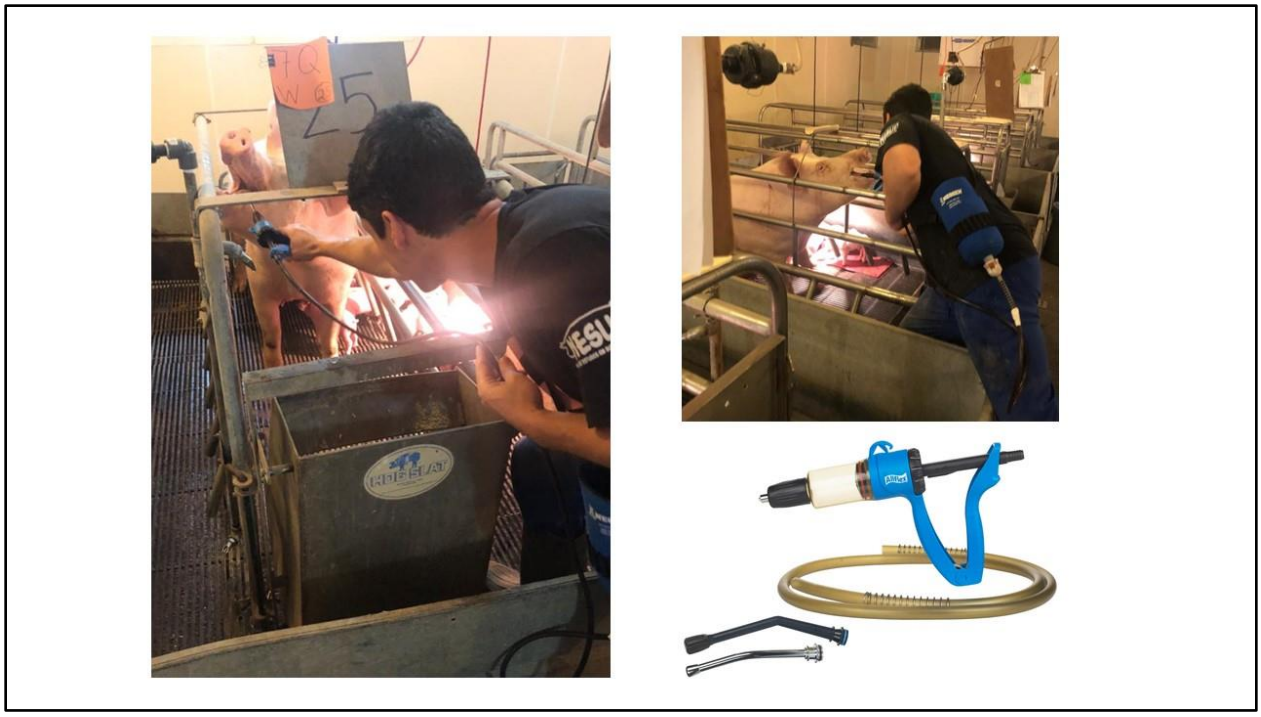

Figure 2.1. The liquid prototype of *Saccharomyces cerevisiae* fermentation product was given once daily using an oral dose gun (Allflex model 30EM-SDP, Kenilworth, NJ, U.S.A).

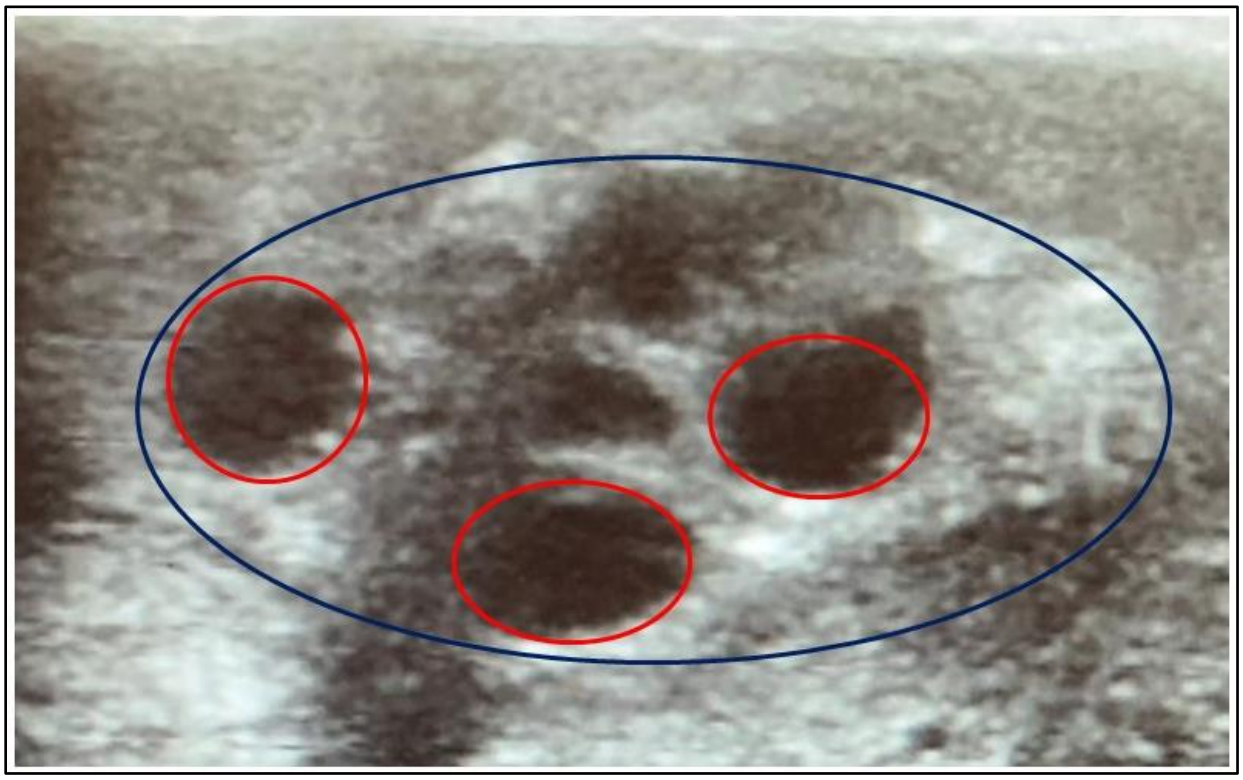

Figure 2.2. Left ovary indicated by the blue circle. Three pre-ovulatory follicles (7.4 mm average size) indicated with red circles. Thin-walled follicles are full of follicular fluid producing an anechoic black image on ultrasonography.

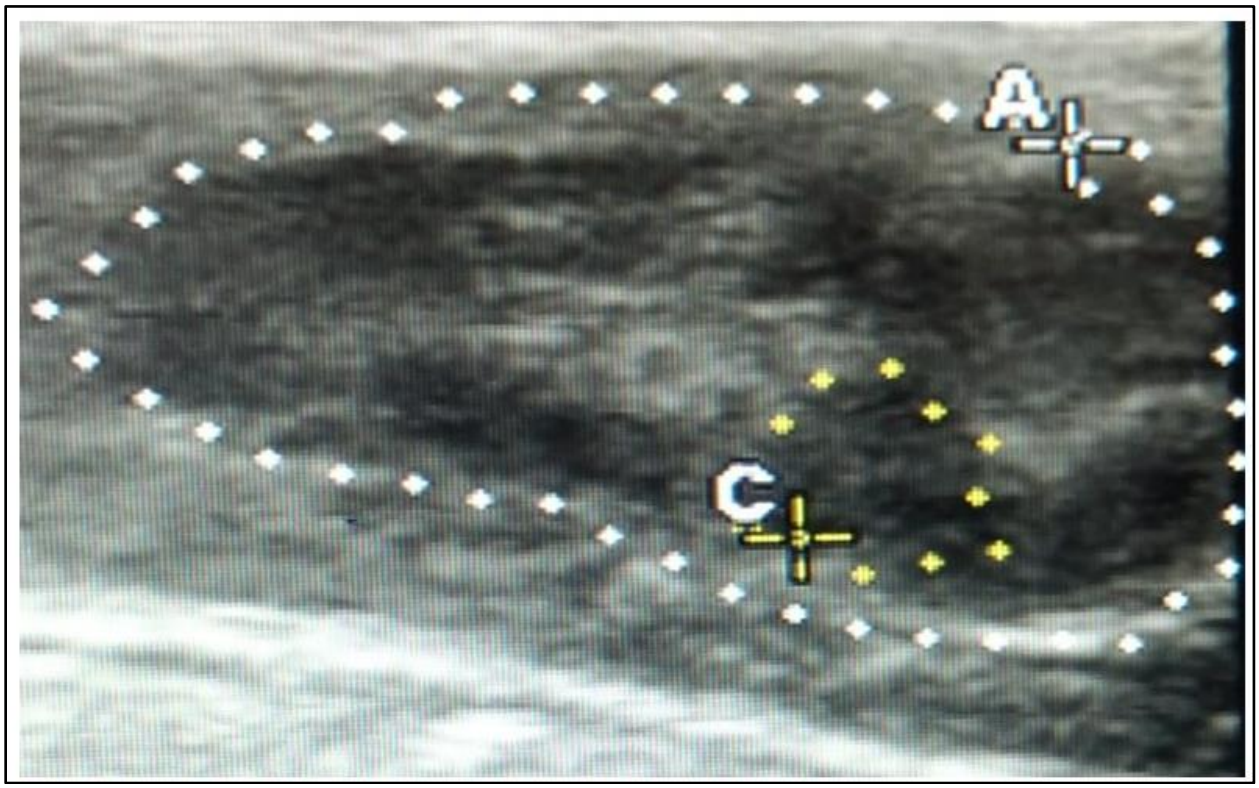

Figure 2.3. Left ovary indicated by the dash circle (A). Hemorrhagic corpus luteum indicated by the yellow dash circle (C). After ovulation, follicles are filled with blood producing an echoic grey scale image on ultrasonography.

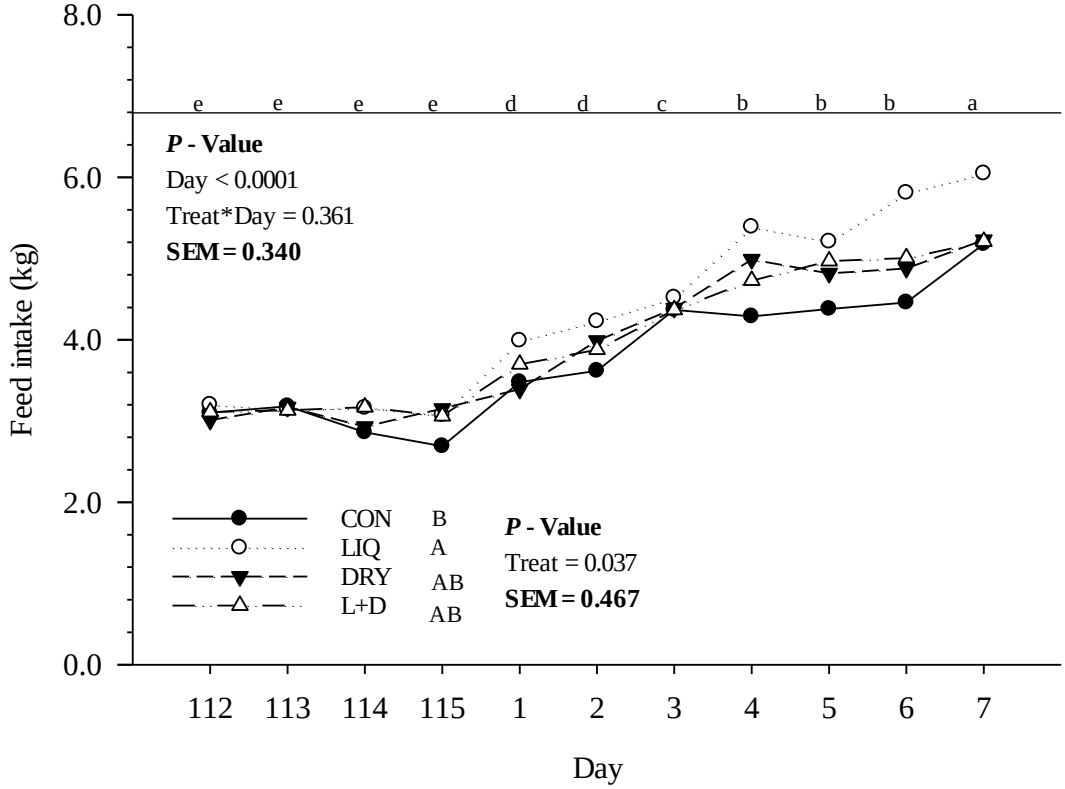

Figure 2.4. Daily feed intake (kg) from day 112 of gestation until day 7 of lactation. Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of *Saccharomyces cerevisiae* fermentation product (SCFP) orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

[a, b, c, d, e] In the top line, daily means with different letters differ by Tukey test ($P < 0.05$).

[A, B, C] Next to the treatment legend, means with different letters differ by Tukey test ($P < 0.05$).

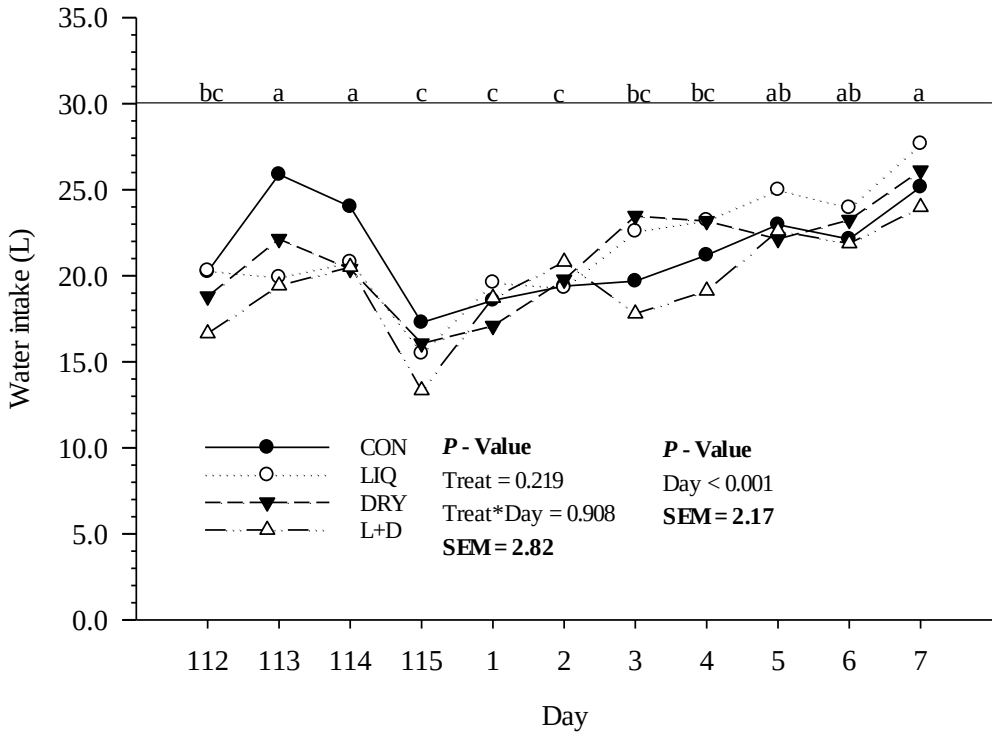

Figure 2.5. Water intake (L) from day 112 of gestation until day 7 of lactation. Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version liquid version of *Saccharomyces cerevisiae* fermentation product (SCFP) orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

[a, b, c] In the top line, daily means with different letters differ by Tukey test ($P < 0.05$).

CHAPTER 3. UTERINE FLUSHING AS A NON-INVASIVE METHOD TO ACCESS INFLAMMATORY CYTOKINES IN SOWS REPRODUCTIVE TRACT AFTER PARTURITION

3.1 Abstract

The objective of this experiment was to evaluate a non-invasive method, not tested before in swine, to assess cytokine profiles from post-partum uterine lavage. The uteri of second and third parity sows (n=14) were flushed with sterile saline solution (0.9%) on days 2, 4, and 14 post-parturition. An artificial insemination (AI) catheter, in its plastic covering, to avoid vaginal contents, was inserted through the vagina into the cervix. A flexible inner catheter was passed through the outer AI catheter and into the uterine horn. Sterile saline solution (30-40 mL) was flushed through the inner catheter into one uterine horn. After inner catheter removal, a tube was attached to the end of the outer AI catheter to allow collection of the natural back-flow of uterine lavage fluid. Uterine fluid collected was immediately centrifuged and the supernatant stored at -20°C. Samples were freeze-dried, re-suspended in sterile saline (2 mL), and stored at -80°C. Cytokine profiles of the uterine fluid were evaluated using a multiplex ELISA panel including interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Cytokine concentrations were calculated relative to protein content (pg/mg of protein). IFN-γ and TNF-α were lower than the limit of detection in most samples (33/38 and 37/38, respectively). IL-4 and IL-10 concentrations did not differ among days of collection ($P>0.14$). IL-8 was greater on day 4 than on days 2 or 14 ($P<0.05$). IL-1β and IL-6 were greater on days 2 and 4 than on day 14 ($P<0.05$). Uterine flushing is a novel non-invasive method to access uterine inflammatory cytokines in sows.

3.2 Introduction

From conception to weaning, many physiological and metabolic changes as well as immunological adaptations take place in modern sow's reproductive tract (Mor et al., 2017). Pregnant sows in mid and late gestation have a great level of oxidative stress indicated by greater concentrations of reactive oxygen species (ROS) while decreasing levels of antioxidants in the serum, and this condition does not rebalance until weaning (Berchieri-Ronchi et al., 2011). Parturition might be the most stressful event in the cycle, like other events such as ovulation and embryo implantation, it leads to an inflammatory process. Right after farrowing, healthy sows can resolve the uterine inflammatory status quickly and return to homeostasis (Jabbour et al, 2009). However, the sterile conditions in the uterus during pregnancy is disrupted at the time of parturition, especially in commercial conditions where interventions are frequent, increasing the chances of infections and welfare issues (Ison et al., 2016).

Uterine involution after farrowing in healthy sows will depend on hormonal shifts with a great amount of prostaglandins being secreted in the uterus and estrogen by the ovaries leading to uterine contractions, repairment, and debris elimination. It is also necessary to have an efficient cellular and humoral immunological response including cytokines secretion, phagocytic activity, and lymphocytes and macrophages migration to the mucosa and lumen, respectively (Jainudeen and Hafez, 2000). This process lasts about two to three weeks depending on the degree of postpartum contamination, placental and piglet retention, and estrogen secretion. In the first week, uterus weight reduces in 65% in healthy sows (Palmer et al., 1965) but it could be delayed if sows had prolonged farrowing, manual palpation, impaired placenta expulsion, and stillborn piglets (Björkman et al., 2018).

The inflammatory process in the uterus during puerperium was considered a passive event due to the decreased expression of inflammatory mediators over time. Yet, other studies had shown

that the inflammatory response to events after parturition are very specific to the re-establishment of homeostasis in the uterine mucosa (Jabbour et al, 2009). Therefore, properly recovering after parturition is crucial to sows to return to homeostasis and to have an adequate subsequent reproductive performance. We hypothesized that evaluating uterine lavage from puerperium sows could be a novel non-invasive methodology to access inflammatory status following parturition. The objective of this experimental pilot was to develop a method to collect and analyze the uterine lavage of sows in the puerperium.

3.3 Materials and Methods

3.3.1 General

The experimental procedures were approved by the Purdue University Animal Care and Use Committee (protocol #1909001949). In general, animal care followed the Guide for Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Sciences Societies, 2010) and individual therapeutic medication treatments were given and recorded when sows exhibited clinical signs of illness following the Purdue Veterinary Care SOP. The experiment was performed in the Animal Sciences Research and Education Center (ASREC) at Purdue University, West Lafayette, IN 47906, U.S.A.

3.3.2 Animals

Second and third parity sows (Landrace x Yorkshire; n=14) were selected three days before the estimated date of farrowing. Selection criteria to be assigned to the trial were body condition, absence of lameness, lesions, and any illness symptoms. Sows were fed a common gestation diet until d 111 of pregnancy and then were moved to the farrowing pens and fed a common lactation diet (Table 3.1). Farrowing stalls were 0.5 x 2.1 m with a stainless steel feeder and stainless steel

water drinker. All sows were allowed to eat 3.1 kg/day upon entry until farrowing and *ad libitum* after farrowing until weaning, with additional feed provided three times per day (0700, 1200, and 1600 h). Sows farrowed naturally, were monitored, and assistance were given and recorded when the birth interval exceeded 40 by palpation of the birth canal, extraction of piglets and/or use of oxytocin. The uteri of selected sows were flushed with sterile saline solution on days 2, 4, and 14 post-parturition.

3.3.3 Uterus flushing procedure

3.3.3.1 Preparation

Before collection, material and supplies were prepared. Commercially available artificial insemination (AI) catheters (SafeBlue, MiniTube, Verona, WI, U.S.A) individually covered with a plastic bag were sterilized. Sterile saline solution (0.9%) was prepared in the laboratory and storage in 50 mL plastic bottles (Fig. 3.1). Latex and powder free gloves were used to connect a conical 15 mL tube to the AI catheter using zip tights (Fig. 3.2).

3.3.3.2 Animal handling

The perianal aera and vulva were initially cleaned and dried. Then, the AI catheter in its plastic covering covered with ultrasound lube was inserted through the vagina into the cervix. The plastic covering was kept in order to avoid any vaginal content contamination. Once in the cervix, the plastic covering was pulled off and discarded. Next, the flexible inner catheter was passed through the outer AI catheter and into the uterine horn, like a normal post-cervix artificial insemination procedure (Fig. 3.3).

3.3.3.3 Uterine flushing and lavage collection

Sterile saline solution (30-40 mL) was flushed through the inner catheter into one uterine horn. After inner catheter removal, a tube was attached to the end of the outer AI catheter to allow collection of the natural back-flow of uterine lavage fluid (Fig. 3.3). The amount of saline solution flushed into the uteri and the amount recovered through natural back-flow was recorded.

3.3.4 Laboratory analysis

Uterine fluid collected was immediately centrifuged (2000 x g for 5 min; E8 Centrifuge, LW Scientific Inc, Lawrenceville, GA, U.S.A) and the supernatant stored at -20°C. Samples were freeze-dried, re-suspended in sterile saline (2 mL), and stored at -80°C until analysis (Fig. 3.4). Samples were analyzed in the Cytokine Reference Laboratory (CRL, University of Minnesota), a CLIA'88 licensed facility (l#24D0931212). Samples were analyzed for total protein content following method described by Bradford (Bradford, 1976). Briefly, a bovine serum albumin standard curve was added to a 96 well microtiter plate. Then, uterine fluid samples were added to each well followed by the Bradford reagent (B6916; Millipore Sigma, Burlington, MA, U.S.A). The plate was gently mixed and read using a BioTek Epoch (BioTek Instruments, Winooski, VT, U.S.A) plate reader at 605 nm. Protein content of the samples were interpolated using a log-log fit standard curve.

The uterine lavage fluid was analyzed for interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) using a porcine cytokine/chemokine panel on the Luminex platform and done as a multi-plex using a magnetic bead set (cat. #PCYTMAG-23K-06; Lot # 3313832; EMD Millipore, St. Charles, MO, U.S.A).

Samples were assayed according to manufacturer's instructions. Fluorescent color-coded beads coated with a specific capture antibody were added to each sample. After incubation, and

washing, biotinylated detection antibody was added followed by phycoerythrin-conjugated streptavidin. The beads were read on a Luminex instrument (Bio-Plex 200; Bio-Rad Laboratories, Inc. Hercules, CA, U.S.A) which is a dual-laser based instrument. One laser determines the analyte being detected via the color coding while the other measures the magnitude of the phycoerythrin signal from the detection antibody which is proportional to the amount of analyte bound to the bead. Samples were analyzed in duplicate, and values were interpolated from 5-parameter fitted standard curves. The lower limit of detection for each analyte were: 42 pg/mL for IL-1β, 20 pg/mL for IL-4, 9 pg/mL for IL-6, 5 pg/mL for IL-8, 9 pg/mL for IL-10, 6 pg/mL for TNF-α, 42 pg/mL for IFN-γ, and 0.08 mg/mL for protein content.

3.3.5 Statistical Analysis

Data were tested for normality using Shapiro-Wilk test before analysis, and any variable that failed to follow a normal distribution a Log10 transformation was applied in SAS (9.4 SAS Inst. Inc., Cary, NC). The sow was considered the experimental unit for all variables. Data were analyzed as repeated measurements using the PROC MIXED procedure in SAS. Day of sampling was considered as the main fixed effect and the sow as a random effect. Cytokine concentrations in the uterine fluid were calculated relative to the total protein content in each sample to standardize the samples based on the success of collection. No significant effect of parity or effects of farrowing manual intervention were found. Therefore, both were removed from the final model. All data are reported as least squares means with standard errors of the means. Means were considered significantly different when $P \leq 0.05$ and trends were discussed when $0.05 < P \leq 0.10$. The Tukey-Kramer adjusted means separation procedure was used.

3.4 Results

Overall, the procedure was successfully performed in all sows. Total born alive from selected sows ranged from 9 to 16 (average 11) and stillborn ranged from 0 to 2 (average 0.4). The farrowing duration ranged from 150 to 488 minutes (average 293). Seven out of fourteen of the selected sows needed farrowing assistance and were sleeved.

3.4.1 Uterine lavage recovery and protein content

Results for total recovery of uterine lavage and total protein content are shown in Table 3.2. The total recovery of uterine lavage after flushing on days 2 (28.95%) and 4 (34.82%) were greater than day 14 (11.17%; $P = 0.0101$). Similarly, the total protein content in individual samples after freeze-dried linearly decreased over time ($P = 0006$). Protein content on day 2 was 9.88 mg, 5.07 mg on day 4, and 1.81 mg on day 14 after parturition.

3.4.2 Cytokines

Results for cytokines concentrations in the uterine fluid are shown in Table 3.3. Means for IFN-γ and TNF-α are not reported as most of the samples (33/38 and 37/38, respectively) were lower than the limit of detection. The IL-4 ($P = 0.368$) and IL-10 ($P = 0.147$) concentrations did not differ among days of collection. Concentrations of IL-1β were greater on days 2 (2345 pg/mg of protein) and 4 (748 pg/mg of protein) than on day 14 (167 pg/mg of protein; $P < 0.001$). Similar, concentrations of IL-6 were greater on days 2 (71 pg/mg of protein) and 4 (30 pg/mg of protein) than on day 14 (17 pg/mg of protein; $P < 0.001$). However, concentrations of IL-8 was greater on day 4 (6178 pg/mg of protein) than on days 2 (3747 pg/mg of protein) or 14 (1716 pg/mg of protein; $P = 0.005$).

3.5 Discussion

The literature regarding uterine inflammation derives from researcher's efforts aiming to understand the inflammatory response to different types of procedures or compounds utilized at artificial insemination as well as the process of maternal recognition in early pregnancy to optimize embryo survival. Yamaguchi et al. (2012) added caffeine to thawing solution and founded that it could inhibit migration of polymorphonuclear (PMN) leukocytes due to downregulation of IL-8 mRNA expression in the endometrium. O'Leary et al. (2004) showed that factors in seminal plasma can program uterine cytokine expression and leukocytes infiltration, regulating pre-implantation embryo development in swine. In both studies, gilts were euthanized for tissue harvest and endometrium evaluated, just like many others evaluating seminal plasma effects on uterine inflammation (Bischof et al., 1994; Hadjisavas et al., 1994; Engelhardt et al., 1997). Indeed, the method described in this thesis chapter, have not been reported in the literature yet. The present experimental pilot consists of non-invasive method to collect uterine fluid in puerperium sows. Considering the reproductive cycle of modern sows having large litters, the parturition might be the most challenging period, involving a great immunological response in the uterus.

The most similar procedure in the literature to evaluate inflammation in the uterus in live animals was described by Rozeboom et al. (1998). They used a similar procedure to the one presented in this chapter to collect the uterine lavage from gilts and sows in estrus to evaluate bacteriological cultures and PMN present in the pellets formed after centrifugation. There was an extensive influx of PMNs into the uterus as a normal consequence to AI (Rozeboom et al., 1998) and components present in the seminal plasma can ameliorate the PMN migration into the uterus following insemination (Rozeboom et al., 1999; Rozeboom et al., 2000). Uterine fluids and secretions have been also collected and analyzed in other species. Gilbert et al. (2005) flushed the uterus of dairy cows to evaluate the presence of inflammatory cells during the post-partum period

for endometritis diagnosis. Boomsma et al. (2009), aspirated endometrial secretions from women prior to embryo transfer undergoing assisted reproduction techniques to evaluate biomarkers of inflammation. Differently, the method described in this chapter was performed in puerperium sows. The plastic covering was used to avoid vaginal contents contamination, supernatant was evaluated instead of the pellets, and a standardization of the material collected was applied by evaluation of the protein content in individual samples.

Each cytokine are known to have a single biological function in the organism, with few exceptions, and many of them have overlapping functions (redundancy), meaning that one single biomarker may not be enough to describe or understand patterns of immune response (Dembic, 2015). Therefore, the cytokine panel chosen in the present study represents a balance between pro-inflammatory (IL-1β, IL-6, IL-8, TNF-α, IFN-ϒ) and anti-inflammatory (IL-4, IL-6, IL-10) cytokines, aiming to understand uterine type of immune response, regulation, and changes over time. Among the major groups of cytokines, those that mainly promote inflammation cascade are considered pro-inflammatory mediators and includes IL-1β, IL-6, IL-8, TNF-α, chemokines, and interferons families. On the other hand, anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1ra), IL-4, IL-6, IL-10, IL-11, and IL-13 are produced to inhibit the process of inflammation by regulating pro-inflammatory cytokines responses (BOSHTAM et al., 2016). Pro-inflammatory cytokines analyzed were generally in greater concentrations on day 2 post-partum and decreasing up to day 14 while anti-inflammatory cytokines did not differ over time. Taken together, these results clearly indicate an inflammatory process in the uterus right after parturition. These findings, set the stage for further investigation regarding strategies to modulate immune response in perinatal sows.

The approach used to analyze inflammatory mediators successfully quantified the analytes in the uterine fluid collected, with exceptions for IFN-γ and TNF-α. Likewise, Boomsma et al. (2009), used a multiplex immunoassay to evaluate a panel of inflammatory mediators present in endometrium secretions prior to embryo transfer of women undergoing *in vitro* fertilization procedures. The cytokine multiplex analysis uses a dual laser to sort and detect analytes through specific antibodies precoated onto color-coded microparticles. Streptavidin-phycoerythrin conjugate binds to the biotinylated detected antibodies, after washing conjugates are resuspended for detection and quantification utilizing a Luminex analyzer. One of the laser specific for the microparticles to identify the analyte detected while the second laser determines the magnitude of the signal directly related to the amount of analyte bound (Luminex). There are some advantages that can be highlighted by using the multiplex approach such as the ability to measure many analytes simultaneously and it requires a small sample volume. That does not apply to other bioassays like ELISA and PCR which measures one analyte at a time and does not detect native protein, respectively (Vignali, 2000).

The total fluid recovered after flushing as well as the total protein content decreased from day 2 post-partum to day 14. That might be an indication of a properly completed uterine involution process occurring by day 14. The uterine involution directly depends on the degree of contamination, placental retention, farrowing duration, manual palpation, and stillborn rate (Palmer et al., 1965; Björkman et al., 2018). The small sample size chosen to run this pilot experiment study, may explain the lack of differences between groups of sows that received or did not receive assistance during farrowing. Yet, the differences on cytokine concentrations over time contributed to understanding the patterns of immune response in the uterus after parturition. The absence of IFN-γ and TNF-α indicated that the sensitivity of the analysis performed was not

enough to detect these analytes and/or a different dilution or processing of the sample was necessary. Therefore, the method described in this chapter can be used to measure the effects of any intervention, nutritional strategy, and management procedure around parturition aiming to improve farrowing efficiency, to modulate immune response, and/or to enhance sows health.

3.6 Conclusion

In conclusion, the experimental procedure developed to access cytokines concentration in the uterine lavage of puerperium sows, successfully achieved the goal of being a novel non-invasive method to be used in swine. The procedure described in this chapter can be used in scientific research to understand uterine immunological response around parturition. Yet, further investigations on the relationship of uterine biomarkers and systemic immune response are still needed.

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

Item	Lactation
Ingredient, %	
Corn	50.303
Soybean meal, 47.5% CP	32.600
Dried distillers grain with solubles	10.000
Swine grease	3.000
Limestone	1.430
Monocalcium phosphate	1.350
Vitamin premix[2]	0.250
Sow premix[3]	0.250
Trace mineral premix[4]	0.125
Se premix[5]	0.050
Phytase[6]	0.100
Salt	0.500
Availa Zn 120[7]	0.042
Total	100.00
Calculated analysis	
ME, Kcal/kg	3353.30
Crude Protein, %	22.33
Total Lysine, %	1.183
SID[7] Lys, %	1.001
SID Met, %	0.311
SID Met+Cys, %	0.615
SID Thr, %	0.693
SID Tryp, %	0.233
SID Iso, %	0.809
SID Val, %	0.889
Calcium, %	0.902
Total P., %	0.732
ATTD P, %	0.501

[1]Diets were fed from day 112 of gestation until weaning.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44.1 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.1 mg and B12 38.6 µg.
[3]Provided per kg of diet: biotin, 0.22 mg; folic acid, 1.65 mg; choline, 16 mg; pyridoxine, 4.96 mg; vitamin E, 22 IU; chromium, 0.20 mg; and carnitine, 49.6 mg.
[4]Provided per kg of diet: Fe, 121.3 mg; Zn, 121.2 mg; Mn, 15.0 mg; Cu, 11.3 mg; and I, 0.46 mg
[5]Provided 0.3 ppm Se.
[6]Phyzyme® (Danisco Animal Nutrition, Morlborough, UK) providing 600 phytase units (FTU)/kg.
[7]Provided per kg of diet: 50 ppm of zinc.
[8]Stndardized ileal-digestible.

Table 3.2 Uterine lavage recovery and total protein content.

Item	Post-parturition day			SEM	*P*-Value
	2	4	14		
Recovery, %[1]	29.0[a]	34.8[a]	11.2[b]	6.00	0.0101
Protein content, mg[2]	9.9[a]	5.1[b]	1.8[c]	1.73	0.0006

[a,b,c]Within a row, means with different letters differ by Tukey test ($P < 0.05$). SEM: Standard error for the mean of non-transformed data.

[1]The saline solution (30-40 mL) flushed in uteri and the amount collected though natural back-flow were recorded to calculate total recovery.

[2]The protein content in each 2 mL sample was evaluated following Bradford methodology.

Table 3.3 Cytokines profile in uterine flushing of sows (n=14) in post-parturition period.

Cytokine[1], pg/mg of protein	**Post-parturition day**			**SEM**	***P*-Value**
	2	4	14		
IL-1β	2345[a]	748[a]	167[b]	626.8	<0.001
IL-4	23	22	21	13.9	0.368
IL-6	71[a]	30[a]	17[b]	14.5	<0.001
IL-8	3747[b]	6178[a]	1716[b]	1364.5	0.005
IL-10	12	10	9	5.7	0.147
IFN-γ	<LLOQ	<LLOQ	<LLOQ	-	-
TNF-α	<LLOQ	<LLOQ	<LLOQ	-	-

[a,b]Within a row, means with different letters differ by Tukey test (P < 0.05). SEM: Standard error for the mean of non-transformed data. P-Values for transformed data (log10).

[1]IL: Interleukin; INF: interferon; TNF: tumor necrosis factor. LLOQ: Lower limit of quantification (pg/mL), IL-1β (42), IL-4 (20), IL-6 (9), IL-8 (5), IL-10 (9), IFN-γ (42), and TNF-α (6).

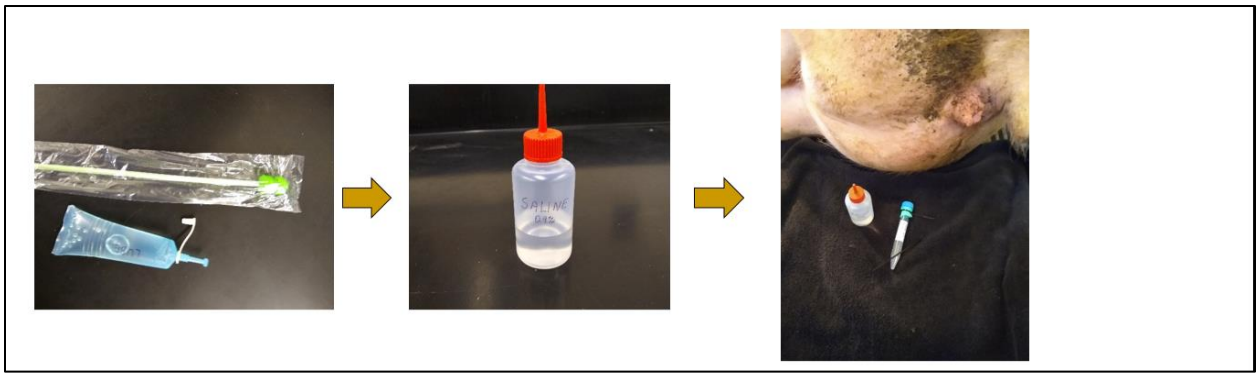

Figure 3.1 Commercially available artificial insemination (AI) catheters individually covered with a plastic bag were sterilized. Sterile saline solution (0.9%) was prepared in the laboratory and storage in 50 mL plastic bottles. Before inserted in the vagina, the AI catheter was lubrified with ultrasound lube.

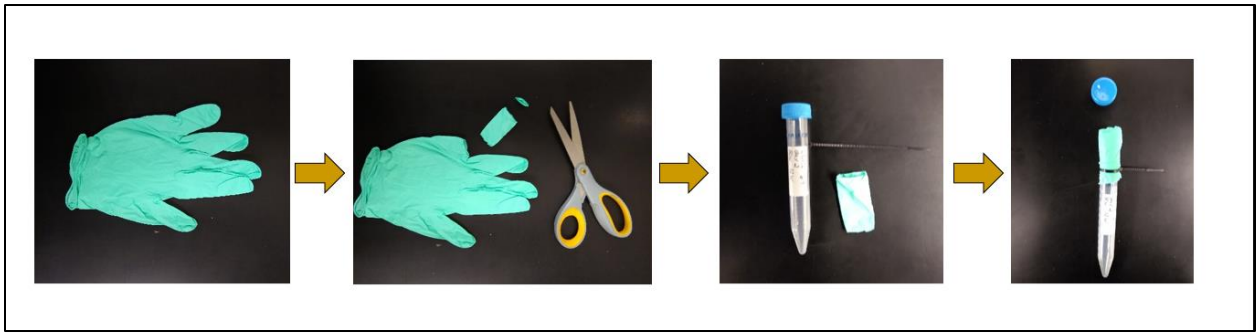

Figure 3.2 Latex and powder free gloves were used to connect a conical 15 mL tube to the AI catheter using plastic zip ties. Tubes were previously identified with sow's tag, pen number, and day of collection. After glove attached, tubes were kept in the refrigerator until the collection.

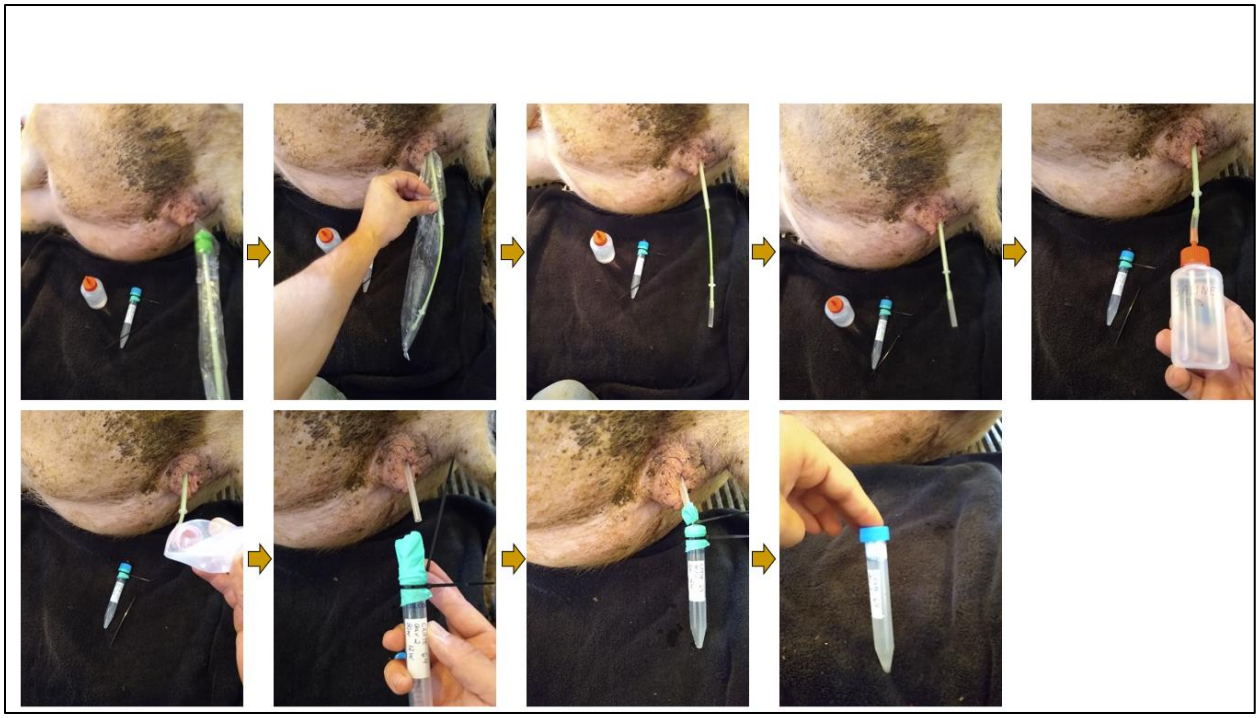

Figure 3.3 The AI catheter in its plastic covering was inserted through the vagina into the cervix. The plastic covering was kept to avoid any vaginal contents contamination. Once in the cervix, the plastic covering was pulled off and discarded. Next, the flexible inner catheter was passed through the outer AI catheter and into the uterine horn like a normal post-cervix artificial insemination procedure. Sterile saline solution (30-40 mL) was flushed through the inner catheter into one uterine horn. After inner catheter removal, a tube was attached to the end of the outer AI catheter to allow collection of the natural back-flow of uterine lavage fluid.

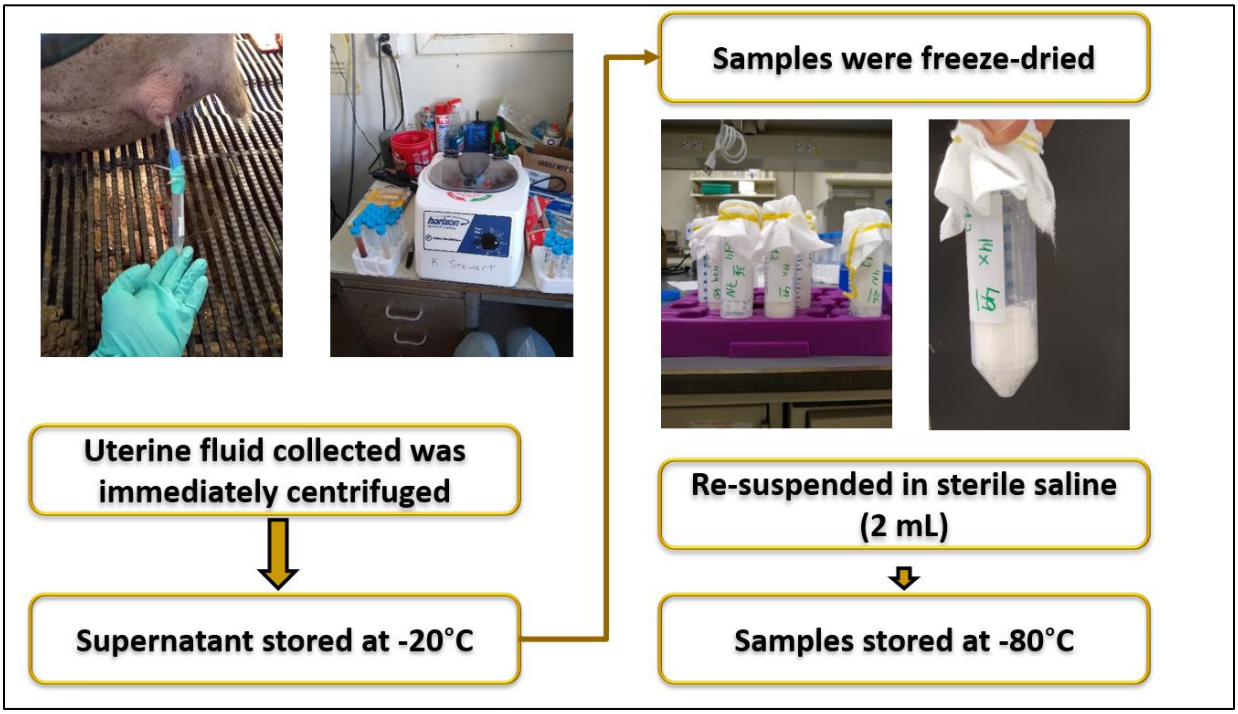

Figure 3.4 Uterine fluid collected was immediately centrifuged (2000 x g for 5 min; E8 Centrifuge, LW Scientific Inc, Lawrenceville, GA, U.S.A) and the supernatant stored at -20°C. Samples were freeze-dried and the dried content was re-suspended in sterile saline (2 mL), and stored at -80°C until analysis.

CHAPTER 4. IMMUNOMODULATORY EFFECTS OF SUPPLEMENTAL YEAST FERMENTATION PRODUCTS ON LACTATING SOWS

4.1 Abstract

This study evaluated the effects of a liquid prototype (LIQP) and dry (XPC®; Diamond V) *Saccharomyces cerevisiae* feed additives on blood and uterine cytokine profiles in sows (n=40). On d 112 of pregnancy sows were allotted to dietary treatments: 1) Control diet (CON), 2) CON + 15 mL/d of LIQP (LIQ), 3) CON + 0.20% of XPC (DRY), and 4) DRY + 15 mL/d of LIQP until d 7 post-farrowing (D+L). Interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were quantified from serum samples collected on d 112 of gestation, d 2 and 6 post-farrowing, and from uterine fluid collected on d 2, 4, and 6 post-farrowing. Serum C-Reactive protein (CRP) and haptoglobin concentrations were also evaluated. No interactions between treatments and day of collection were observed ($P>0.13$). LIQ and D+L sows had the greatest serum IL-10 concentration ($P<0.001$) and sows fed CON tended to have lower concentration of IL-8 ($P<0.06$) vs. other treatments. Serum CRP concentrations were greatest on d 2 ($P<0.001$), serum IL-10 ($P<0.04$) and IL-4 ($P<0.07$) linearly decreased while serum haptoglobin ($P<0.02$) and IFN-γ ($P<0.001$) linearly increased post-farrowing. In the uterine fluid, LIQ and D+L sows had greater IFN-γ ($P=0.04$) concentrations and CON tended to have the least concentration of TNF-α ($P=0.08$). Uterine fluid IL-1 tended to linearly increase ($P<0.07$), and IL-6 linearly decrease ($P<0.01$) post-farrowing. Serum pro-inflammatory cytokines on d 6 were positively correlated with uterine pro-inflammatory cytokines and negatively correlated with uterine anti-inflammatory cytokines on d 2. LIQ sows had the greatest daily feed intake and CON the least during the first week of lactation ($P=0.04$). Providing LIQP pre and post-farrowing to

sows modified immune response increasing both pro- and anti-inflammatory cytokines in serum and uterine fluid, allowing animals a quicker recovery and increased feed intake.

4.2 Introduction

Porcine immune system has been the target of studies in many different areas like vaccination response to viral infections (Balasch et al., 2019; Wu et al., 2021; Zhuo et al., 2021), in-utero heat stress effects on offspring (Johnson et a., 2020), acute heat stress in finishing pigs (Kpodo et al., 2021), post-weaning disorders (Bissonnette et al., 2016), and intestinal barrier function (Celi et al., 2019). All these research studies have in common the evaluation of cytokines profiles as biomarkers of inflammation due to different types of pathogenic or non-pathogenic stressors in pigs. Moreover, acute phase proteins have been applied in many types of studies to evaluate acute phase reaction due to different conditions such as tail biting (Heinonen et al., 2010), health and well-fare status (Hennig-Pauka et al., 2019), effects of weaning (Segalés et al., 2004), and transportation (Salamano et al., 2008).

Cytokines are non-structure molecules (polypeptides, glycoproteins, and proteins) that function as cell-to-cell messengers with immunomodulator effects through multiple biological (pleiotropism) functions in the organism (Dinarello, 2007; Dembic, 2015). Among cytokines, those which promote inflammation cascade are considered as pro-inflammatory mediators including interleukins (IL)-1β, IL-6, IL-8, TNF-α, chemokine, and interferon (IFN) families. On the other hand, anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1ra), IL-4, IL-6, IL-10, IL-11, and IL-13 are produced to inhibit the process of inflammation by regulating pro-inflammatory cytokines responses (Boshtam et al., 2016).

Cytokines act mainly in the microenvironment that they were secreted and increased level of these molecules in the serum are likely to be related to a spillover from sites of inflammation

and infection into the blood (Dembic, 2015). Indeed, many of inflammatory process in swine are related to local inflammation like the mucosa of the gastro-intestinal tract, the respiratory tract, and the reproductive tract. We hypothesized that immune response in the uterus of sows after parturition can be reflected by systemic inflammation and the overall outcomes of lactation performance depend on the degree of post-partum inflammation and recovery. Therefore, the objectives of this study were to evaluate and describe the cytokines profiles in the serum and uterus of puerperium sows and to evaluate the immunomodulatory effects of *Saccharomyces cerevisiae* fermentation product (SCFP) fed to sows.

4.3 Materials and Methods

4.3.1 General

Experimental procedures and animal handling were approved by the Purdue University Animal Care and Use Committee (protocol #1909001949). In general, animal care followed the Guide for Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Sciences Societies, 2010) and individual therapeutic medication treatments were given and recorded when sows or piglets exhibited clinical signs of illness following the Purdue Veterinary Care SOP. The experiment was performed in the Animal Sciences Research and Education Center (ASREC) at Purdue University, West Lafayette, IN 47906, U.S.A. The two SCFP tested were provided by Diamond V (Cedar rapids, IA, U.S.A) and contain a variety of compounds from the fermentation processes such as mannan-oligosaccharides, β-glucans, yeast metabolites, yeast cell wall fragments, as well as the media utilized in the fermentation. One of the sources is a liquid SCFP prototype source (LIQP) and the second one is a commercially available SCFP dry source (XPC®; Diamond V).

4.3.2 Animals and treatments

Forty pregnant sows (Landrace x Yorkshire; BW 253.5 ±12.2 kg; parity 3.0 ±0.62) were blocked by parity and body condition score approximately on day 109 of gestation and allotted to one of four treatments: 1) Control diet (corn-soybean meal basal diet; CON), 2) CON + 15 mL/d of LIQP from d 112 to weaning (LIQ), 3) CON + 0.20% of XPC from d 112 to weaning (DRY), and 4) DRY + 15 mL/d of LIQP from d 112 to d 7 post-farrowing (L+D). The LIQP was given once daily using an oral dose gun (Allflex model 30EM-SDP, Kenilworth, NJ, U.S.A) and XPC was included in the feed. Sows were fed a common gestation diet until d 109 of pregnancy and then were moved to the farrowing barn and fed a common lactation diet (CON) until day 112 of pregnancy when dietary treatments were fed (Table 4.1). Farrowing stalls were 0.5 x 2.1 m with one stainless steel feeder and stainless steel water drinker. The farrowing pens were 1.52 x 2.64 m and the creep space for the piglets was 0.46 m wide on each side of the sow stalls. All sows were allowed to eat 3.1 kg/day upon entry until farrowing and ad libitum after farrowing until weaning with feed additions occurring three times per day (0700, 1200, and 1600 h). Feed left over was weighed every morning before the first meal to calculate daily feed intake. Each crate was equipped with a water meter (Assured Automation, model WM-Pd-050, Roselle, NJ, U.S.A) and daily water intake (DWI) until d 7 of lactation and weekly average DWI (ADWI) were calculated.

4.3.3 Animals handling and measurements

Sows were weighed on day 1, day 7 of lactation, and on weaning day using a calibrated scale (IQ plus 355-2A; 227 g precision; Rice Lake, WI, U.S.A). Backfat (BF) and loin depth (LD) were evaluated on day 112 of gestation and on weaning day using ultrasound (Aloka SSD 500V, Aloka Co., Ltd., Tokyo, Japan). The BF and LD measurements were performed parallel to the spine from the last rib forward, 6.5 cm away from the vertebral column (P2 point) on each side to

calculate the means. Sows farrowed either naturally or were induced on day 115 of gestation (2 cc prostaglandin; Lutalyse®, Zoetis Inc., Parsippany-Troy Hills, NJ, U.S.A). Farrowing was monitored and assistance were given when birth interval exceeded 40 min. The reproductive performance was evaluated as the number of total piglets born, piglets born alive, stillborn piglets, and mummified fetuses.

Pigs were weighed on day 1 (before cross-fostering) using a calibrated scale (RL-DBS-2; 5 g precision; Rice Lake, WI, U.S.A), on day 7 and weaning day using a calibrated scale (IQ plus 390-DC; 45 g precision; Rice Lake, WI, U.S.A) to calculate individual pig and litters ADG. After 24 h of birth and up to 48 h, litters were equalized within the same treatments according to the number of functional teats. Piglet processing was performed within the first 24 h which encompass receiving supplemental iron injection (1 cc, Uniferon 200, Pharmacosmos A/S, Holbaek, Denmark), ear notching, tail docking, teeth clipping, and castration for males. All pigs were weaned on the same day (18.1 ±0.30 days of age).

4.3.4 Blood collection

Sows had blood samples (n = 40) collected from a jugular vein on day 112 of gestation, on day 2 and 6 of lactation in tubes for serum biomarkers of inflammation analysis. Blood samples were allowed to clot stored in refrigerators (5°C) for as much time as was needed to collect samples from all sows. Then in the same order blood was collected, serum was further separated by centrifugation (E8 Centrifuge, LW Scientific Inc, Lawrenceville, GA, U.S.A) at 4°C and 2500 rpm for 15 min, aliquoted and stored at -80°C until shipment to the Cytokine Reference Laboratory (CRL, University of Minnesota).

4.3.5 Uterine fluid collection

The uterine fluid collection followed method described in Chapter 2. Briefly, the uteri were flushed with sterile saline solution (0.9%) on days 2, 4, and 6 post-parturition. An artificial insemination (AI) catheter was inserted through the vagina into the cervix. A flexible inner catheter was passed through the outer AI catheter and into the uterine horn and sterile saline was flushed into one uterine horn. After the inner catheter was removed a tube was attached to the end of the outer AI catheter to allow collection of the natural back-flow of uterine lavage fluid. Uterine fluid collected was immediately centrifuged (2000 x g for 5 min; E8 Centrifuge, LW Scientific Inc, Lawrenceville, GA, U.S.A) and the supernatant stored at -20°C. Samples were freeze-dried, re-suspended in sterile saline (1 mL), and stored at -80°C until shipment to the Cytokine Reference Laboratory (CRL, University of Minnesota). Two modifications from the method described in Chapter 2 were applied. The third day of collection was sooner on day 6 post-partum rather than day 14 and freeze-dried uterine fluid samples were re-suspended in 1 mL sterile saline instead of 2 mL.

4.3.6 Laboratory analysis

Cytokine profiles in the uterine fluid and serum from sows around parturition were evaluated using a multiplex ELISA panel including interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) using a porcine cytokine/chemokine panel on the Luminex platform and done as a multi-plex using a magnetic bead set (cat. #PCYTMAG-23K-06; Lot # 3313832; EMD Millipore, St. Charles, MO, U.S.A). Uterine fluid samples were analyzed for total protein content following the method described by Bradford (Bradford, 1976). Cytokine profiles in uterine samples were calculated relative to protein content (pg/mg of protein).

Samples were assayed according to manufacturer's instructions. Fluorescent color-coded beads coated with a specific capture antibody were added to each sample. After incubation, and washing, biotinylated detection antibody was added followed by phycoerythrin-conjugated streptavidin. The beads were read on a Luminex instrument (Bio-Plex 200; Bio-Rad Laboratories, Inc. Hercules, CA, U.S.A) which is a dual-laser fluidics based instrument. One laser determines the analyte being detected via the color coding while the other measures the magnitude of the phycoerythrin signal from the detection antibody which is proportional to the amount of analyte bound to the bead. Samples were analyzed in duplicate, and values were interpolated from 5-parameter fitted standard curves. The lower limit of detection for each analyte were: 42 pg/mL for IL-1β, 9 pg/mL for IL-6, 5 pg/mL for IL-8, 20 pg/mL for IL-4, 9 pg/mL for IL-10, 6 pg/mL for TNF-α, 42 pg/mL for IFN-γ, and 0.08 mg/mL for protein content.

Additionally, the acute phase proteins haptoglobin and C-reactive protein (CRP) were analyzed in the serum samples. Samples were analyzed using porcine specific commercially available ELISA kits (Eagle Biosciences, Nashua, NH, U.S.A). Samples were assayed according to manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. The absorbance was measured on the microtiter plate reader (EPOCH BioTek Instruments, Inc, Winooski, VT, U.S.A) and the intensity of the color formed was used to calculate the analyte concentration of the sample. Samples were run in duplicate, and values were interpolated from 4-parameter fitted standard curves. The lower limit of detection for these analytes were 0.1 mcg/mL for CRP and 3.1 ng/mL for haptoglobin.

4.3.7 Statistical Analysis

All variables measured were tested for normality using Shapiro-Wilk test before analysis, and any variable that failed to follow a normal distribution was transformed through the RANK

procedure in SAS (9.4 SAS Inst. Inc., Cary, NC). The sow and its litter were considered the experimental unit for the lactation performance and data were analyzed using the PROC MIXED procedure in SAS. For performance data, pre-planned orthogonal contrasts were used to compare the different sources of SCFP since one of them is a prototype. The daily water and feed intake in the first week of lactation were analyzed as repeated measurements.

Biomarkers of inflammation were analyzed as repeated measurements with dietary treatments and day of collection as main effects using PROC MIXED procedure in SAS. Parity and total number of pigs born were tested as covariates, if significant, were kept in the final model. For each analyte, samples lower than the limit of quantification were considered missing values. Marginal correlations between day of collection, uterine cytokines, and serum cytokines were analyzed as Pearson correlation coefficients using the CORR procedure in SAS. Variables were considered highly correlated if correlation coefficient were greater or equal to 0.7, moderately correlated between 0.4 and 0.7, and not correlated if lower than 0.4.

All data are reported as least squares means with standard errors of the means. Means were considered significantly different when $P \leq 0.05$ and trends are discussed when $0.05 < P \leq 0.10$. The Tukey-Kramer adjusted means separation procedure was used.

4.4 Results

4.4.1 Sow body weight and tissue mobilization

Sows had similar BW ($P > 0.69$), backfat (BF; $P > 0.64$), and loin depth (LD; $P > 0.30$) at the beginning of the study. On day 7 of lactation, BW and BW change until d 7 did not differ among treatments ($P > 0.20$; Table 4.2). The sow BW at weaning did not differ among treatments ($P > 16$) but sows from LIQ group tended to have less BW losses (-2.2%) throughout the lactation

period compared to CON (-5.9%; P = 0.09) and similar to DRY (-3.8%; P = 0.45). No difference among treatments were observed in the final BF thickness and LD (P > 0.65) as well as in the overall change in both tissues (P > 0.14).

4.4.2 Sow feed and water intake

As expected, no differences were observed in ADFI before parturition as sows were restricted fed (P > 0.16). The ADFI in the first week of lactation of sows fed SCFP treatments did not differ from CON (P = 0.50; Table 4.3) but LIQ sows tended to have greater ADFI than CON (P = 0.06) and DRY (P = 0.07). No differences among treatments were observed on ADFI in the second week of lactation (P > 0.13). From d 15 until weaning, SCFP treatments had greater ADFI than CON (P < 0.01), LIQ sows had greater ADFI than CON (P < 0.01) and DRY (P = 0.02). The overall lactation ADFI was greater for sows fed LIQ compared to CON (P <0.01) and DRY (P = 0.04) but SCFP treatments did not differ from CON (P = 0.12). Sows fed LIQ tended to have greater DWI than DRY (P = 0.08) on day 114 of gestation and greater DWI than DRY in the first week of lactation (P = 0.02). In the first week of lactation, sows fed LIQ tended to have greater DWI than CON (P < 0.10). No treatment effects were observed for DWI in any other period evaluated (Table 4.4; P > 0.22).

4.4.3 Reproductive performance

The number of total born, born alive, stillborn, and mummified fetus, did not differ from SCFP treatments and CON (P > 0.74; Table 4.5). Sows given the LIQ treatment had fewer mummies than the DRY treatment (P < 0.01). The number of pigs sows were allowed to nurse after cross-fostering (P = 0.04) and litter weight on d 1 (P = 0.05) was greater for CON sows but the number of weaned pigs per sow did not differ among treatments (P > 0.70). Total litter weights

on d 7 and at weaning, as well as the total litter gain did not differ among treatments (P > 0.13). Litter ADG and individual pig weights in any period evaluated were not influenced by dietary treatments (P > 0.36). The proportion of pigs weaned lighter than 3.5 kg from sows fed SCFP treatments (P = 0.03) or sows fed LIQ (P = 0.05) were lower than CON. The body weight coefficients of variation within the litters on d 1 and the overall change from d 1 until weaning were not influenced by treatments (P > 0.12). At weaning, litters from sows fed SCFP treatments tended to have lower variation than CON (P = 0.06) and litters from LIQ sows were more uniform than CON (P = 0.02). The pre-weaning mortality rates in the first week as well as the overall lactation period were not affected by treatments (P > 0.20).

4.4.4 Serum biomarkers of immune response

No interactions of the main effects of day of collection and treatment were detected for any immune response biomarker in the serum (Table 4.6; P > 0.10). C-reactive protein had the least concentration prior to farrowing, the greatest on day 2 post-farrowing and returned to an intermediate level on day 6 post-farrowing (Fig 4.1A; P < 0.001). No difference among treatments were detected for CRP concentration in the serum (Fig 4.1B; P = 0.644). Haptoglobin linearly increased from prior to farrowing to day 6 post-farrowing (Fig 4.2A; P = 0.018) but did not differ among dietary treatments (Fig 4.2B; P = 0.481). Serum IFN-ϒ prior to farrowing did not differ from day 2 post-farrowing but had the greatest concentration on day 6 post-farrowing (Fig 4.3A; P < 0.001) but did not differ among dietary treatments (Fig 4.3B; P = 0.253). The concentration of TNF-α in most of the samples (76.7%) was lower than the limit of quantification using the method described previously. The day of collection did not influence IL-1β (Fig 4.4A; P = 0.628) and IL-6 (Fig 4.5A; P = 0.345) serum concentrations. Also, dietary treatments did not affect IL-1β (Fig 4.4B; P = 0.565) and IL-6 (Fig 4.5B; P = 0.519) concentrations in the serum. Serum IL-8 was not

affected by day of collection (Fig 4.6A; P = 0.662), but SCFP treatments tended to have greater concentration than CON (Fig 4.6B; P = 0.058). Concentration of IL-4 tended to linearly decrease from prior to farrowing to day 6 post-farrowing (Fig 4.7A; P = 0.069) and was not influenced by dietary treatments (Fig 4.7B; P = 0.116). Serum IL-10 concentration prior to farrowing was similar to day 2 post-farrowing and decreased on day 6 (Fig 4.8A; P = 0.038). Sows that were fed both LIQ and L+D had greater IL-10 concentration than sows fed the CON and DRY regardless of the day of collection (Fig 4.8B; P = 0.010).

4.4.5 Uterine biomarkers of immune response

No interactions of the main effects of day of collection and treatment were detected for any immune response biomarker in the uterine fluid (P > 0.10). The protein content in the uterine fluid after freeze-drying and re-suspending in saline solution was greater on day 2 post-farrowing and decreased on days 4 and 6 (Fig 4.9A; P = 0.007) but did not differ among dietary treatments (Fig 4.9B; P = 0.607). Concentrations of IFN-ϒ was not influenced by the day of collection (Fig 4.10A; P = 0.188). Sows that were fed LIQ and L+D had greater IFN-ϒ concentration than sows fed CON and DRY regardless of the day of collection (Fig 4.10B; P = 0.040). No difference was observed in TNF-α concentrations among days of collection (Fig 4.11A; P = 0.185). Sows fed LIQ tended to have greater TNF-α concentration than CON with DRY and L+D being intermediate (Fig 4.11B; P = 0.070). The concentration of IL-1β tended to be greater on day 6 post-farrowing but did not differ between day 2 and day 4 (Fig 4.12A; P = 0.061). Dietary treatments did not affect IL-1β concentration in the uterine fluid (Fig 4.12B; P = 0.479). Concentration of IL-6 did not differ between day 2 and day 4 pos-farrowing but significantly decreased on day 6 (Fig 4.13A; P = 0.001) and was not affected by dietary treatments (Fig 4.13B; P = 0.780). The day of collection did not influence IL-8 (Fig 4.14A; P = 0.518), IL-4 (Fig 4.15A; P = 0.442), and IL-10 (Fig 4.16A; P =

0.849) concentrations in the uterine fluid. Concentrations of IL-8 (Fig 4.14B; P = 0.376), IL-4 (Fig 4.15B; P = 0.461), and IL-10 (Fig 4.16B; P = 0.531) were not affect by dietary treatments.

4.4.6 Correlation between serum and uterine biomarkers

Pearson correlation coefficients and P-values from cytokines concentrations on days 2 and 6 post-farrowing in the uterine fluid and serum of lactating sows are shown in Table 4.8 and Table 4.9, respectively. Variables were considered highly correlated if correlation coefficient were greater or equal to 0.7, moderately correlated between 0.4 and 0.7, and not correlated if lower than 0.4.

4.4.6.1 Correlations between cytokines in the serum and in the uterus on day 2 post-farrowing

On day 2 post-partum, serum IL-6 was strongly correlated with uterine IL-8 (r = 0.84; P = 0.02). Serum IL-4 had a moderate correlation with uterine IL-10 (r = 0.53; P = 0.06). Serum IL-8 was correlated with uterine IL-1β (r = 0.52; P = 0.07) and IL-8 (r = 0.51; P = 0.09) while a weak negative correlation was found with IL-4 (r = -0.35; P < 0.10). Serum IL-10 was negatively correlated uterine IL-4 (r = -0.46; P = 0.01) while positively correlated with IL-1β (r = 0.46; P < 0.10), IL-6 (r = 0.57; P = 0.01), and IL-8 (r = 0.64; P = 0.01). Serum IFN-ϒ was correlated with IL-1β (r = 0.66; P = 0.02) while a weak negative correlation with IL-10 in the uterus was detected (r = -0.13; P = 0.01). TNF-α in the serum was strongly correlated to uterine IL-1β (r = 0.96; P < 0.01), moderately correlated with uterine IL-6 (r = 0.50; P = 0.03), IL-8 (r = 0.56; P = 0.04), and negatively correlated to IL-4 (r = -0.33; P = 0.09).

4.4.6.2 Correlations between day 2 post-farrowing serum cytokines and day 6 post-farrowing uterus cytokines

Day 2 post-partum serum IL-1β was strongly correlated with day 6 uterine IFN-ϒ (r = 0.70; P = 0.05). Serum IL-4 was correlated with uterine IL-1β (r = 0.69; P = 0.07) and strongly correlated with IL-6 (r = 0.81; P = 0.01). Serum IL-6 was correlated with uterine IL-4 (r = 0.69; P = 0.01) and strongly correlated with IL-6 (r = 0.85; P = 0.01). Serum IL-10 was correlated with uterine IL-6 (r = 0.58; P = 0.04). Serum IFN-ϒ was correlated with uterine IL-4 (r = 0.46; P = 0.02), IL-8 (r = 0.51; P < 0.10), and IFN-ϒ (r = 0.49; P = 0.06). TNF-α in the serum was strongly correlated to uterine IL-6 (r = 0.80; P < 0.01) and correlated with uterine IFN-ϒ (r = 0.62; P < 0.01).

4.4.6.3 Correlations between day 6 post-farrowing serum cytokines and day 2 post-farrowing uterus cytokines

Day 6 post-partum serum IL-1β was strongly correlated with day 2 uterine IL-6 (r = 0.70; P < 0.01), IL-8 (r = 0.98; P < 0.01), IFN-ϒ (r = 0.97; P < 0.01) and correlated with IL-1β (r = 0.69; P = 0.02). A weak correlation was observed between serum IL-4 and uterine IL-10 (r = 0.35; P = 0.09). Serum IL-6 was correlated with uterine IL-8 (r = 0.66; P = 0.02) and IFN-ϒ (r = 0.67; P = 0.01). Serum IL-10 was weakly negative correlated to uterine IL-4 (r = -0.37; P = 0.06) while positively correlated with IL-1β (r = 0.69; P = 0.01), IL-6 (r = 0.60; P = 0.01), IL-8 (r = 0.63; P = 0.02), and IFN-ϒ (r = 0.48; P = 0.09). IFN-ϒ in the serum was strongly correlated to uterine IL-6 (r = 0.99; P < 0.01) and IFN-ϒ (r = 0.93; P < 0.01) and correlated with uterine IL-1β (r = 0.69; P = 0.01) and IL-8 (r = 0.65; P = 0.01). Serum TNF-α was negatively correlated with uterine IL-4 (r = -0.42; P = 0.01) while positively correlated with IL-1β (r = 0.53; P = 0.05), IL-6 (r = 0.60; P = 0.01), and IL-8 (r = 0.59; P = 0.02).

4.4.6.4 Correlations between cytokines in the serum and in the uterus on day 6 post-farrowing

On day 6 post-partum, serum IL-1β was strongly correlated with uterine IL-6 ($r = 0.75$; $P < 0.01$) and weakly correlated with IL-10 ($r = 0.39$; $P < 0.05$). Serum IL-4 was correlated with uterine IFN-ϒ ($r = 0.46$; $P = 0.09$) and weakly correlated with IL-1β ($r = 0.34$; $P = 0.10$). A moderate correlation was observed between serum IL-8 and uterine IFN-ϒ ($r = 0.42$; $P = 0.09$). Serum IL-10 was correlated with uterine IL-6 ($r = 0.51$; $P = 0.07$) and IFN-ϒ ($r = 0.49$; $P = 0.06$). TNF-α in the serum was correlated with IL-6 ($r = 0.61$; $P = 0.03$) and IFN-ϒ ($r = 0.43$; $P = 0.07$).

4.5 Discussion

The group of sows used in this study as a subsample of the study described in chapter 2 and showed a very similar response to the dietary treatments. Sows fed the liquid prototype of SCFP had less body weight loss than the other treatments, reflecting their greater feed intake in the first week of lactation and overall. Different than the entire group of sows evaluated, in this study litter performance was not affected by dietary treatments although the numerical differences followed the same responses observed in chapter 2, indicating that the sample size was crucial to detect the performance differences. The modification in the method to evaluate uterine cytokines profiles in puerperium sows, described in chapter 3, resulted in some improvements in uterine cytokines discovery. Changing from d 14 to 6 post-partum allowed a better understanding of the acute phase reaction after farrowing, as it is discussed next. Resuspending freeze-dried samples in 1 mL of saline solution instead of 2 mL, resulted in a greater number of samples within the range of detection.

Serum concentrations of IL-1β on day 6 post-partum and TNF-α on days 2 and 6 post-partum were positively correlated with all pro-inflammatory cytokines in the uterine fluid on day

2 post-partum. Therefore, these two pro-inflammatory cytokines in the serum can be considered good indicators of degree of uterine inflammation right after parturition. Given the fact that uterine IL-1β, IFN-ϒ, and TNF-α increased from day 2 to day 6 post-partum, it is reasonable to infer that a lower concentration of IL-1β, IFN-ϒ, and TNF-α in the serum after parturition will equate to lower concentrations of major pro-inflammatory cytokines in the uterus right after parturition. That is important because these cytokines can exert adverse effects when circulating in other systems other than where they were secreted. Peripheral cytokines can reach and have effects in the central nervous system through a variety of mechanisms such as passive transport via the blood-brain barrier. The anorexigenic effects of pro-inflammatory cytokines are part of the acute phase response during inflammation (Johnson, 1998) which can lead the sows to reduce feed intake.

IL-4 pro-inflammatory functions are related to its ability to stimulate Th2 while inhibiting Th1 and Th17 immune responses as well as through direct suppression of IL-1β and TNF-α secretion (Dembic, 2015). Serum concentrations of TNF-α on days 2 and 6 post-partum were negatively correlated to IL-4 concentrations in uterus on day 2 post-partum. Considering that serum IL-4 and IL-10 decreased from prior to farrowing until day 6 post-partum and that uterine IL-1β, IFN-ϒ, and TNF-α increased from day 2 to day 6 post-partum, it clearly indicates a shift from Th2 type of immune response to Th1 after farrowing. Interesting, serum concentrations of IL-10 on days 2 and 6 post-partum were also negatively correlated to IL-4 concentrations in uterus on day 2 post-partum. IL-4 and IL-10 have different but complementary functions to keep Th2 immune response predominant during gestation, which is important to the establishment and maintenance of pregnancy (Chatterjee et al., 2014). Yet, one type of the IL-10 secreting cells are B cells in the later stages of activation (Dembic, 2015), meaning that IL-4 might be suppressed as early acute phase reactions take place. In this study, SCFP had immunomodulatory effects on IL-

4 and IL-10 concentrations. Regardless of the day, IL-4 was numerically greater in the serum and in the uterus while IL-10 was significantly greater in the serum and numerically greater in the uterus of sows fed LIQ. Although no interaction between day of collection and treatment were detected, it can be inferred that even though the type of immune response predominant after farrowing was similar among treatments, sows fed LIQ had greater concentrations of anti-inflammatory molecules in both tissues, allowing them to have a quicker recovery post-partum and return to homeostasis faster.

Immune modulation can be defined as a range of effects that stimulates or suppresses the immune system to achieve control, stabilization, and potential elimination of inflammatory processes and diseases (Naidoo et al., 2014). In this study, additionally to LIQ modulation on anti-inflammatory cytokines aforementioned, pro-inflammatory cytokines were also influenced by the SCFP. Despite the increase of uterine TNF-α and IFN-ϒ on LIQ sows, serum IL-1β, IL-6 and IFN-ϒ did not differ among treatments. It can be speculated then, the activation of induction and effectors sites of immune response in the mucosa were stimulated to resolve post-partum uterine inflammation with minimum systemic effects. IL-6 and IL-8, the other two pro-inflammatory cytokines, followed different behaviors. Serum IL-8 increased in the blood of sows fed SCFP regardless of the day of evaluation and did not differ in the uterus. IL-8 can be quickly secreted at sites of inflammation to recruit and to activate cells, maintaining stability at the site of acute inflammation, like mucosa, even for several days (Remick, 2005). To the best of our knowledge, there is no reference in the literature for cytokines concentrations in the uterine fluid of sows post-partum. Yet, the immunomodulatory effect of SCFP in the uterus seems to affect the chemotaxis processes despite the day of evaluation. On the other hand, IL-6 did not show any clear pattern of response due to farrowing and dietary treatments other than few correlations with other cytokines.

IL- 6 is a major representative of cytokines with pleiotropic effects on inflammation, immune response, and hematopoiesis (Tanaka et al., 2014). Therefore, among the biomarkers evaluated in the different tissues and days, IL-6 seems to be the least promising molecule to understand systemic and local immune response in puerperium sows. Although, it was demonstrated in gilts that IL-6 have an important role in early phase of pregnancy during embryo implantation (Chabot et al., 2004).

Along with pro-inflammatory cytokines like serum IFN-ϒ, the shift in the type of immune response after farrowing from Th1 to Th2 up to day 6 post-partum was also confirmed by the linear increase on serum concentration of acute phase proteins. Haptoglobin and CRP are non-specific biomarkers of acute phase reaction (Hennig-Pauka et al., 2019) but have clear effects during inflammation such as fever (Jain et al., 2011). In addition to their systemic effects possibly resulting in reduced appetite because of fever and neutrophilia, acute phase proteins might have negative effects on litter growth. Hiss-Pesch et al. (2011) showed that haptoglobin can pass to the offspring via colostrum and its concentration was negatively correlated to litter growth. These findings corroborate with the concept that greater the degree of inflammation after farrowing, the greater are the impacts on sow's health and litter performance. Moreover, acute phase proteins did not differ among the dietary treatments used in this study despite the day of evaluation. It can be inferred that SCFP fed to sows were not able to directly modulate acute phase proteins as they are non-specific biomarkers. Additionally, the acute phase protein serum amyloid A has been demonstrated to be useful on the diagnosis and prediction of milk production disorders in sows due to poor health condition and other inflammatory causes before parturition (Wierzchosławskiet al., 2018). Conversely, serum haptoglobin and CRP were not correlated to rectal and vaginal

temperatures and to post-partum disorders in sows (Stiehler et al., 2016) indicating that selected acute phase proteins might have different purposes in diagnosis for swine.

The effects of SCFP demonstrated in this study are likely to be related to its major components direct effects in the microbiota and intestinal immune system. One of the major components well known to have immunomodulatory effects are the mannooligosaccharides (MOS) present in the cell wall of *Saccharomyces cerevisiae*. Yet, it must be considered that there are characteristics of yeast products derived from the cells wall that confer different effects like the types of linkages between mannans polymers, the mannan structure, the polymerization degree, and the distribution and proportion between MOS and β-glucan, another major component in the yeast cell wall (Che et al., 2012). Duan et al. (2016) fed MOS to sows and their offspring and observed that regardless of the route of supplementation, through the sows or directly to the offspring, piglets from MOS treatments had greater growth performance possibly because of improvements in innate humoral components status. Zanello et al., (2012) demonstrated that feeding live yeast to sows creates a stimulation of the intestinal mucosa immune system resulting on greater concentration of IgG and IgA in their colostrum and milk, respectively.

Antioxidant effects of MOS and other soluble components commonly present in SCFP are well described in the literature (Kogan et al., 2005; Yang et al., 2016; Yao et al., 2014). Within the mucosal-associated lymphoid tissues, MOS and β-glucan can stimulate macrophage activity (Djeraba and Quere, 2000; Davis et al., 2004) increasing the level of mucosal Th1 cytokines like IFN-ϒ but not influencing its blood circulating levels, as observed in young pigs (Shen et al., 2009). That is an indication that SCFP can stimulate innate non-specific response in the mucosa level, through phagocytic activity for instance, while not altering the type of systemic immune response. In this study, this pattern of immunomodulatory effects of SCFP was observed in the uterus of

puerperium sows. Linked to their direct effects on the immune system, SCFP also modulates the immune system indirectly by modulating the microbiome of pigs through enhancement of beneficial fermentative bacterial populations in the gut (Hasan et al., 2018). Mechanistically, MOS can attach to pathogens in the intestinal lumen preventing them to adhere and translocate in the mucosa barrier, thus it decreases pathogen colonization (Liu et al., 2008). In this study, the effects of SCFP on the gut microbiome of sows was not evaluated. Modulating the microbiome, the following effects are likely to be related to the production of short-fatty acids and other metabolites involved in energy metabolism of sows (Liu et al., 2020).

Besides the influence of feed additives on the regulation of immune response in pigs, the body condition of sows plays an important role in inflammation. Zhou et al. (2019) have studied the effects of late gestation backfat on placental antioxidant parameters and pro-inflammatory cytokines. It was demonstrated that greater is the backfat the lower was the antioxidant capacity and greater were the mRNA expression of toll-like receptors (TLR) 2, TLR4, TNF- α, IL-1β, and IL-6 while IL-10 and IL-8 did not differ. This low-grade inflammatory process during late gestation due to obesity (or body condition) has also been reported in sheep (Zhu et al., 2010), dairy cows (Liang e al., 2020), and in women (Huang et al., 2014). In this study, sows had similar body weight and dietary treatments were balanced by body condition score at late gestation as well as parity. Moreover, sows under heat stress conditions are susceptible to greater oxidative stress (Zhao and Kim, 2020), to have poor lactation performance (Vilas Boas Ribeiro et al., 2018), and to have the offspring immune response altered (Johnson et al., 2020). This study was conducted in the fall with environmental temperatures within the thermoneutral zone ranges for sows. Therefore, immunomodulatory effects observed are likely to mainly be related to the farrowing processes and dietary treatments. Clearly, the serum concentrations of cytokines on day 2 and 6 were more

correlated to the uterine cytokines profiles on day 2 compared to day 6. Therefore, inferences can be taken evaluating inflammation biomarkers either early in the uterine fluid or later in the serum, regardless of the effects of treatments. Indeed, the method applied here to evaluate uterine inflammation is much less invasive than the blood collection for serum evaluation. To our knowledge, this was the first study evaluating post-partum uterine inflammation in live sows.

4.6 Conclusion

Overall, the correlations between the cytokines evaluated in the uterus and in the serum showed a reasonable pattern following the type of immune response established and predominant after farrowing. The immunomodulatory effects expected from SCFP fed to sows were demonstrated systemically and locally, in the serum and uterine fluid respectively. Sows fed SCFP, especially LIQ, had pro- and anti-inflammatory cytokines concentrations increased and these profiles were successfully evaluated in the samples through the methods proposed. In conclusion, the farrowing process was marked by the shift of type of immune response towards inflammation. Sows fed SCFP build a more robust immune response to resolve faster the consequences of acute phase reaction allowing them to recovery from puerperium quicker and to improve the lactation performance.

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Table 4.1 Lactation diet composition (as-fed basis)[1]

Item	CON	LIQ	DRY	L+D
Ingredient, %				
Corn	50.303	50.303	50.103	50.103
Soybean meal, 47.5% CP	32.600	32.600	32.600	32.600
Dried distillers grain with solubles, 7% fat	10.000	10.000	10.000	10.000
Swine grease	3.000	3.000	3.000	3.000
Limestone	1.430	1.430	1.430	1.430
Monocalcium phosphate	1.350	1.350	1.350	1.350
Vitamin premix[2]	0.250	0.250	0.250	0.250
Sow premix[3]	0.250	0.250	0.250	0.250
Trace mineral premix[4]	0.125	0.125	0.125	0.125
Se premix[5]	0.050	0.050	0.050	0.050
Phytase[6]	0.100	0.100	0.100	0.100
Salt	0.500	0.500	0.500	0.500
Availa Zn 120[7]	0.042	0.042	0.042	0.042
XPC[8]	0.000	0.000	0.200	0.200
Total	100.00	100.00	100.00	100.00
Calculated analysis				
ME, Kcal/kg	3353.30	3353.30	3353.30	3353.30
Crude Protein, %	22.33	22.33	22.33	22.33
Total Lysine, %	1.183	1.183	1.183	1.183
SID[9] Lys, %	1.001	1.001	1.001	1.001
SID Met, %	0.311	0.311	0.311	0.311
SID Met+Cys, %	0.615	0.615	0.615	0.615
SID Thr, %	0.693	0.693	0.693	0.693
SID Tryp, %	0.233	0.233	0.233	0.233
SID Iso, %	0.809	0.809	0.809	0.809
SID Val, %	0.889	0.889	0.889	0.889
Calcium, %	0.902	0.902	0.902	0.902
Total P., %	0.732	0.732	0.732	0.732
ATTD P, %	0.501	0.501	0.501	0.501
Analyzed composition[10]				
Ash, %	5.71	5.71	6.17	6.17
Crude Protein, %	20.52	20.52	21.07	21.07
Crude Fiber, %	2.84	2.84	2.85	2.85
Crude Fat, %	5.12	5.12	5.58	5.58
NDF, %	9.31	9.31	9.42	9.42
ADF, %	4.56	4.56	4.87	4.87
Calcium, %	1.03	1.03	1.19	1.19

(Table continues)

Total P, %	0.665	0.665	0.716	0.716
Lysine, %	1.10	1.10	1.11	1.11
Methionine, %	0.29	0.29	0.31	0.31
Cysteine, %	0.31	0.31	0.33	0.33
Threonine, %	0.74	0.74	0.74	0.74
Tryptophan, %	0.25	0.25	0.26	0.26
Isoleucine, %	0.93	0.93	0.94	0.94
Leucine, %	1.81	1.81	1.82	1.82
Valine, %	1.06	1.06	1.06	1.06

[1]Diets were fed from day 112 of gestation until weaning.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44.1 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.1 mg and B12 38.6 µg.
[3]Provided per kg of diet: biotin, 0.22 mg; folic acid, 1.65 mg; choline, 16 mg; pyridoxine, 4.96 mg; vitamin E, 22 IU; chromium, 0.20 mg; and carnitine, 49.6 mg.
[4]Provided per kg of diet: Fe, 121.3 mg; Zn, 121.2 mg; Mn, 15.0 mg; Cu, 11.3 mg; and I, 0.46 mg
[5]Provided 0.3 ppm Se.
[6]Phyzyme® (Danisco Animal Nutrition, Morlborough, UK) providing 600 phytase units (FTU)/kg.
[7]Provided per kg of diet: 50 ppm of zinc.
[8]Saccaromyches cerevisiae fermentation product (Diamond V, Cedar Rapids, IA, U.SA).
[9]Standardized ileal-digestible
[10]Analysis conducted by University of Missouri Experiment Station Chemical Laboratories.

Table 4.2 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sow (n=40) body weight, backfat, and loin depth.

Performance	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Parity	3.3	2.8	3.1	2.9	0.62	0.591	0.625	0.876
Lactation length, days	18.2	17.7	18.3	18.0	0.37	0.552	0.272	0.253
Body weight (BW)								
Initial BW (d 1), kg	250.3	252.1	251.8	259.7	12.20	0.694	0.892	0.982
BW (d 7), kg	246.8	251.4	251.1	259.2	12.33	0.505	0.727	0.984
BW change until d 7, %	-1.46	-0.41	-0.27	-0.32	0.935	0.203	0.334	0.897
Final BW (weaning), kg	233.0	247.7	243.0	249.1	12.91	0.166	0.223	0.699
Overall BW change, %	-5.9	-2.2	-3.8	-4.7	1.76	0.187	0.088	0.445
Backfat (BF)								
Initial BF (d 112), mm	23.5	22.9	23.2	24.4	2.68	0.931	0.642	0.727
Final BF (weaning), mm	17.7	20.3	19.0	21.2	2.02	0.317	0.523	0.934
BF change, %	-21.7	-11.9	-18.5	-13.7	4.57	0.196	0.141	0.318
Loin depth (LD)								
Initial LD (d 112), mm	54.3	54.4	59.1	59.9	2.89	0.299	0.971	0.262
Final LD (weaning), mm	51.2	50.7	51.9	52.9	2.21	0.779	0.835	0.647
LD change, %	-7.6	-7.4	-9.7	-9.5	3.67	0.969	0.684	0.437

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 4.3 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on sow (n=40) gestation and lactation feed intake.

Feed Intake, kg	Treatments[1]				SEM	Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D		1	2	3
Gestation period								
d 112, kg	2.96	3.12	2.94	2.97	0.092	0.522	0.182	0.165
d 113, kg	3.36	3.32	3.45	3.35	0.205	0.691	0.951	0.464
d 114, kg	3.49	3.77	3.21	3.75	0.341	0.679	0.371	0.168
d 115 (farrowing day), kg	2.72	3.27	2.53	2.71	0.558	0.663	0.287	0.209
Lactation period								
d 1 – 7, kg	4.80	5.63	4.83	4.63	0.476	0.504	0.059	0.069
d 8 - 14, kg	6.01	6.73	6.03	5.91	0.520	0.580	0.136	0.147
d 15 - weaning, kg	5.67	7.63	6.45	6.56	0.478	0.004	<0.001	0.015
Overall, kg	5.40	6.49	5.65	5.55	0.453	0.117	0.008	0.038

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 4.4 Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sow (n=40) average daily water intake.

Water intake, L/day	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Gestation period								
d 114, L/day	22.20	29.07	16.88	14.96	4.322	0.439	0.585	0.079
d 115 (farrowing day), L/day	14.30	14.44	12.72	11.63	2.365	0.353	0.766	0.620
Lactation period								
d 1 - 7, L/day	21.58	28.12	20.27	20.37	3.676	0.748	0.097	0.023
d 8 - 14, L/day	31.08	35.76	35.38	25.55	4.107	0.951	0.291	0.425
d 15 - weaning, L/day	34.00	38.82	37.29	30.61	3.953	0.494	0.193	0.679
Overall, L/day	28.39	32.41	30.64	24.61	4.161	0.883	0.251	0.453

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.

Table 4.5 Effect of *Saccharomyces cerevisiae* fermentation product (SCFP) on lactating sow (n=40) and offspring performance.

Item	Treatments[1]					Contrast *P*-value[2]		
	CON	LIQ	DRY	L+D	SEM	1	2	3
Total born	13.2	12.4	14.4	11.6	1.17	0.978	0.836	0.266
Born alive	12.5	12.1	13.3	10.8	1.14	0.895	0.945	0.566
Still born, %	4.1	2.0	3.2	5.4	1.65	0.826	0.420	0.602
Mummies, %	1.4	0.0	4.9	0.7	1..16	0.740	0.316	0.002
Number after cross-fostering[3]	13.7	13.1	13.6	12.3	0.63	0.044	0.056	0.312
Weaned, n	10.6	10.2	10.5	10.3	0.48	0.931	0.722	0.701
Litter weight d 1, kg	21.0	20.7	19.5	16.5	0.91	0.054	0.831	0.331
Litter weight d 7, kg	30.3	30.5	28.7	25.4	1.81	0.258	0.946	0.416
Litter weight at weaning, kg	61.4	61.0	62.9	56.4	4.13	0.902	0.906	0.887
Litter gain, kg	44.0	46.1	46.0	47.4	1.61	0.131	0.291	0.942
Litter ADG d 1 -7, kg	1.88	1.86	1.78	1.58	0.138	0.369	0.926	0.674
Litter ADG d 8 -weaning, kg	2.87	2.92	3.10	2.84	0.195	0.813	0.784	0.754
Litter ADG, kg	2.48	2.47	2.58	2.34	0.166	0.796	0.995	0.748
Pig weight d 1, kg	1.54	1.62	1.47	1.37	0.075	0.519	0.457	0.135
Pig weight d 7, kg	2.75	2.87	2.70	2.43	0.130	0.583	0.519	0.361
Pig weight at weaning, kg	5.78	6.02	6.03	5.47	0.278	0.809	0.462	0.987
PBW[3] at weaning <3.5 kg, %	7.4	1.9	1.0	4.8	1.92	0.034	0.048	0.739
PBW CV d 1, %	21.2	16.6	21.7	23.1	1.98	0.719	0.148	0.120
PBW CV at weaning, %	21.4	13.4	15.6	20.3	2.19	0.059	0.015	0.497
PBW CV change d 1-weaning, %	0.2	-3.2	-5.9	-2.6	2.64	0.205	0.402	0.578
d 1 -7 mortality, %	13.3	8.4	13.4	9.7	3.66	0.460	0.258	0.203
d 1- weaning mortality, %	17.2	12.9	15.1	10.8	4.16	0.307	0.380	0.548

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[2]Pre-planned orthogonal contrasts were: 1) CON vs all SCFP treatments; 2) CON vs LIQ; and 3) LIQ vs DRY.
[3]The number of cross-foster pigs are greater than born alive because of the change in sow numbers and the need to cross-foster more piglets onto sows.
[4]PBW: pig body weight, CV: coefficient of variation.

Table 4.6 Effect of *Saccharomyces cerevisiae* fermentation product sources fed to lactating sow (n=40) on serum inflammatory response.

Item[2]	Treatments[1]													*P*-value		
	CON	LIQ	DRY	L+D	CON	LIQ	DRY	L+D	CON	LIQ	DRY	L+D	SEM	Treat	Day	Treat * Day
	Prior to farrowing				Day 2 post-farrowing				Day 6 post-farrowing							
CRP, mg/mL	159	97	84	112	318	324	342	363	251	229	188	203	56.4	0.644	<0.001	0.819
Haptoglobin, mg/mL	2072	2766	2927	2479	2821	2581	3035	2417	3462	2939	3337	2698	389.2	0.481	0.018	0.496
IFN-ϒ, pg/mL	17887	9805	6261	39245	19458	17778	9958	42909	36188	23501	13869	47630	11082	0.253	<0.001	0.138
IL-1β, pg/mL	1165	3051	517	2002	1048	2488	507	2098	1306	1804	579	1814	1021.5	0.565	0.628	0.958
IL-4, pg/mL	4570	12988	1342	6708	3573	9091	1310	7284	5096	7009	1290	6340	4068.6	0.128	0.069	0.857
IL-6, pg/mL	536	2854	236	1208	480	1536	219	1366	567	976	24	1133	1119.4	0.510	0.345	0.331
IL-8, pg/mL	62	107	86	133	76	108	128	131	81	106	132	120	61.8	0.058	0.662	0.217
IL-10, pg/mL	3062	9060	936	5844	2374	7624	842	6725	2970	4537	972	5519	3390.2	0.010	0.038	0.538

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

[2]Interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were evaluated using multiplex ELISA kits. C-reactive protein (CRP) and haptoglobin were evaluated individually using ELISA kits.

Table 4.7 Effect of *Saccharomyces cerevisiae* fermentation product sources fed to lactating sow (n=40) on uterine inflammatory response.

Item[2]	Treatments[1]													*P*-value		
	CON	LIQ	DRY	L+D	CON	LIQ	DRY	L+D	CON	LIQ	DRY	L+D	SEM	Treat	Day	Treat * Day
	Day 2 post-farrowing				Day 4 post-farrowing				Day 6 post-farrowing							
Protein, mg	25.8	19.9	28.4	30.3	16.0	14.3	24.6	14.2	18.2	13.9	12.6	13.4	5.09	0.607	0.007	0.808
IFN-ϒ, pg/mg of protein	21.2	366.1	29.7	318.8	176.5	994.8	96.5	476.9	229.3	573.3	40.3	432.1	517.59	0.040	0.188	0.888
TNF-α, pg/mg of protein	2.4	15.2	9.1	2.5	6.6	11.8	11.8	18.3	4.51	49.9	4.7	19.8	12.34	0.082	0.185	0.497
IL-1β, pg/mg of protein	292	235	867	652	377	588	435	651	1130	1001	1368	927.0	383.15	0.479	0.061	0.739
IL-4, pg/mg of protein	33.1	159.2	7.7	58.7	18.2	173.4	29.6	0.0	25.5	230.1	2.1	13.1	67.19	0.461	0.442	0.848
IL-6, pg/mg of protein	40.3	39.7	55.1	57.0	51.6	48.9	59.7	71.0	36.2	35.3	14.9	26.1	15.83	0.780	0.001	0.799
IL-8, pg/mg of protein	1406	937	1737	1159	1994	874	899	1116	2776	1599	3429	57.6	1056.6	0.376	0.518	0.919
IL-10, pg/mg of protein	24.0	60.2	14.3	31.5	16.8	79.9	14.3	9.1	25.2	113.0	8.1	8.3	27.72	0.531	0.849	0.889

[1]Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

[2]Interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were evaluated using multiplex ELISA kits.

Table 4.8 Pearson correlation coefficients of cytokines[1] concentrations on days 2 and 6 post-farrowing in the uterine fluid and serum of lactating sow (n=40).

		Cytokines concentrations in the serum on day 2 post-farrowing							Cytokines concentrations in the serum on day 6 post-farrowing						
		IL-1β	IL-4	IL-6	IL-8	IL-10	IFN-ϒ	TNF-α	IL-1β	IL-4	IL-6	IL-8	IL-10	IFN-ϒ	TNF-α
Cytokines concentrations in the uterine fluid on day 2 post-farrowing	IL-1β	0.526	0.381	-0.119	0.519	0.461	0.658	0.956	0.690	0.557	0.273	0.336	0.689	0.620	0.529
	IL-4	-0.164	0.344	-0.202	-0.347	-0.456	-0.020	-0.329	-0.053	0.065	-0.175	0.208	-0.366	-0.104	-0.416
	IL-6	0.064	0.350	-0.195	0.083	0.570	0.005	0.497	0.700	-0.057	0.336	0.123	0.599	0.988	0.602
	IL-8	0.203	0.565	0.840	0.512	0.636	-0.031	0.560	0.976	-0.144	0.656	0.282	0.631	0.653	0.587
	IL-10	0.055	0.527	0.200	-0.091	-0.207	-0.132	-0.052	0.013	0.349	-0.055	0.183	-0.058	-0.002	-0.142
	IFN-ϒ	-0.236	0.316	0.561	0.243	0.289	-0.046	0.069	0.974	-0.171	0.673	0.086	0.476	0.928	0.358
	TNF-α	-0.403	0.279	-0.284	-0.095	-0.202	0.464	-0.005	0.200	-0.207	-0.176	0.070	-0.005	0.006	-0.134
Cytokines concentrations in the uterine fluid on day 6 post-farrowing	IL-1β	-0.111	0.687	0.059	-0.197	-0.175	0.012	-0.080	-0.059	0.343	-0.155	0.080	-0.117	-0.029	-0.172
	IL-4	-0.294	0.411	0.690	-0.224	-0.248	0.455	-0.184	-0.043	0.023	-0.133	0.182	-0.263	-0.055	-0.250
	IL-6	0.271	0.809	0.845	0.324	0.581	0.380	0.796	0.752	0.241	0.244	0.028	0.514	0.592	0.612
	IL-8	-0.261	0.212	0.310	-0.146	0.025	0.513	0.082	0.060	0.004	-0.030	0.061	-0.107	0.094	-0.054
	IL-10	-0.247	0.123	0.383	-0.225	-0.088	0.464	-0.037	0.387	-0.097	0.243	0.105	0.066	0.160	-0.069
	IFN-ϒ	-0.704	0.123	0.373	0.196	0.298	0.491	0.620	0.256	0.456	0.148	0.423	0.493	0.319	0.431
	TNF-α	-0.058	0.284	0.323	-0.123	0.013	-0.198	0.060	0.443	-0.028	0.273	0.107	0.152	0.270	0.048

Correlations between day of collection, uterine cytokines and serum cytokines were analyzed as Pearson correlation coefficients using the CORR procedure in SAS (SAS Institute Inc., Cary, NC, U.S.A). Variables were considered highly correlated if correlation coefficient were greater or equal to 0.7, moderately correlated between 0.4 and 0.7, and not correlated if lower than 0.4.

[1]Interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were evaluated using multiplex ELISA kits.

Table 4.9 P-values for Pearson correlation coefficients of cytokines[1] concentrations on days 2 and 6 post-farrowing in the uterine fluid and serum of lactating sow (n=40).

		Cytokines concentrations in the serum on day 2 post-farrowing							Cytokines concentrations in the serum on day 6 post-farrowing						
		IL-1β	IL-4	IL-6	IL-8	IL-10	IFN-ϒ	TNF-α	IL-1β	IL-4	IL-6	IL-8	IL-10	IFN-ϒ	TNF-α
Cytokines concentrations in the uterine fluid on day 2 post-farrowing	IL-1β	0.146	0.277	0.742	0.069	0.097	0.020	<0.001	0.019	0.602	0.391	0.286	0.009	0.014	0.052
	IL-4	0.477	0.250	0.490	0.097	0.013	0.929	0.087	0.799	0.748	0.460	0.340	0.061	0.592	0.014
	IL-6	0.821	0.321	0.643	0.760	0.011	0.987	0.031	0.001	0.847	0.240	0.663	0.005	<0.001	0.005
	IL-8	0.506	0.113	0.018	0.089	0.014	0.928	0.038	<0.001	0.656	0.021	0.375	0.015	0.008	0.022
	IL-10	0.817	0.064	0.494	0.673	0.280	0.012	0.793	0.950	0.087	0.823	0.403	0.774	0.992	0.429
	IFN-ϒ	0.574	0.317	0.073	0.423	0.278	0.871	0.806	<0.001	0.527	0.006	0.762	0.085	<0.001	0.158
	TNF-α	0.219	0.649	0.496	0.756	0.437	0.110	0.985	0.458	0.519	0.606	0.830	0.984	0.981	0.562
Cytokines concentrations in the uterine fluid on day 6 post-farrowing	IL-1β	0.641	0.007	0.840	0.345	0.354	0.957	0.682	0.769	0.087	0.501	0.705	0.552	0.878	0.323
	IL-4	0.222	0.145	0.006	0.271	0.186	0.022	0.339	0.838	0.912	0.566	0.383	0.176	0.772	0.154
	IL-6	0.371	0.008	0.008	0.281	0.038	0.278	0.001	0.002	0.451	0.400	0.934	0.072	0.226	0.026
	IL-8	0.266	0.466	0.280	0.487	0.896	0.096	0.674	0.769	0.981	0.899	0.773	0.587	0.615	0.758
	IL-10	0.280	0.688	0.219	0.291	0.643	0.549	0.852	0.046	0.646	0.301	0.624	0.735	0.399	0.692
	IFN-ϒ	0.051	0.705	0.288	0.466	0.263	0.063	0.008	0.399	0.088	0.629	0.103	0.062	0.197	0.074
	TNF-α	0.857	0.496	0.397	0.627	0.953	0.431	0.797	0.181	0.911	0.367	0.694	0.512	0.212	0.817

Correlations between day of collection, uterine cytokines and serum cytokines were analyzed as Pearson correlation coefficients using the CORR procedure in SAS (SAS Institute Inc., Cary, NC, U.S.A). Variables were considered highly correlated if correlation coefficient were greater or equal to 0.7, moderately correlated between 0.4 and 0.7, and not correlated if lower than 0.4.

[1]Interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were evaluated using multiplex ELISA kits.

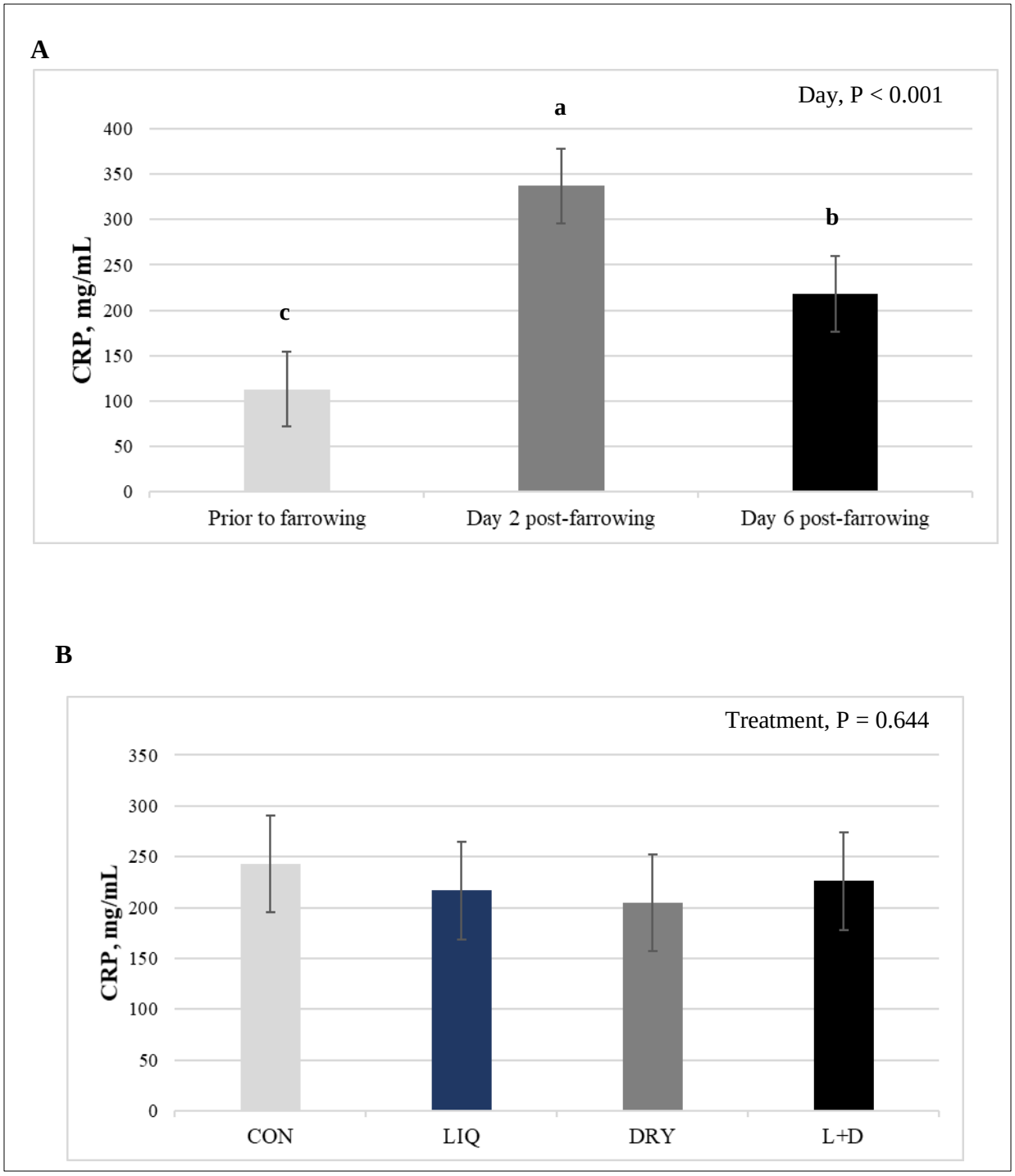

Figure 4.1 C-reactive protein (CRP, mg/mL) concentrations in the serum of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b,c]Means with different letters differ by Tukey test (P < 0.05).

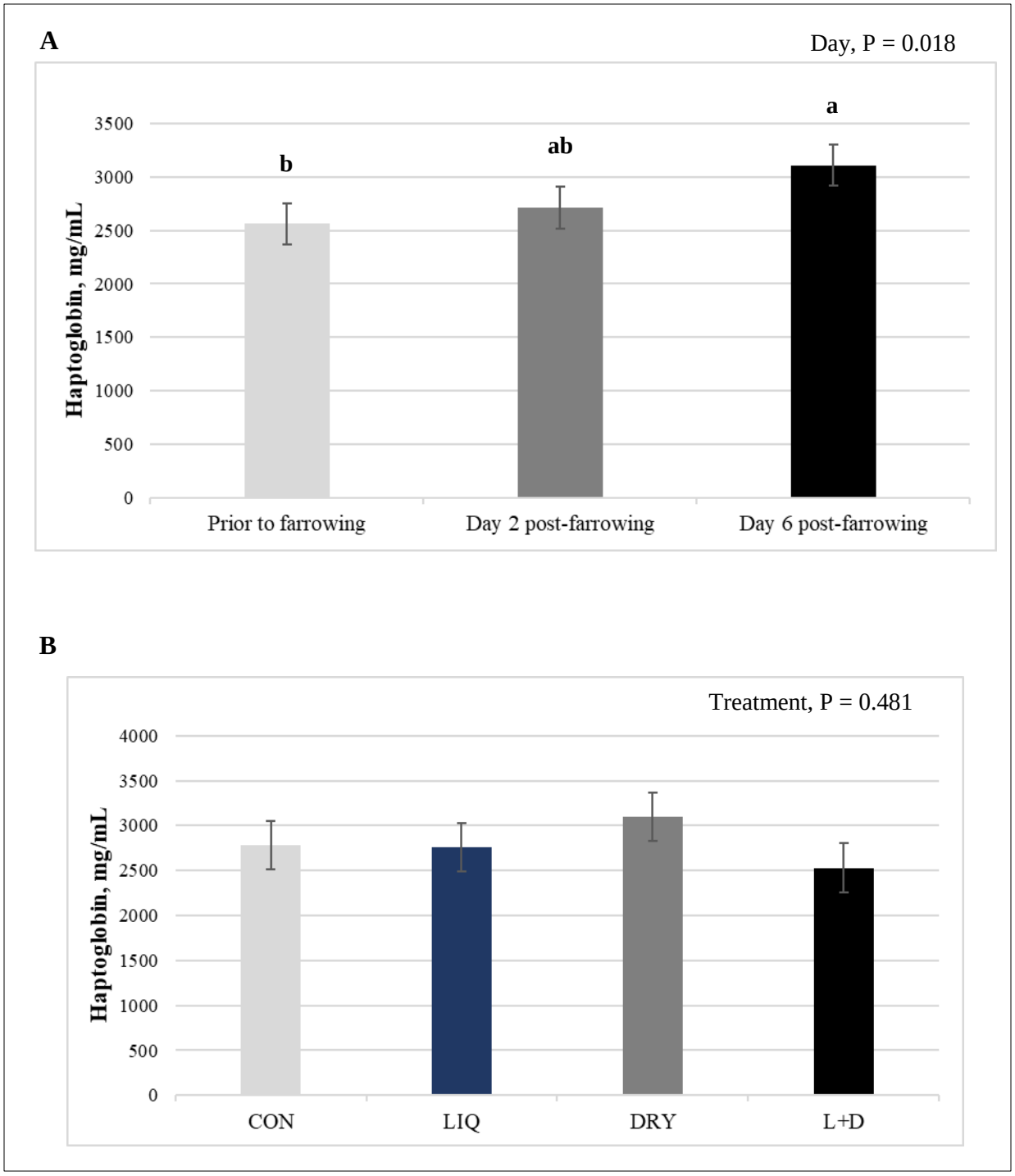

Figure 4.2 Haptoglobin (mg/mL) concentrations in the serum of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b]Means with different letters differ by Tukey test (P < 0.05).

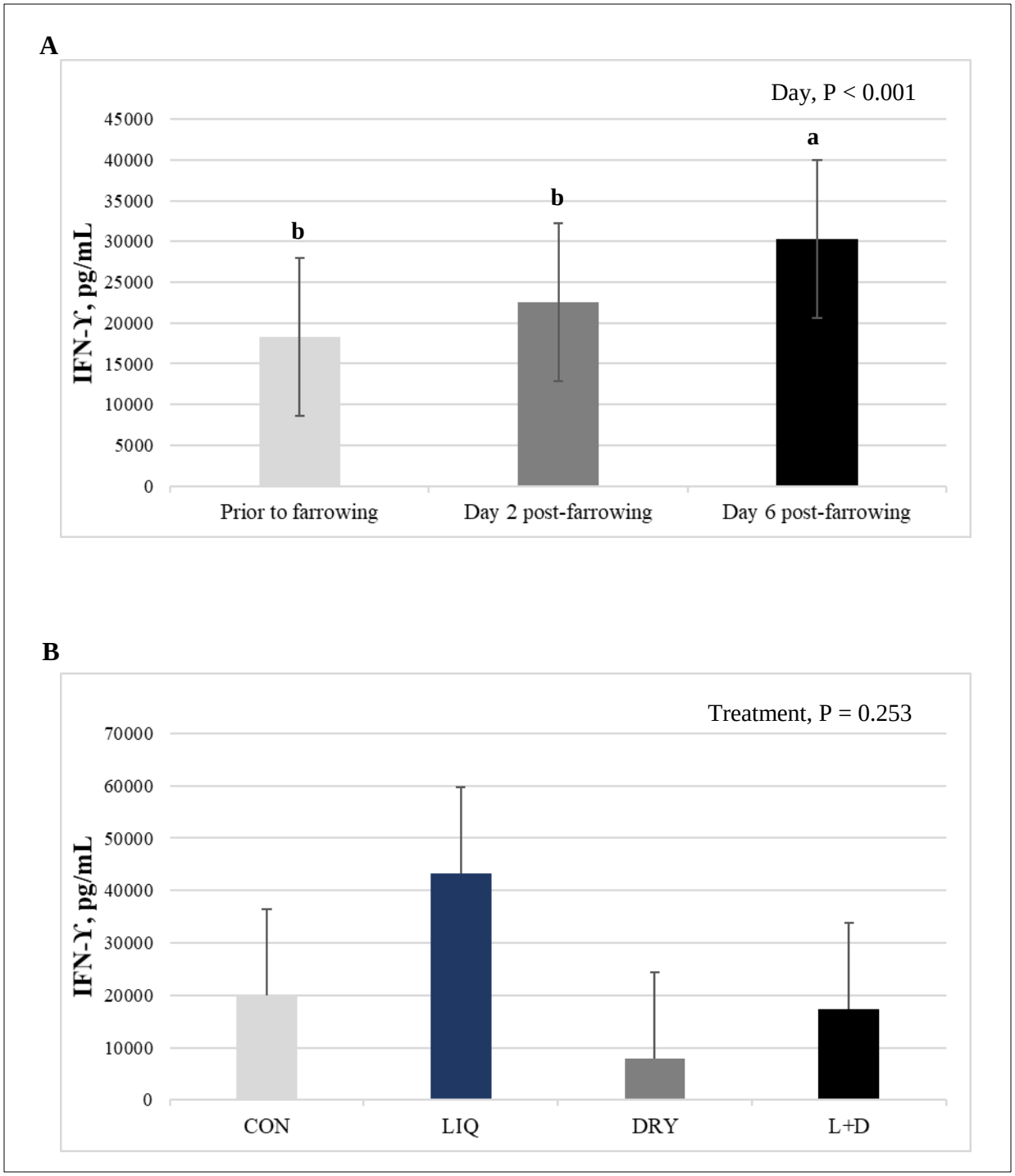

Figure 4.3 Interferon-ϒ (IFN-ϒ, pg/mL) concentrations in the serum of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b]Means with different letters differ by Tukey test (P < 0.05).

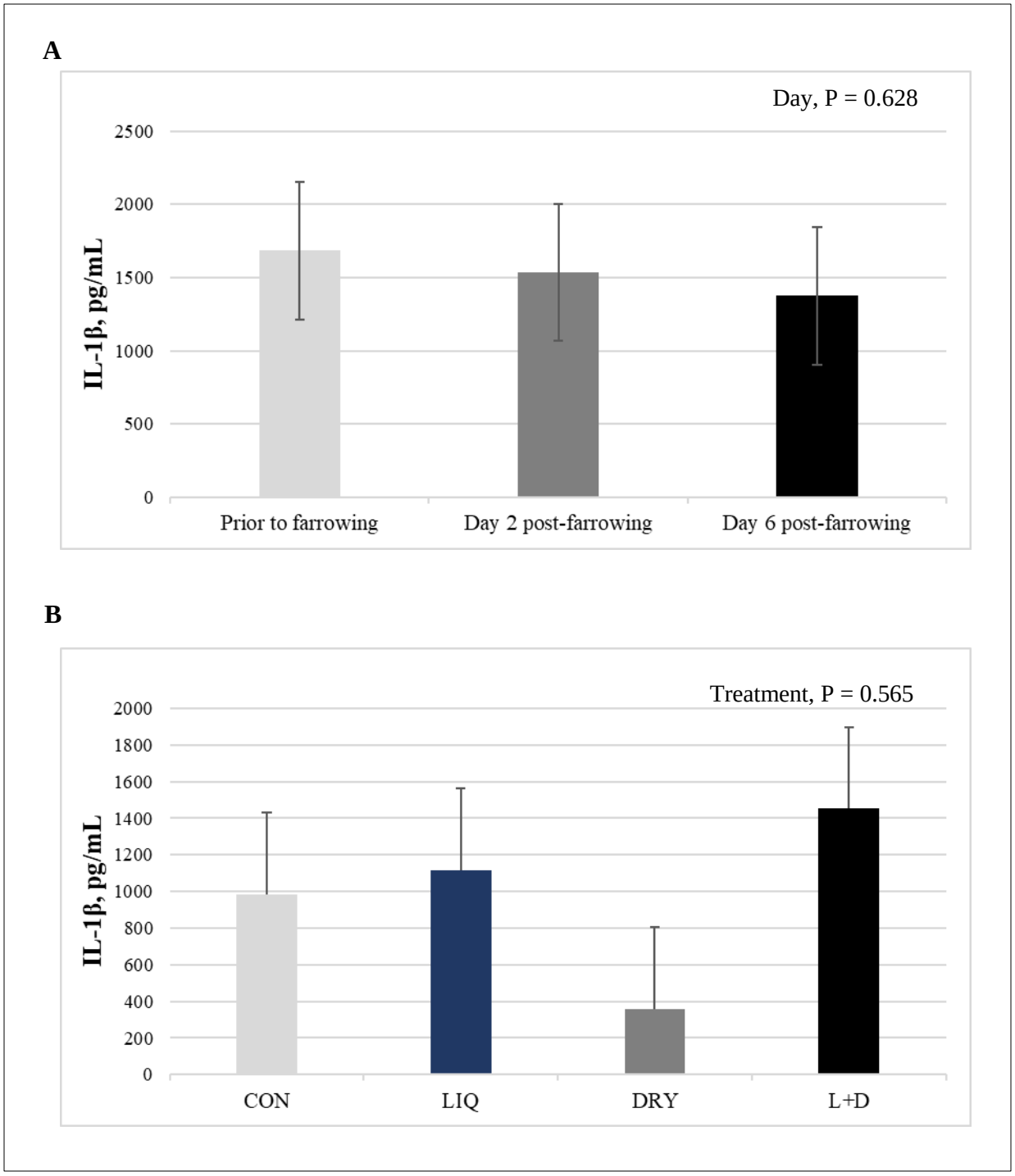

Figure 4.4 Interleukine-1β (IL-1β, pg/mL) concentrations in the serum of sows (n=40). No effects of treatment, day of collection or their interaction were detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

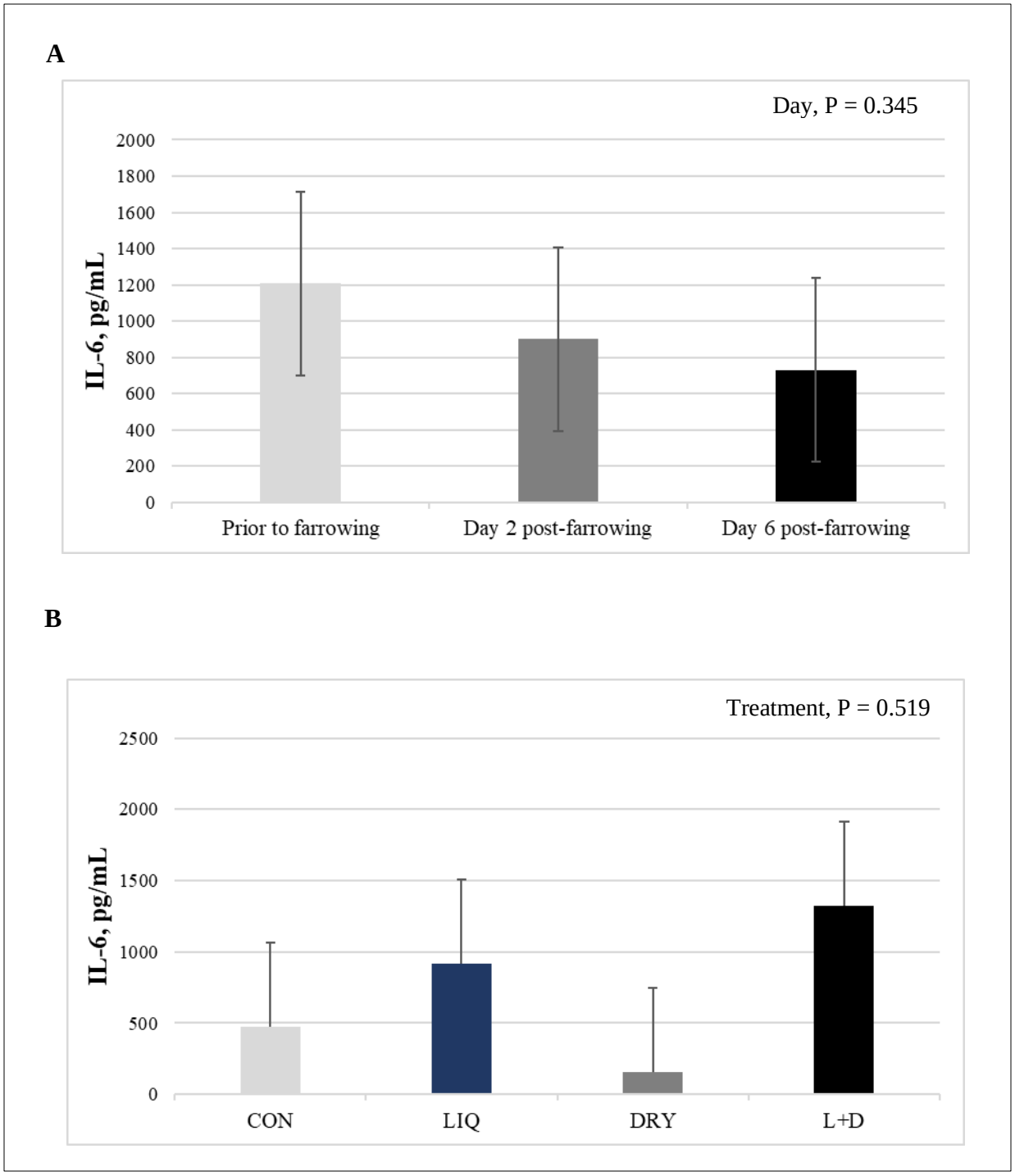

Figure 4.5 Interleukine-6 (IL-6, pg/mL) concentrations in the serum of sows (n=40). No effects of treatment, day of collection or their interaction were detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

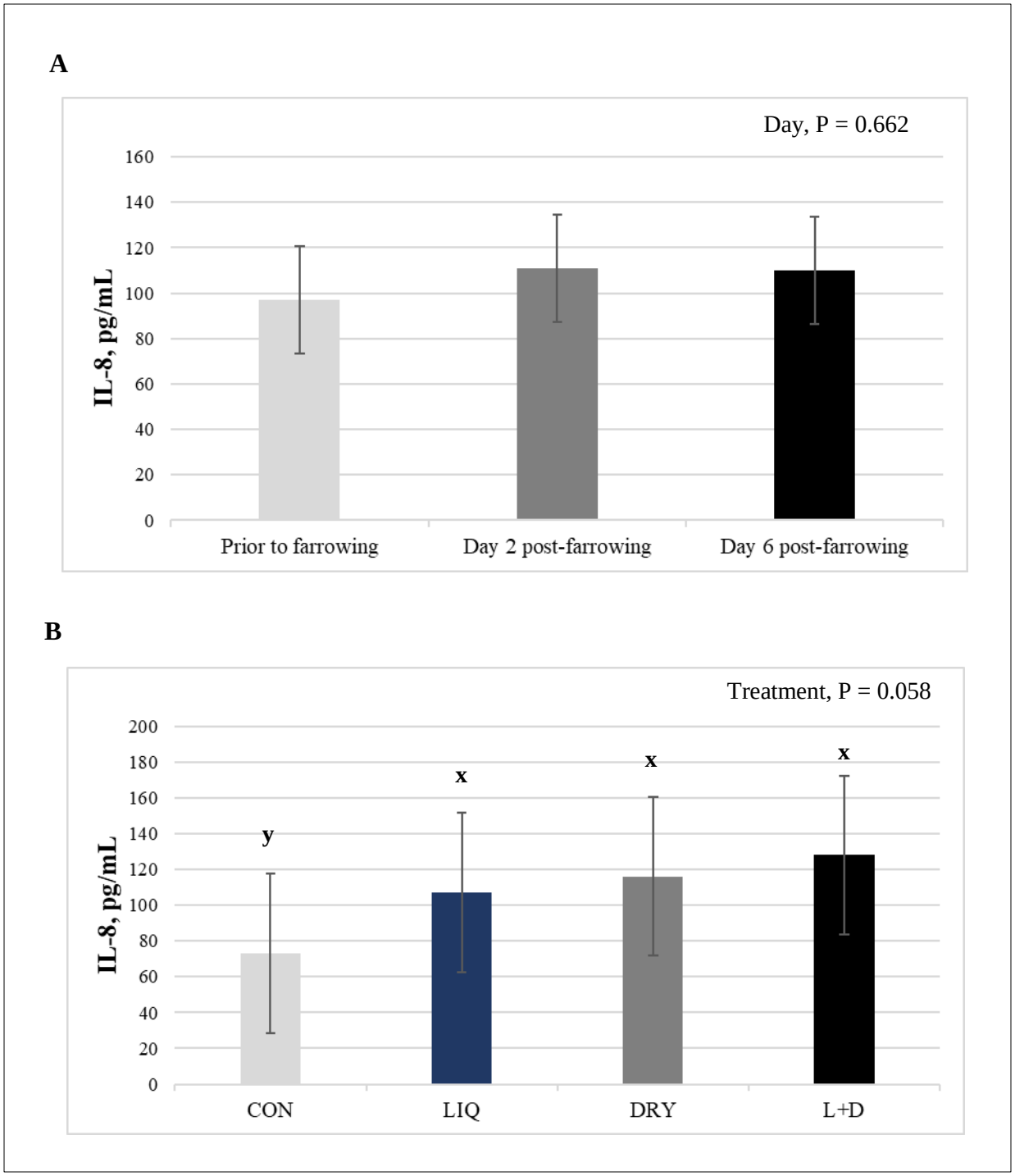

Figure 4.6 Interleukine-8 (IL-8, pg/mL) concentrations in the serum of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[x,y]Means with different letters differ by Tukey test (P ≤ 0.10).

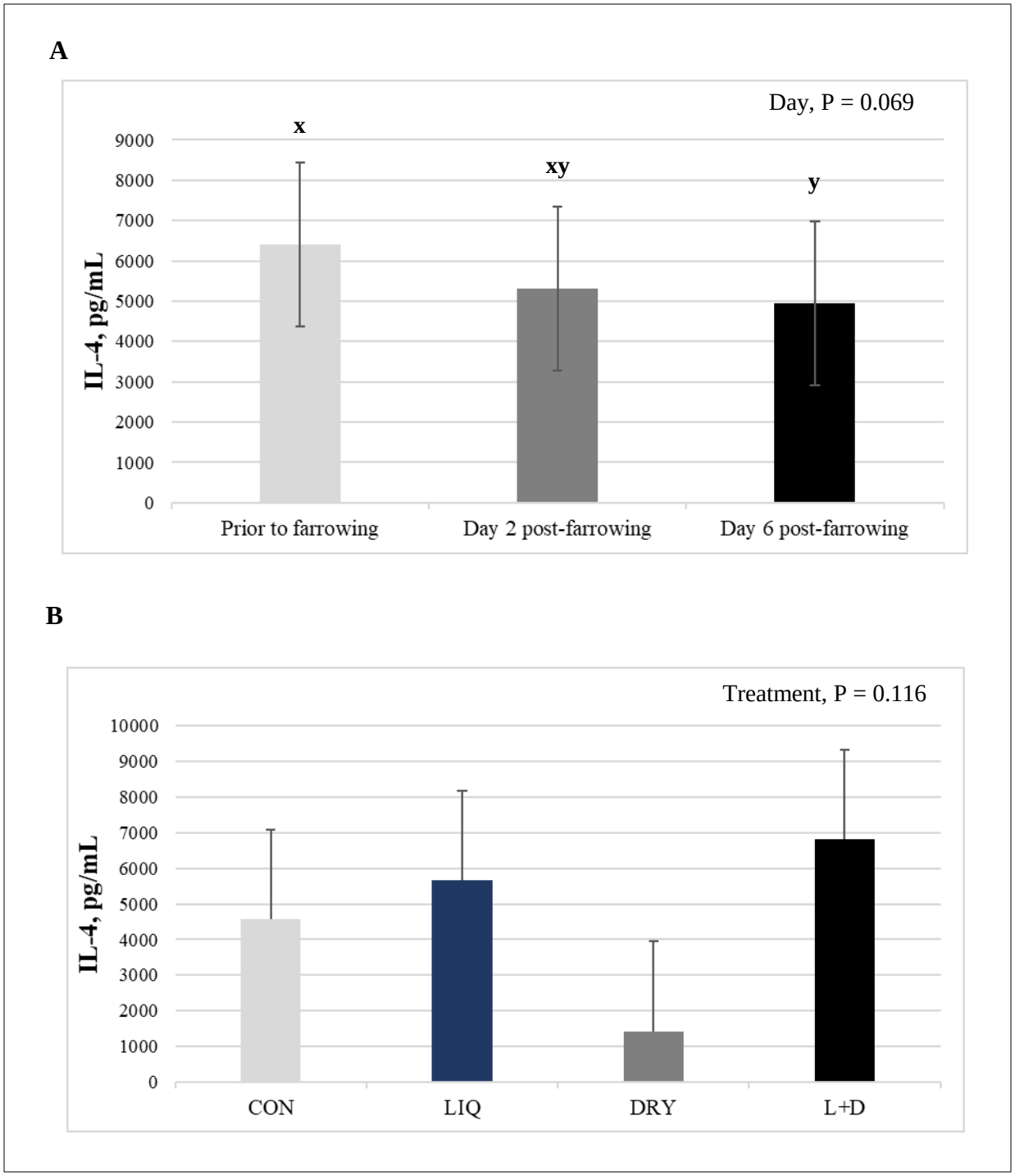

Figure 4.7 Interleukine-4 (IL-4, pg/mL) concentrations in the serum of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[x,y]Means with different letters differ by Tukey test (P ≤ 0.10).

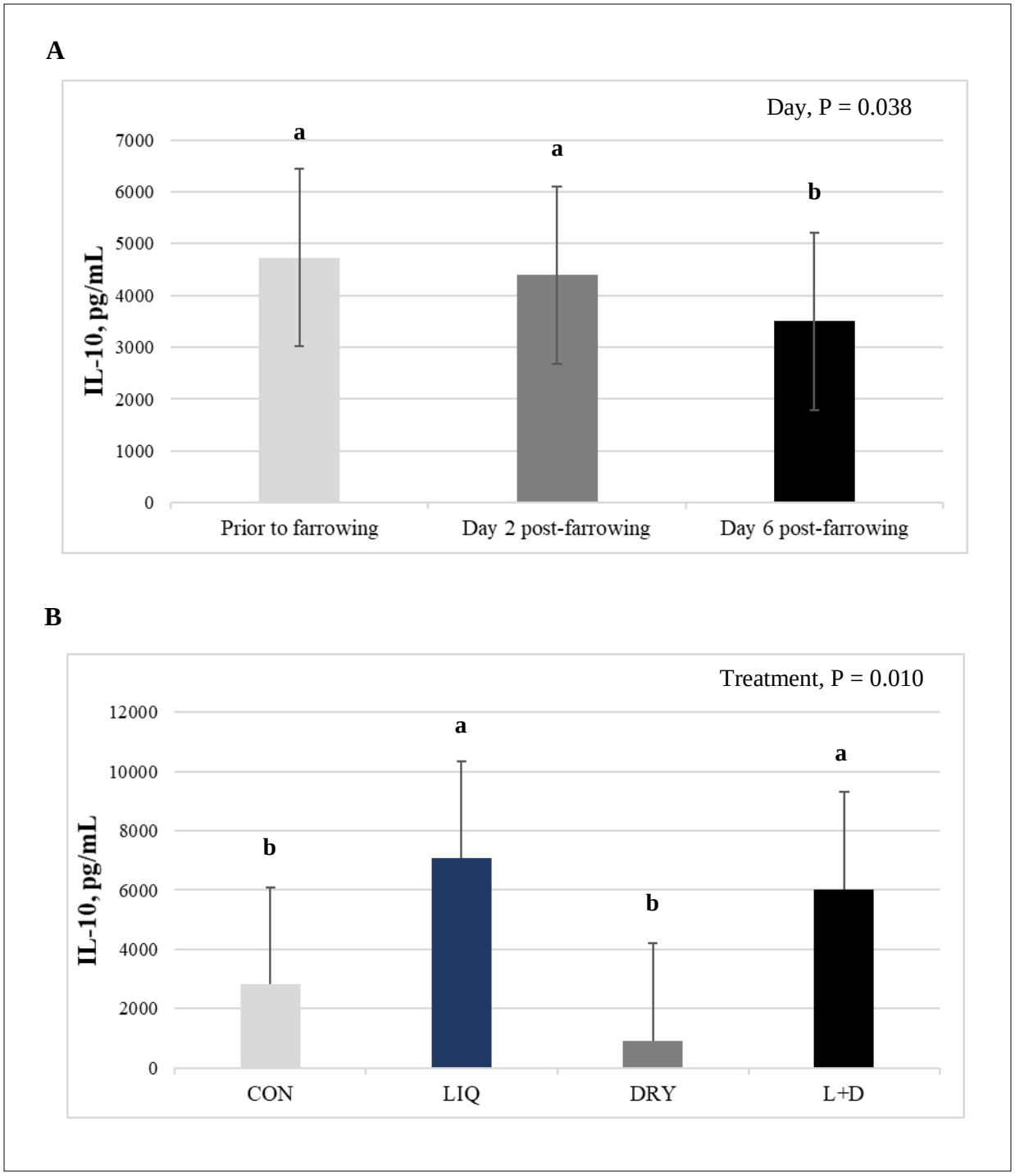

Figure 4.8 Interleukine-10 (IL-10, pg/mL) concentrations in the serum of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were prior to farrowing (d 112 of gestation), d 2 and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b]Means with different letters differ by Tukey test (P < 0.05).

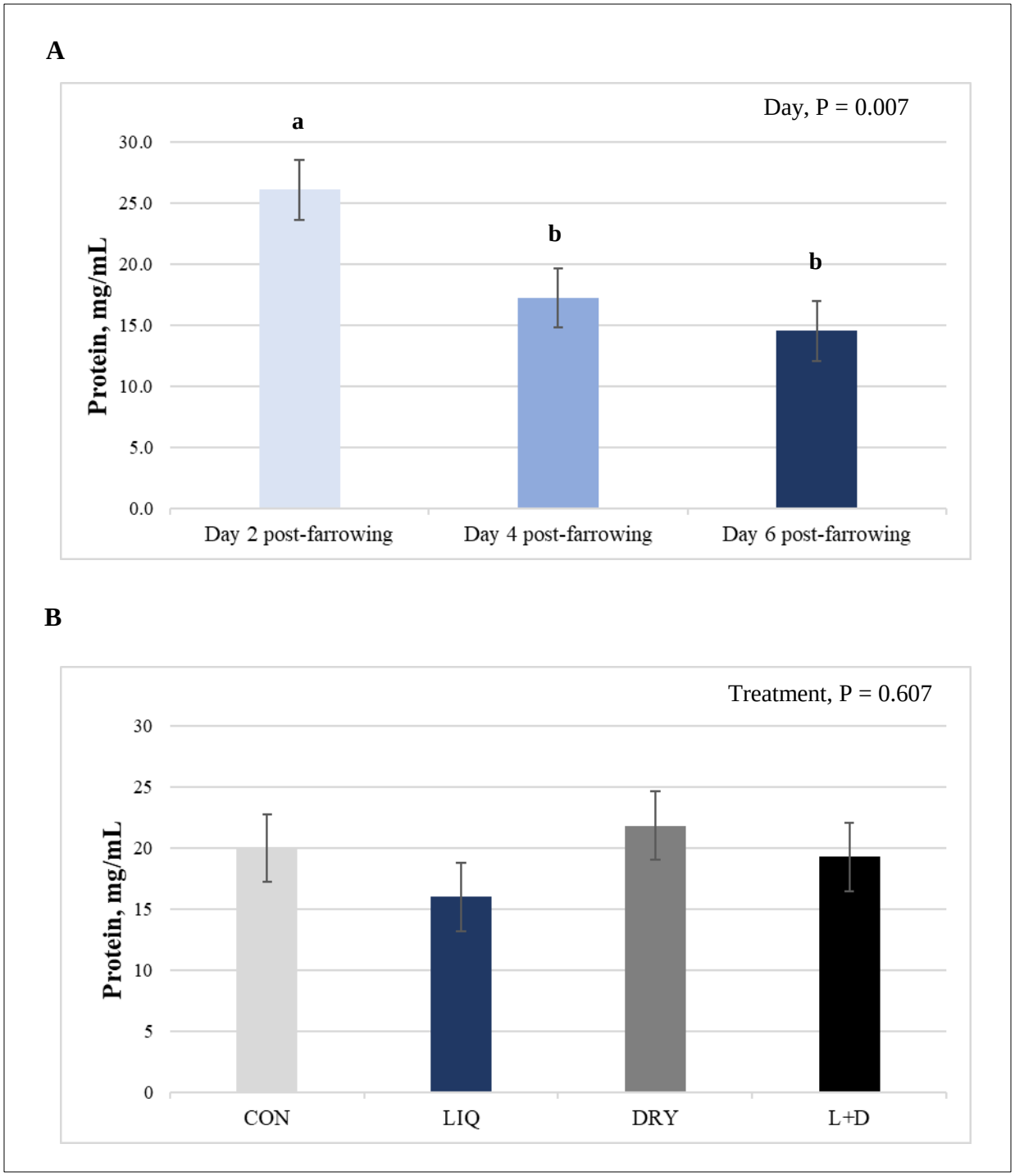

Figure 4.9 Protein content (mg/mL) in the uterine fluid after freeze-drying and re-suspending in sterile saline (1 mL). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b]Means with different letters differ by Tukey test (P < 0.05).

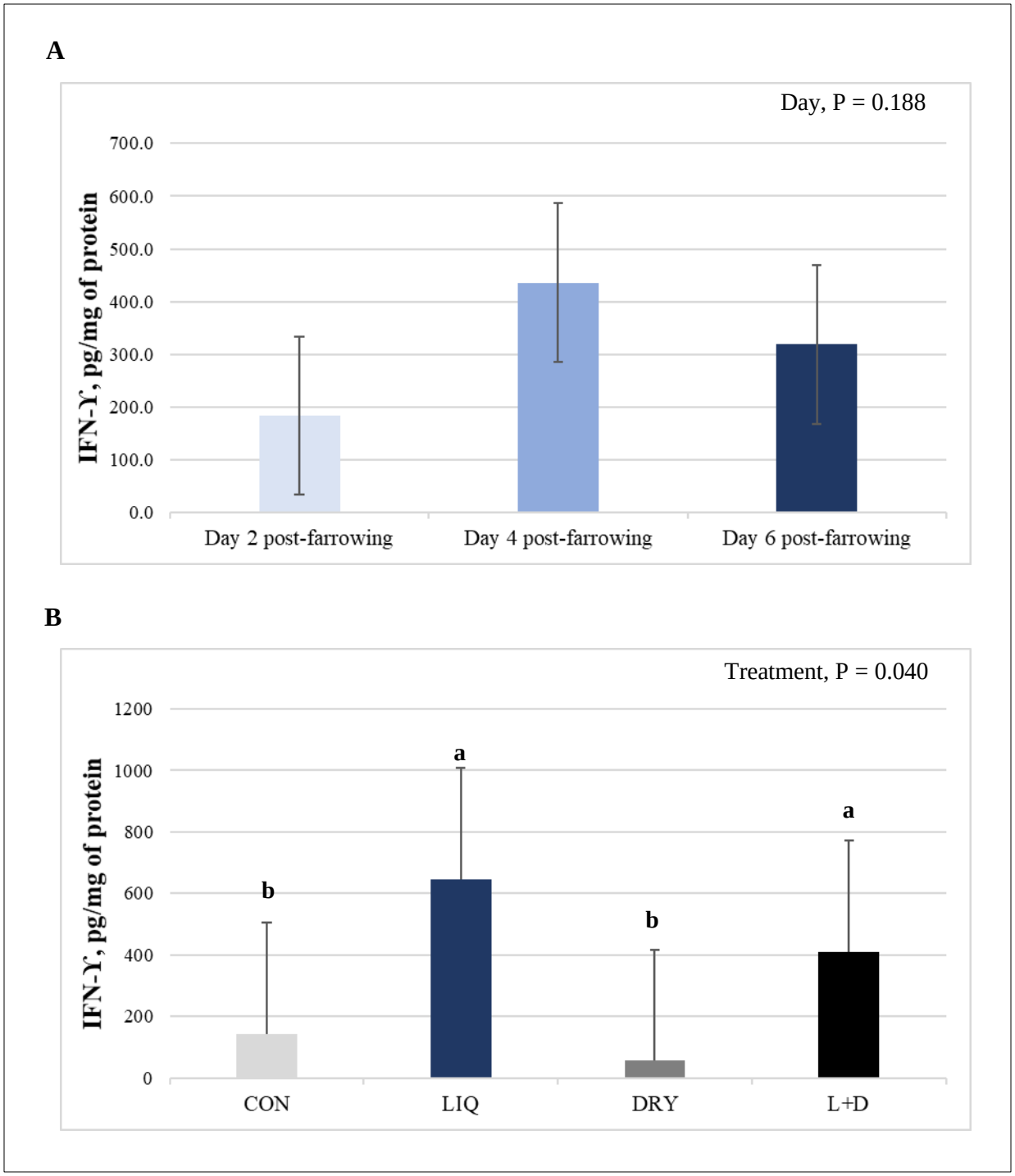

Figure 4.10 Interferon-ϒ (IFN-ϒ, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b]Means with different letters differ by Tukey test (P < 0.05).

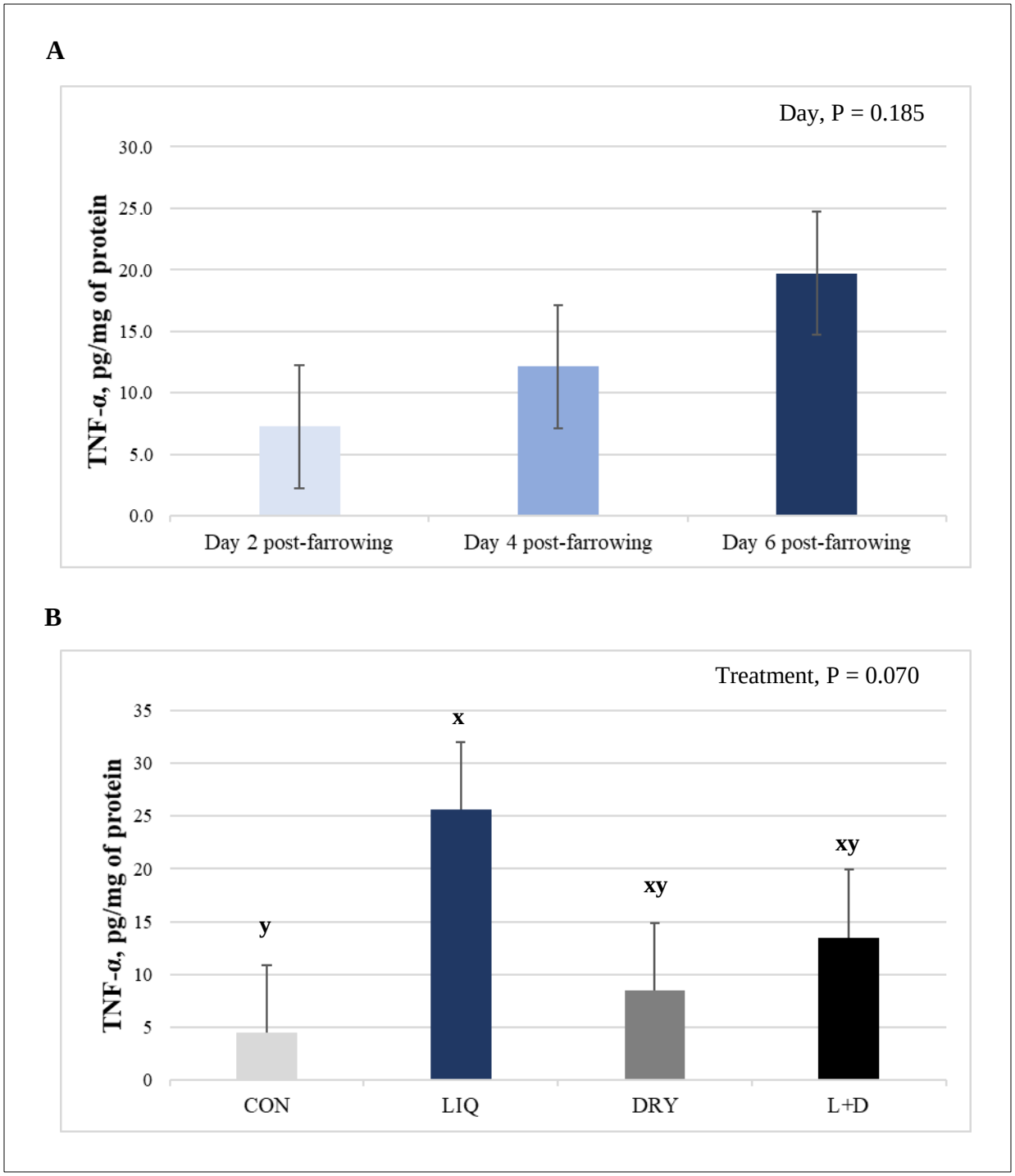

Figure 4.11 Tumour necrosis factor-α (TNF-α, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[x,y]Means with different letters differ by Tukey test (P ≤ 0.10).

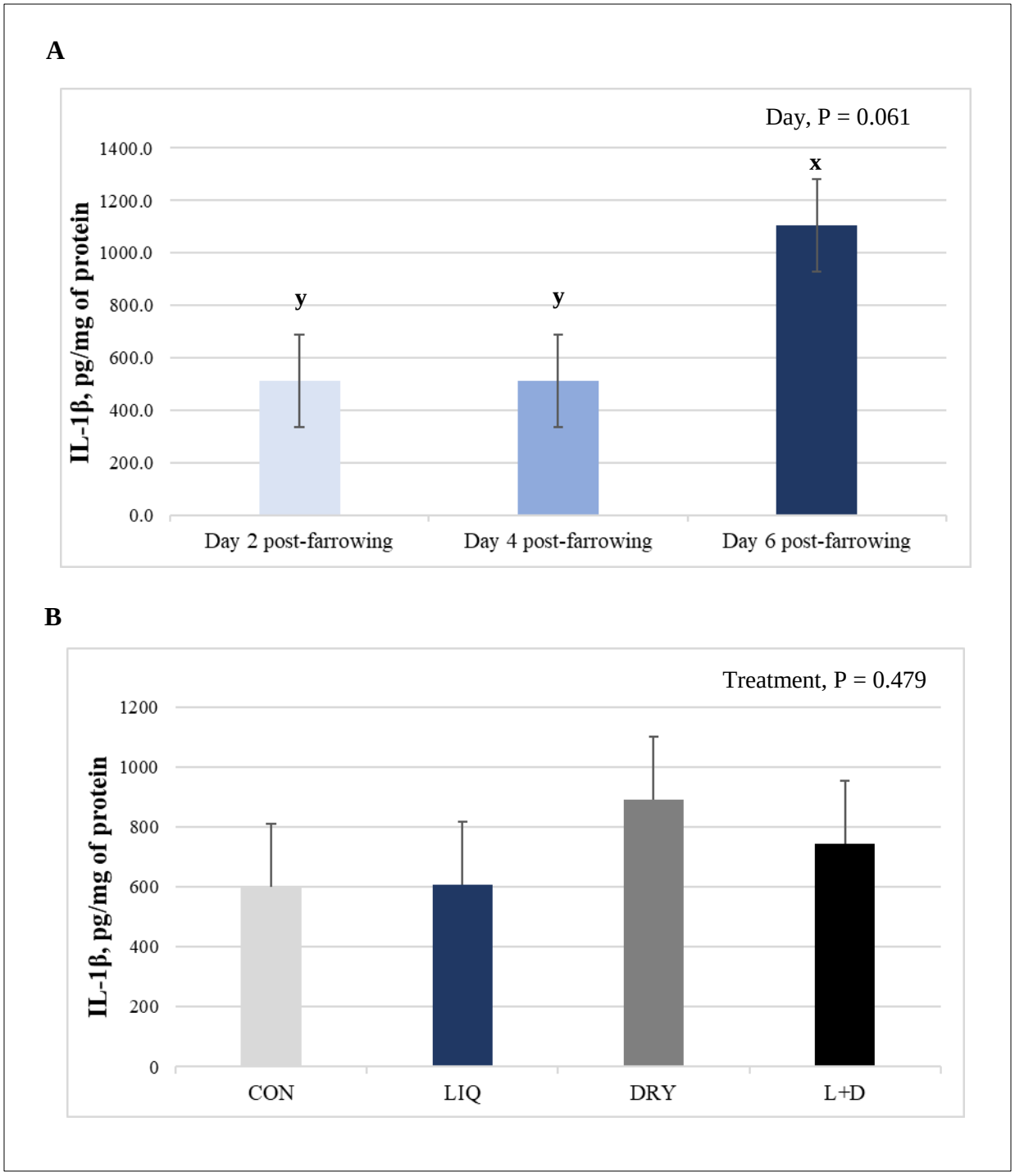

Figure 4.12 Interleukine-1β (IL-1β, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[x,y]Means with different letters differ by Tukey test (P ≤ 0.10).

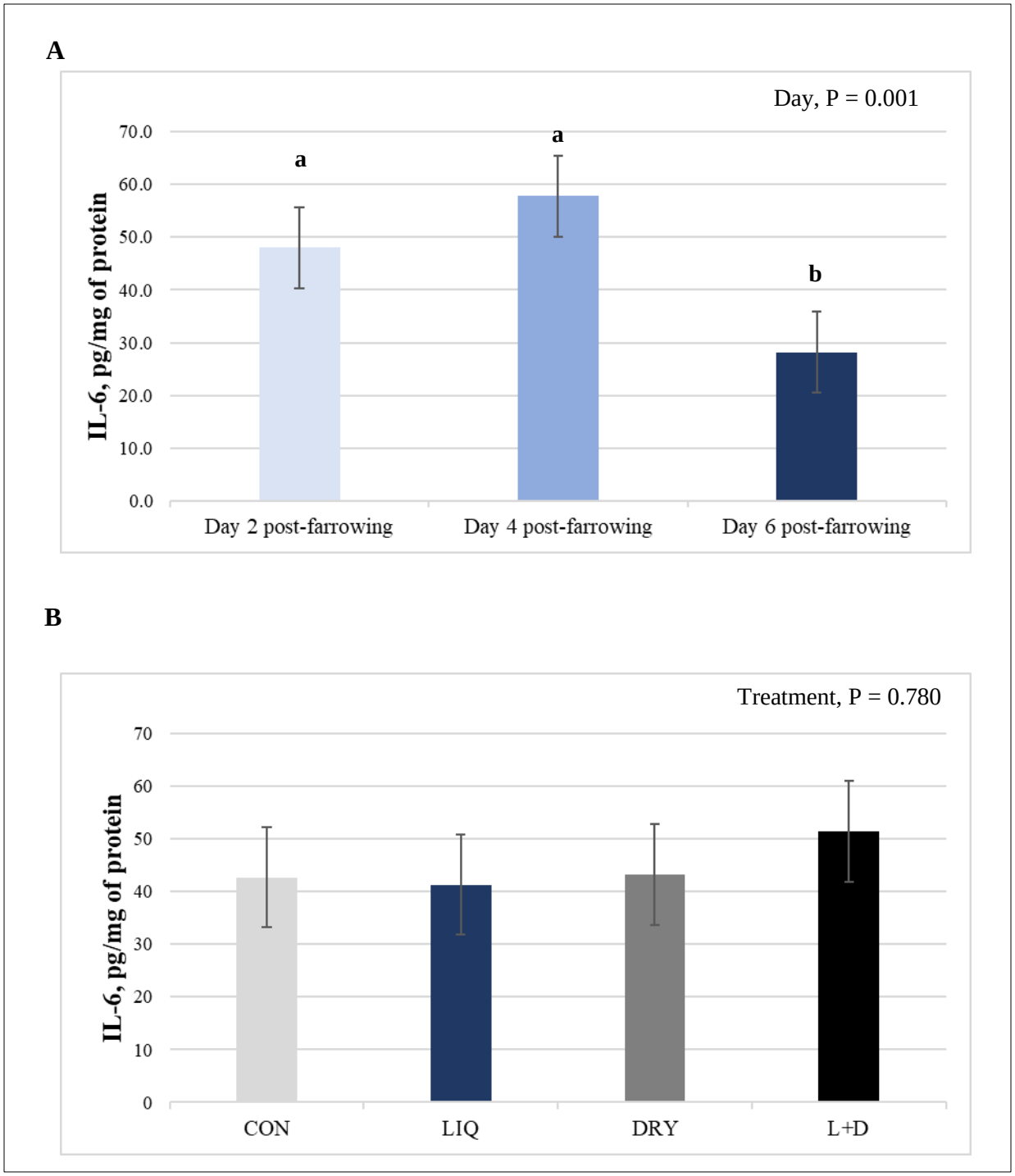

Figure 4.13 Interleukine-6 (IL-6, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No treatment by day of collection interaction was detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.
[a,b]Means with different letters differ by Tukey test (P < 0.05).

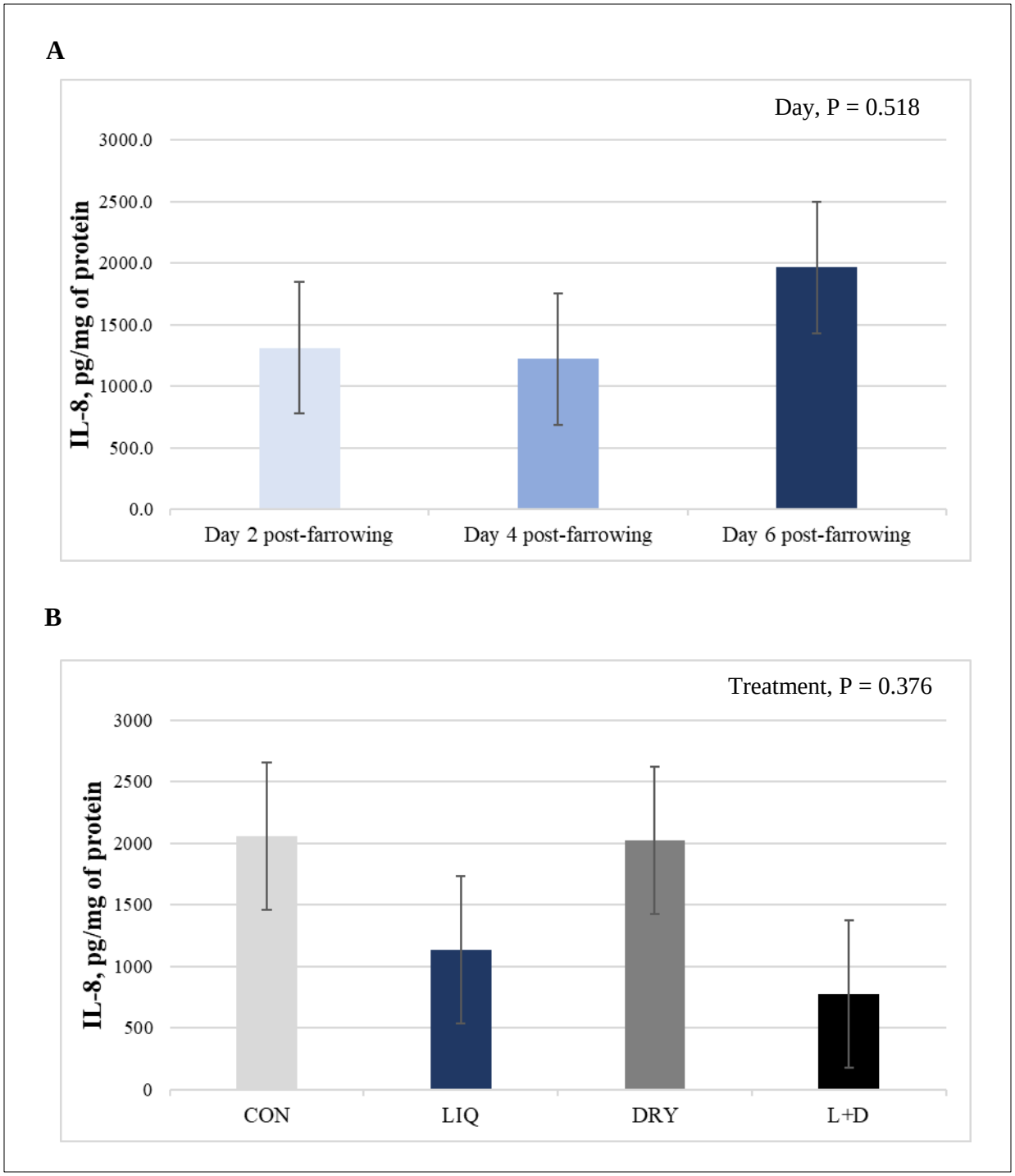

Figure 4.14 Interleukine-8 (IL-8, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No effects of treatment, day of collection or their interaction were detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

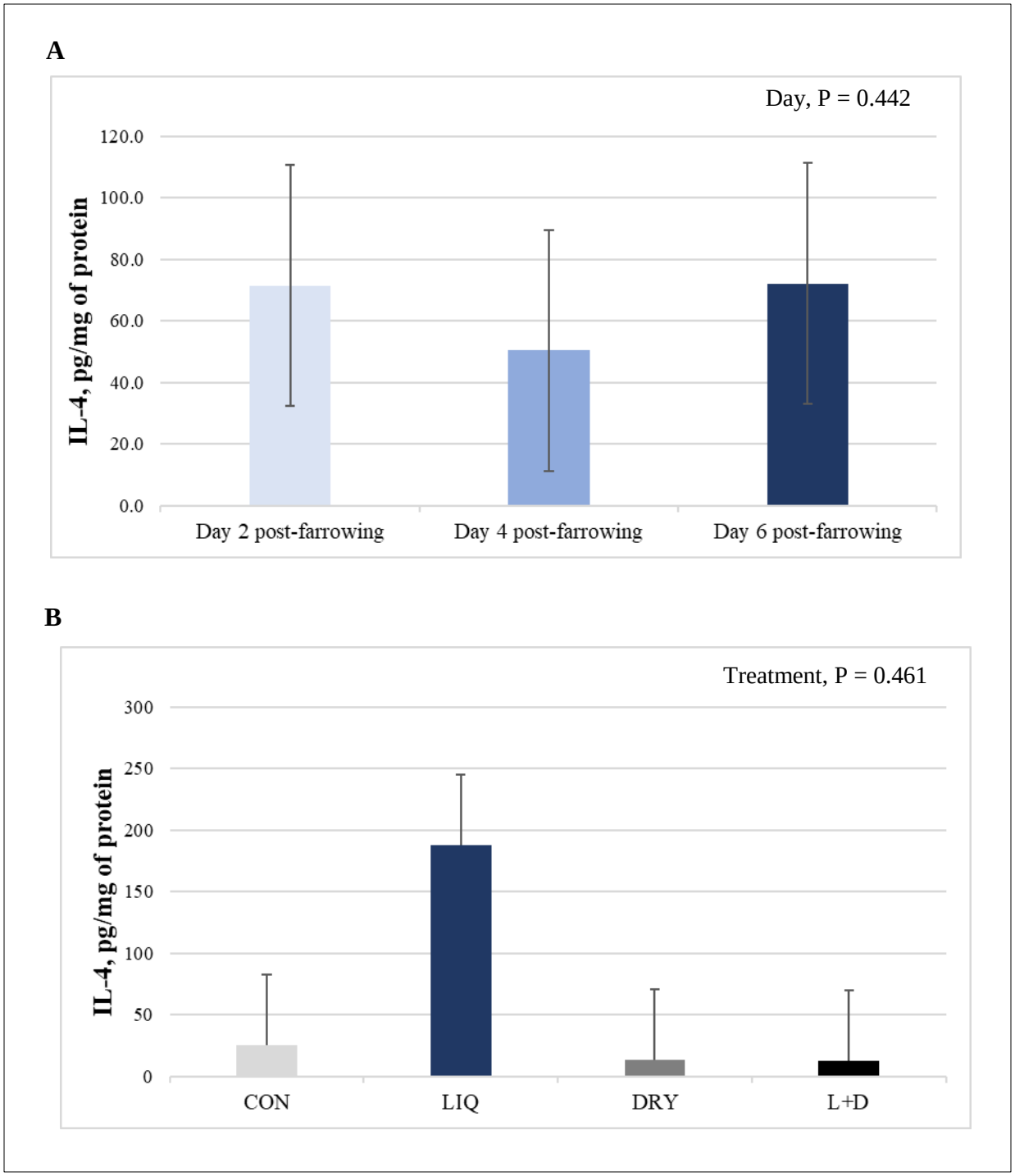

Figure 4.15 Interleukine-4 (IL-4, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No effects of treatment, day of collection or their interaction were detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

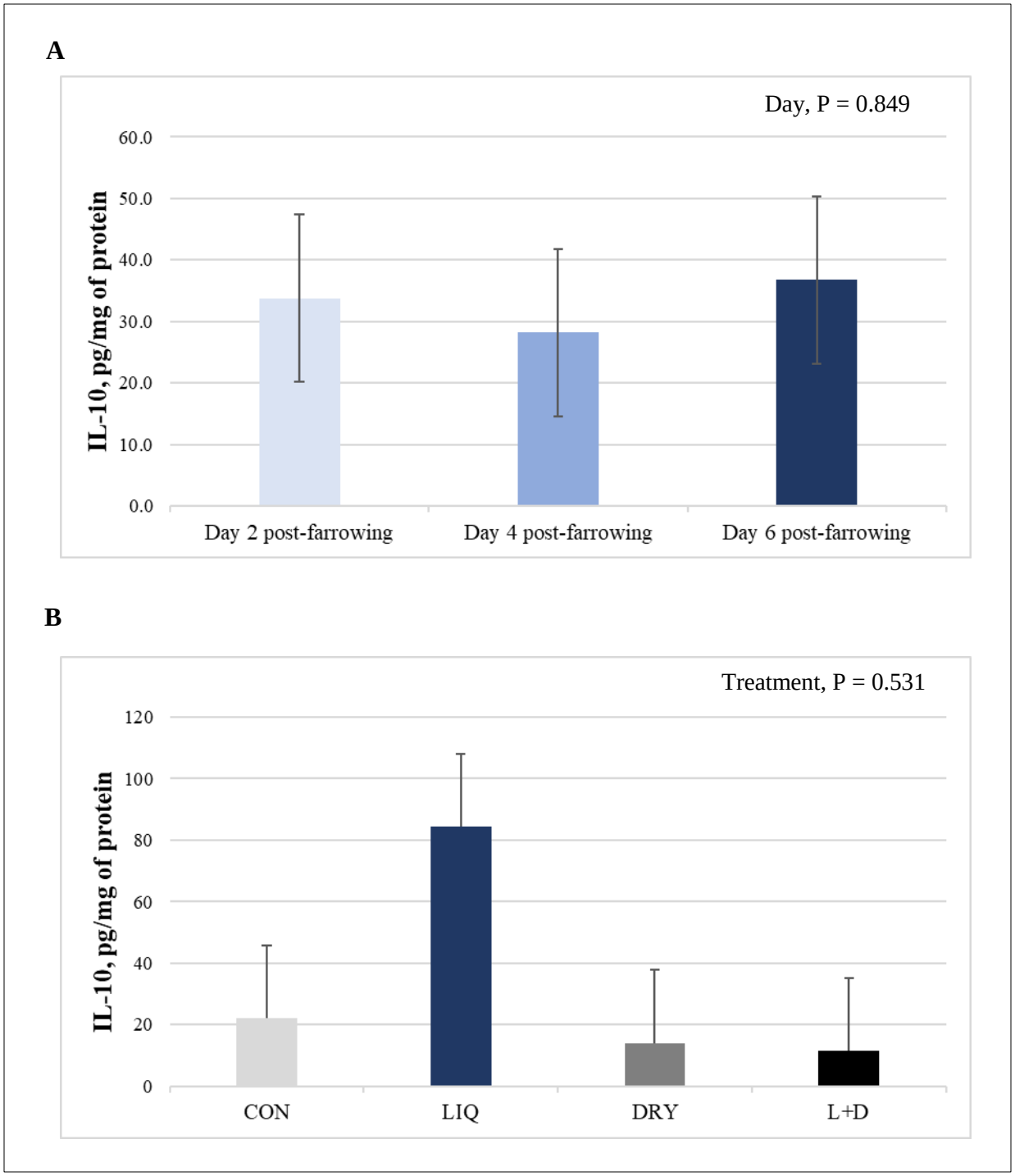

Figure 4.16 Interleukine-10 (IL-10, pg/mg of protein) concentrations in the uterine fluid of sows (n=40). No effects of treatment, day of collection or their interaction were detected (P > 0.10). **(A)** Days of collection were d 2, d 4, and d 6 post-farrowing. **(B)** Experimental treatments were: 1) CON, lactation control diet, 2) LIQ, liquid version of SCFP orally given for the entire experimental period, 3) DRY, dried version of SCFP in the feed given for the entire experimental period, and 4) L+D: dried version of SCFP in the feed given for the entire experimental period + liquid version of SCFP orally given from day 112 of gestation until d 7 of lactation.

CHAPTER 5. SUPPLEMENTAL YEAST FERMENTATION PRODUCTS EFFECT ON SOW LACTATION PERFORMANCE AND POST-PARTUM RECOVERY BASED ON UTERINE FLUIDS AND BLOOD PARAMETERS

5.1 Conclusions

Feeding SCFP to lactating sows from prior to farrowing until weaning improved feed intake, especially in the first week of lactation, and litter growth while not affecting colostrum quality, immunoglobulins in the offspring, and milk output. Pig weight at weaning was improved in sows given the liquid source of SCFP, pig ADG and litter uniformity at weaning were improved for all the sources of SCFP. Indeed, sows fed SCFP had significantly lower number of pigs below the payable industry cut point (3.5 kg). Sows given the liquid source of SCFP had the greatest early and overall lactation feed intake, less body weight mobilization, and a reduction in WEI. The liquid prototype evaluated in this study seems to be a promising additive for lactating sows. Feeding SCFP to sows improved lactation and litter performance possibly through promoting a quicker recovery after farrowing as demonstrated by greater DFI in the first week post-farrowing.

Given that many feed additives commercially available in the industry claims immunomodulatory effects in swine, direct or indirectly, we set the objective to develop a method to access reproductive tract inflammation in sows. The methodology developed to access cytokines concentration in the uterine lavage of puerperium sows, successfully achieved the goal of being a novel non-invasive procedure to be applied in puerperium sows. This experimental procedure can be used in scientific research to describe and measure uterine immunological response following parturition, specially evaluating strategies to improve lactation performance.

Lastly, the methodology developed to access cytokines concentration in the uterine lavage of puerperium sows was further improved and applied in a research trial to evaluate the

immunomodulatory effects of SCFP. The relationship between uterine fluid cytokines profiles and serum cytokines profiles was accessed. Overall, the correlations between the uterine and serum cytokines showed a clear pattern resulted from the type of immune response developed in the puerperium period. The immunomodulatory effects of SCFP were demonstrated by the cytokine shifts observed in the serum and in the uterine fluids in response to the farrowing process. Feeding SCFP to lactating sows stimulates pro- and anti-inflammatory mechanisms, possible through innate activation, allowing sows to mount a more desirable immune response. Thus, sows had a quicker resolution of acute phase reaction ensuring them to recovery from puerperium faster and to improve the lactation performance.

5.2 Future Direction

Feeding SCFP to lactating sows provided very insightful results. More research can be done evaluating the entire gestating period. As one of the mechanisms are likely to be directly the modulation of the sows microbiome, evaluating the shifts and characterizing the microbiota of pregnant and lactating sows at different time points might be useful to elucidate other mechanisms not explored in the study. Also, the liquid prototype demonstrated to have slightly different effects compared to the other source. Identifying the main differences in the composition might be important to have future evaluations. Likewise, more research should be done on the liquid protype to evaluate its stability and physical properties to be applied in medicators through water lines. The approach used in this study, individually orally given, was because it was aimed to provide better precision on the amount given to the sows which is not as applicable in commercial field conditions.

The subsequent reproductive performance was evaluated in this study by measuring follicular development on a subsample of sows. Sows fed the liquid prototype had 0.5 day

reduction on wean to estrus interval and greater average daily follicle growth. It is reasonable to infer that this was a result from the greater feed intake and reduced body weight loss. Considering that evaluation of follicle development in sows is time consuming and not easy to perform, future research could use some biomarkers such as IGF, leptin, FSH, and LH.

Postbiotics have some advantages over probiotics. Probiotics effects depend on the viability of the bacteria when they reach certain parts of the intestine as well as on the metabolic activity of them in the gut. Postbiotics, are probiotic-related compounds that are sufficient to promote the desirable outcomes without needing the original live microorganisms. New studies should be conducted to elucidate principal components within these products that are responsible for its effects in sows. Here, it was speculated that it was related to its major cell wall components, MOS and β-glucans. However, SCFP had a variety of compounds because it also contains the medium that was used for yeast fermentation. In future research with SCFP, a clear definition of the composition might be very insightful to the industry.

Sows used in the study were balanced by parity and body condition score to avoid confounding factors. Indeed, fat sows are likely to be more oxidative and inflamed. Future research with SCFP could be done on large scale to evaluate interaction effects in different parities as well as with over conditioned sows, which is very common in the field.

The simple colostrum analysis performed in this study is very applicable in commercial conditions. Yet, several maternal immunological molecules can pass through the colostrum to the offspring such as cytokines (including the various growth factors), leukocytes, and immunoglobulins. Therefore, future studies should focus on determining if colostrum and milk components can be altered by feeding SCFP to sows and the effects on the offspring. Given the

clear effect on feed intake and litter growth in sows fed SCFP, future research should evaluate milk yield with more precise methodology like DO as well as a larger number of sows.

The work presented in this dissertation had focused on the correlations between uterine immune response in the post-partum period to the systemic immune response by analyzing serum biomarkers of inflammation. Up to this date, no references for cytokines concentration in uterine fluids of puerperium sows can be found in the literature. Therefore, it set the stage for future research in this area. Gestating and lactating sows are very susceptible to inflammation and oxidative stress. Therefore, a better understanding of antioxidant capacity and its relationship with uterine inflammation is needed. Moreover, it is known that mucosa immune systems crosstalk occurs. Gut associated lymphoid tissues are the center of investigation as it is the larger immunological tissues in organisms. Nevertheless, digging into reproductive-respiratory-gastrointestinal tracts crosstalk may reveal a large open field for research, as it can influence responses to diseases, vaccination, and feed additives.

Cytokines are final products of many activated cells during immune response. In this study the cytokines profiles were the center of the research. However, other information could contribute even more to understand and characterize the immune responses following parturition. For instance, polymorphonuclear cells could be counted, marked, and clustered in the uterine fluids to indicate the extent of migration and infiltration into the endometrium mucosa. Ratios of $CD4^+/CD8^+$ cells in the serum could also help to explain the patterns of immune response as well as a simple leukogram analysis.

The approach used in the studies presented in this dissertation to develop non-invasive method to access biological responses in sows might be applied in another field. As mentioned above, the importance of gut associated lymphoid tissues is well known. Thus, there is large space

to search for methods and biomarkers of biological response in the gut, like inflammation, barrier disruption, and antioxidant capacity. Translational studies might be an optimal way to do so, as human medicine is advanced in the area. Additionally, field conditions research is more applicable to the industry while a greater number of animals can be used. For instance, non-invasive methods could be applied to identify many biological markers in hair, saliva, feces, urine, and uterine fluids. Other than biological markers, animal behavior evaluation is also a great potential field in animal research.

Epigenetics have gained attention in swine production. This area of research could also be applied in immunology studies in sows. As discussed in this dissertation, during pregnancy sows shift the type of immune response to predominate the tolerance status for the maintenance of pregnancy. However, in commercial situations, many factors can challenge the sows to respond with Th1 or Th17 type of immune response. For instance, immune response occurs due to leg, skin and feet lesions, vaccination, diseases outbreaks, dietary changes, heat stress, mycotoxins, and feed additives. Therefore, inflammation may have direct effects on the fetal development but also may modify the DNA as some inflammatory molecules can cross the placenta. It could be expected that maternal inflammatory process could impact several aspects in the offspring like nervous system development, immunological responses, and susceptibility to different stressors later in their lives. For instance, if that occurs in the window of muscle fiber development it could impact the growth potential of offspring or even carcass traits.

Finally, the SCFP in the liquid form used in this study potentially can be tested in other challenging situations in pig production. The well-known post-weaning disorders in young pigs might be a great example for future research with SCFP via drinking water as these pigs are likely to drink even though not eating. These SCFP might also have interesting effects in vaccination

responses. For instance, the product could be tested in gestating gilts evaluating vaccination efficacy and the effects in the offspring. Like other eubiotics, SCFP can potentially have positive effects in finishing pigs, especially at the windows of antibiotics withdraw before marketing. Additionally, as sows had benefit from SCFP during lactation, immunomodulatory effects of SCFP may also be beneficial to mature boars.